Origin, evolution, and biodiversity of the Neotropical herpetofauna

Patterns and processes of the world's richest and most threatened biota

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To my family, to my friends

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ABSTRACT

The biological diversity of tropical America (the Neotropics) is astonishing. However, even among terrestrial vertebrates, most biogeographical patterns are not fully described nor understood, especially for many Neotropical reptiles and amphibians (the herpetofauna). To understand the evolutionary processes that gave rise to this incredible diversity, it is necessary to map the geographical distribution of multiple species. Furthermore, biogeographical analyses that integrate phylogenetic information provide the means to disentangle the roles of geography and environment in shaping biodiversity patterns. Herpetofaunal groups are very diverse in the region, occupying a wide range of habitats and niches, making them key organisms to understanding the origins of Neotropical biodiversity. My goal in this thesis is to understand biogeographical patterns and processes underlying this diversity. For this, I aim to: (1) provide novel taxonomic assignments and mapping of the distribution of snakes in the region, (2) test the role of geographical and environmental distances in the patterns of phylogenetic regionalization in reptiles and amphibians in the Cerrado savannas, (3) map endemism patterns for all Neotropical snakes, (4) investigate the origins and assembly of Neotropical savannas, and (5) apply biodiversity indices to guide conservation. The results indicate that in the Cerrado savannas, geography and environment affect the distribution of reptile and amphibian lineages in different ways, resulting in distinct patterns of phylogenetic regionalization. Also, biodiversity patterns in the Cerrado region were shaped in the context of a much more recent appearance of savanna ecosystems in comparison to tropical forests, as shown from several lines of evidence. The main contribution of this thesis is the mapping of narrowly distributed snake diversity in Central America, the Andean mountains, the Caribbean Islands, and the Atlantic Forest. The topographical complexity of these regions is the main predictor of both ancient and recent endemism. By describing diversity patterns of the Neotropical herpetofauna, I hope to contribute to the understanding of critical biogeographical patterns and processes underlying one of the world's richest biotas.

Keywords

Amazonia, Cerrado, phylogenetic endemism, phylogenetic turnover, integrative taxonomy, biodiversity indices.

SVENSK SAMMANFATTNING

Den biologiska mångfalden i tropiska Amerika (neotropikerna) är häpnadsväckande. För att förstå de evolutionära processerna som gav upphov till denna otroliga mångfald är det nödvändigt att kartlägga den geografiska spridningen av flera arter. Biogeografiska analyser som integrerar fylogenetisk information gör det dessutom möjligt att reda ut geografins och miljöns roller i den biologiska mångfaldens utveckling. Dock är dessa biogeografiska mönster inte fullt beskrivna eller kända för många organismgrupper, till och med för landlevande ryggradsdjur, och speciellt för många neotropiska reptiler och amfibier (herpetofaunan). Grupper inom herpetofaunan i regionen är mycket artrika och upptar ett brett spektrum av livsmiljöer och nischer, och de blir därmed nyckelgrupper för att förstå ursprunget till den neotropiska mångfalden. Mina mål med den här avhandlingen är att: (1) bidra med nya taxonomiska tilldelningar och kartläggningar av organismer i regionen (ormar), (2) testa vilken roll geografiska och miljömässiga avstånd spelar för fylogenetiska regionaliseringsmönster (reptiler och amfibier), (3) kartlägga var koncentrationerna av snävt spridda arter finns över hela neotropikerna (ormar), och (4) undersöka ursprunget och sammansättningen av neotropiska ekosystem (tropiska savanner), samt (5) applicera olika biodiversitets mått för att guida artbevarande. Resultaten indikerar att geografin och miljön på Cerrado-savannen påverkar spridningen av reptila och amfibiska släktlinjer på olika sätt, vilket resulterar i distinkta mönster av fylogenetisk regionalisering. Dessutom formades den biologiska mångfalden i Cerrado-regionen i samband med en mycket senare uppkomst av savannens ekosystem jämfört med tropiska skogar, vilket framgår av flera bevislinjer. Huvudbidraget i denna avhandling är kartläggningen av snävt fördelad ormdiversitet i Centralamerika, Anderna, Karibiska öarna och i Atlantskogen. Den topografiska komplexiteten i dessa regioner är den främsta prediktorn för både forntida och nutida endemism. Genom att beskriva mönstren av biologisk mångfald i den neotropiska herpetofaunan hoppas jag kunna bidra till förståelsen av kritiska biogeografiska mönster och processer som ligger till grund för en av världens rikaste flora och fauna.

MANUSCRIPTS IN THIS THESIS

On the Cerrado savannas

I – Josué A. R. Azevedo, Rosane G. Collevatti, Carlos A. Jaramillo, Caroline A. E. Strömberg, Thaís B. Guedes, Pável Matos-Maraví, Christine D. Bacon, Juan D. Carrillo, Søren Faurby, Alexandre Antonelli. In: Rull, V., Carnaval, A. Neotropical Diversification. *On the young savannas in the land of ancient forests*. Springer. In press.

II – Josué A. R. Azevedo, Cristiano de C. Nogueira, Paula H. Valdujo, Søren Faurby, Alexandre Antonelli. *Contrasting patterns of phylogenetic turnover in amphibians and reptiles are driven by environment and geography in Neotropical savannahs*. Manuscript.

On Neotropical snakes

III – Daniel F. Gomes, **Josué A. R. Azevedo**, Roberta Murta-Fonseca, Søren Faurby, Alexandre Antonelli, Paulo Passos. *Taxonomic review of the genus Xenopholis Peters, 1869 (Serpentes: Dipsadidae), integrating morphology with ecological niche*. Manuscript.

IV – Josué A. R. Azevedo, Thaís B. Guedes, Cristiano de C. Nogueira, Paulo Passos, Ricardo J. Sawaya, Ana L. C. Prudente, Fausto E. Barbo, Christine Strüssmann, Francisco L. Franco, Vanesa Arzamendia, Alejandro R. Giraudo, Antônio J. S. Argôlo, Martin Jansen, Hussam Zaher, João F. R. Tonini, Søren Faurby, Alexandre Antonelli. *Museums and cradles of diversity are geographically coincident for narrowly distributed Neotropical snakes*. Manuscript re-submitted to Ecography (minor revision).

Conservation and herpetofauna elsewhere

V – Harith Farooq, **Josué A. R. Azevedo**, Francesco Beluardo, Cristóvão Nanvonamuquitxo, Dominic Bennett, Justin Moat, Amadeu Soares, Søren Faurby, Alexandre Antonelli. *WEGE: a new metric for ranking locations for biodiversity conservation*. Manuscript submitted to Conservation Biology.

MANUSCRIPTS AND PUBLICATIONS NOT INCLUDED IN THIS THESIS

VI – Josué AR Azevedo, Paula H Valdujo, Cristiano de C. Nogueira (2016) *Biogeography of anurans and squamates in the Cerrado hotspot: coincident endemism patterns in the richest and most impacted savanna on the globe*. Journal of Biogeography.

VII – Alexandre Antonelli, María Ariza, James Albert, Tobias Andermann, **Josué A. R. Azevedo**, Christine Bacon, Søren Faurby, Thais Guedes, Carina Hoorn, Lúcia G. Lohmann, Pável Matos-Maraví, Camila D. Ritter, Isabel Sanmartín, Daniele Silvestro, Marcelo Tejedor, Hans ter Steege, Hanna Tuomisto, Fernanda P. Werneck, Alexander Zizka, Scott V. Edwards. (2018) *Conceptual and empirical advances in Neotropical biodiversity research*. PeerJ.

VIII – Alexander Zizka, Daniele Silvestro, Tobias Andermann, **Josué A. R. Azevedo**, Camila D. Ritter, Daniel Edler, Harith Farooq, Andrei Herdean, María Ariza, Ruud Scharn, Sten Svantesson, Niklas Wengström, Vera Zizka, Alexandre Antonelli (2018) *CoordinateCleaner: Standardized cleaning of occurrence records from biological collection databases*. Methods in Ecology and Evolution.

IX – Alexander Zizka, **Josué A. R. Azevedo**, Elton Leme, Beatriz Neves, Andrea Ferreira da Costa, Daniel Caceres, Georg Zizka. *Biogeography and conservation status of the pineapple family (Bromeliaceae)*. Manuscript submitted to Diversity and Distributions.

X – Paulo Passos, **Josué A. R. Azevedo**, Cristiano C. Nogueira, Ronaldo Fernandes, Ricardo J. Sawaya. *An Integrated Approach to Delimit Species in the Puzzling Atractus emmeli Complex (Serpentes: Dipsadidae)*. Herpetological Monographs. In press.

XI – Thaís Guedes, **Josué A. R. Azevedo**, Christine Bacon, Diogo Provete, Alexandre Antonelli. In: Rull, V., Carnaval, A. Neotropical Diversification. *Diversity, endemism, and evolutionary history of montane biotas outside the Andean region*. Springer. In press.

INTRODUCTION

In an expedition to Amazonia in 1848, A. R. Wallace noticed that closely related species of monkeys, birds, and butterflies sometimes occur on different sides of large rivers. From this observation, he asked several questions that we are still trying to answer: *"Are very closely allied species ever separated by a wide interval of country? What physical features determine the boundaries of species and of genera? (...) What are the circumstances which render certain rivers and certain mountain ranges the limits of numerous species, while others are not? None of these questions can be satisfactorily answered till we have the range of numerous species accurately determined"* (Wallace, 1852). With the increasing knowledge about species distribution over the last century, many of these questions can be now more accurately addressed, and even long-standing hypotheses such as the riverine barriers in Amazonia are being tackled (Oliveira et al. 2017, Santorelli et al. 2018).

The wonderful biological diversity of the tropical America both arouses the curiosity of naturalists as well as challenges the limits of our knowledge. The **Neotropical region** is one of the eight (or so) biogeographical realms, roughly spanning from central Mexico to Argentina, including the Caribbean (Olson et al. 2001, Morrone 2018, Fig. 1). Within this region, there are some of the richest and most threatened ecosystems on Earth. The Amazon rainforest and the Andean associated ecoregions concentrate up to 25% of all species of vascular plants (Govaerts 2001). The Cerrado savannas in South America harbour the highest number of plants species when compared to any other savanna worldwide (Cole 1986, Klink and Machado 2005). The tropical forests in Central America are well known for the extremely high levels of species endemism for many terrestrial organisms (Zuloaga et al. 2019). To answer how these extreme biodiversity numbers came to be, it is necessary to analyse biogeographical patterns of organisms beyond what is currently known for plants, mammals and birds.

Reptiles and amphibians – collectively referred to as the **herpetofauna** – may provide essential clues on the evolution of the Neotropical biota due to their astonishing diversity in the region. Herpetofaunal groups are generally sampled and studied together by the same set of investigators. This system is in part a historical coincidence but at the same time provides an opportunity for comparative studies of extremely different groups of organisms. This thesis is about the biogeographical patterns of Neotropical squamate reptiles (lizards, amphisbaenians, and snakes) and anuran amphibians (frogs and toads).

The number of Neotropical reptile and amphibian species is larger than in anywhere else in the world (as it is known to this date). The diversity of frogs in the region is proportionally the most extreme in terms of the total number of species than any other herpetofaunal group, with more than 3,000 species, roughly 50% of the world' amphibians (Bolaños et al. 2008, Frost 2019). The diversity of Neotropical lizards (non-snake squamates) is only surpassed by the Australasia region (Roll et al. 2017). Also, the Neotropical region harbours about one-third of all described species of snakes (~1,300 of ~3,800). Finally, most species of these groups are distributed in the Central American forests, Amazon basin, Andean associated ecoregions, the Atlantic Forest, and the Cerrado savannas (Buckley and Jetz 2007, Roll et al. 2017, Guedes et al. 2018) (Fig. 2a for snakes).

Due to their incredible ecological diversity and rich natural history, the distribution patterns of snake species in the Neotropics encompasses most of the biogeographical patterns found for the other herpetofaunal groups. For example, there are widespread species like *Boa constrictor,* found across virtually all tropical South America (Card et al., 2016). Several species of pit vipers can be found all over the entire American continent with varying range-sizes, from species limited to a single small island (e.g., *Bothrops insularis*) to the entire Amazonia and the Atlantic Forest (e.g., *Epicrates cencria*). Not only islands or biomes limit the distribution of snakes, as there are also several species restricted to small mountains or plateaus (e.g., *Apostolepis* spp. in the Cerrado savannas) (Azevedo et al., 2016). All this variety of range sizes and habitat-use may have resulted in uneven patterns of diversity still to be determined on a continental scale.

High levels of species diversity are not only restricted to the tropical forests in the region. A single locality in the Cerrado savannas may harbour more than 61 species of snakes, 54 species of frogs, and 26 species of lizards (França et al. 2008, França and Braz 2013, Santoro and Brandão 2014, Colli et al. 2016). Although a lot has been described concerning local diversity of reptiles and amphibians, explanations for the evolution of the entire Neotropical diversity may lie in the turnover of species from site to site, and in patterns of endemism, which are still to be fully described.

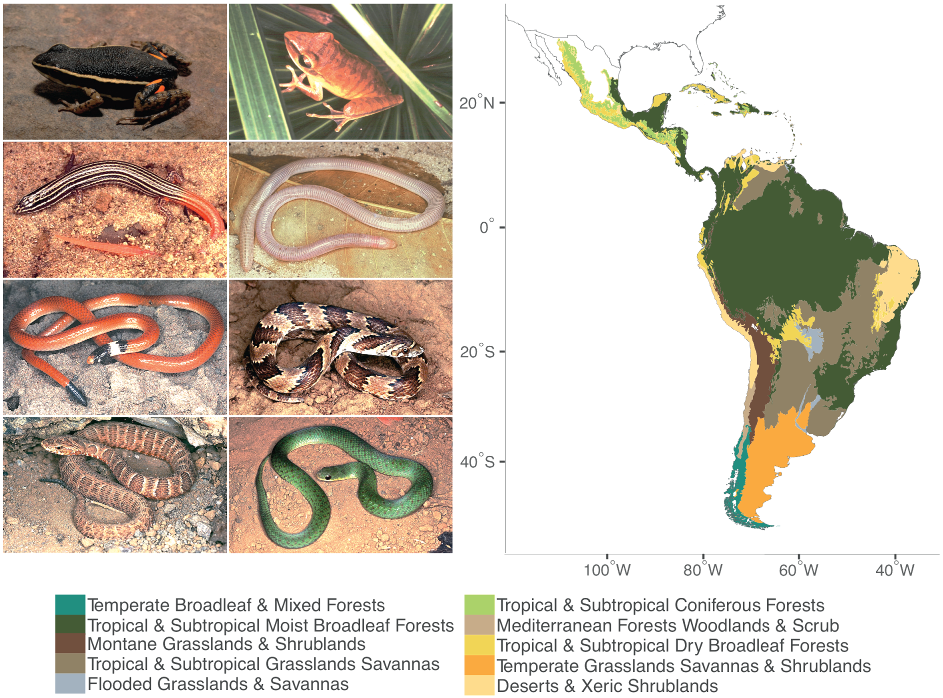


Figure 1. The distribution of biomes in the Neotropical region and representatives of the Neotropical herpetofauna. Photos by Cristiano Nogueira and Paula H. Valdujo.

From left-to-right, top-to-bottom: *Ameerega berohoca*, *Hypsiboas albopunctatus*, *Vanzosaura savanicola*, *Amphisbaena ibijara*, *Apostolepis cearensis*, *Xenodon nattereri*, *Bothrops itapetiningae* & *Erythrolamprus typhlus*. All these species are present in the Cerrado savannas (Chapter II).

The origins of the Neotropical region

The geological and climatic histories of the Neotropics are key to understanding its astonishing diversity (Antonelli and Sanmartín 2011). First, South America remained isolated from all the other landmasses throughout most of the Tertiary (~60 - 10 Ma), evolving a unique highly endemic biota (Simpson 1980, Bacon et al. 2016). This part of the Neotropics remained mostly situated in the tropical zone since the Gondwana breakup, supporting the continued existence of vast extents of forested biomes (Mittelbach et al. 2007). Second, the most highlighting aspect of the South American topography is the Andes, with an extensive geological history since the Cretaceous (Antonelli et al. 2009). The presence of the Andes shaped the evolution of South American biotas by increasing habitat-isolation, habitat-heterogeneity, and faunal duplication on both sides of the range (Hoorn et al. 2010). Additionally, the Guiana Shield and the Brazilian

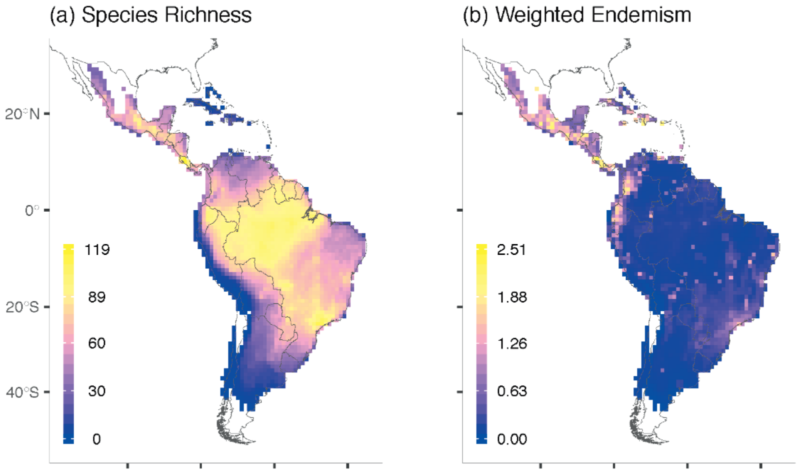
Highlands by the Atlantic coast are even more ancient than most parts of the Andes, possibly providing stable orographic precipitation during the entire Cenozoic (66-0 Ma), thus locally buffering the effects of climate changes (Safford 1999).

The geological history of the northern portion of the Neotropics is very complex and not fully understood. This region is the result of the junction of four main tectonic plates (Marshall 2007). The northernmost parts of the Neotropics (i.e., central Mexico) are geologically part of North America, and have remained close to subtropical zones since the Cretaceous (van Hinsbergen et al. 2015). This area also contains ancient mountain ranges oriented in the latitudinal axis, making it an environmentally heterogeneous region where several relictual taxa are found (Mastretta-Yanes et al. 2015). On the other hand, the lower Central America is composed of several independent geological units, some of them submerged until the Miocene (~23 Ma). The current geological configuration of this region as a narrow stretch of land between two seas, intersected by a central chain of mountains and volcanos is probably one of the primary causes of the extreme levels of endemism found in this region (Zuloaga et al. 2019).

Another important factor explaining the high biodiversity of the Neotropics is the interchange between South, Central, and North American biotas – the Great American Biotic Interchange – contributing to the formation of an enormous pool of lineages (20 - 2.5 Ma; Bacon et al. 2015). Different from what is known for mammals and birds, the continental interchange of reptiles and amphibians did not involve the direct colonization of species that were typical from North America into South America and vice versa, but it was instead a process of diversification of lineages typical of each landmass in Central America, followed by diversifications of the resulting Central American lineages and only then, the dispersal to the other landmasses (Vanzolini and Heyer 1985).

The Origins of the Neotropical herpetofauna

The Neotropical herpetofauna have a mixed history of origins, with part of the current lineages being **autochthonous**, that is, derived from taxa originally present in the landmasses that were part of Gondwana (or Laurentia in the Mexican portion), whereas other lineages reached the American continent through land connections with Eurasia or long-distance dispersal across the Atlantic Ocean.


(a) Species Richness

(b) Weighted Endemism

Figure 2 – Patterns of species distribution in Neotropical snakes (From Chapter IV, Supplementary Information).

Most lineages of Neotropical frogs have probably been present in the landmasses that gave rise to the current Neotropical region since the Mesozoic Era (> 66 Ma, e.g., the Gondwanan Pipidae and the South American Leptodactylidae) with very few Eurasian colonizers (e.g., Ranidae) (Duellman 1979). The history of success of Neotropical frogs can be better exemplified by the toads of the family Bufonidae, which dispersed from South America and diversified in all continents but Australia in 10 million years (Pramuk et al. 2008). On the other hand, the two other amphibian groups are generally less representative in terms of species diversity and are not studied in this thesis: Caecilians, which are an ancient Gondwanan group (Pyron 2014), and Salamanders, with only one family represented in the Neotropics (Plethodontidae) (Duellman 1999).

Lizards are a paraphyletic group of Squamata reptiles (in relation to Serpentes - snakes), of which most families and subfamilies are autochthonous to the Neotropics (Vanzolini and Heyer 1985). Similar to frogs, very few (if any) lineages of lizards colonized the Neotropics coming from Asia through North America during the Tertiary (Estes and Báez 1985). Instead, the non-autochthonous stock of lizards in the region was originated from notable cases of long-distance dispersal. As an example, all South American skinks (Scincidae) are derived from an African ancestor that crossed the Atlantic Ocean some 30 million years ago (Pereira and Schrago 2017). Also, some Neotropical geckos are derived from African clades that arrived in

South America around the timing of the arrival of monkeys and caviomorph rodents (Gamble et al. 2011). Lizards thrive not only in forests but also in dry biomes (Pianka and Vitt 2003). This wide range of habitats is reflected in the presence of closely related lizard species in both savannas (e.g., Cerrado) and forests (e.g., Amazonia, Fig. 3).

Figure 3 – *Kentropyx calcarata*. Lizards of the genus *Kentropyx* are an example of taxa with closely related species found both in the Cerrado savanna as well as in Amazonia and in the Atlantic Forest. Drawing by Lisa Selin.

The Neotropical snakes

The global origin of snakes dates back to the Early Cretaceous (~128 Ma) (Hsiang et al. 2015), and one of the first typical Neotropical snakes appeared in the fossil record of the Paleocene with *Titanoboa cerrejonensis* (Boidae, 58 Ma), the largest snake ever recorded. However, this early fauna of Neotropical snakes was mostly composed of clades that do not dominate current assemblages (e.g., anilioids, tropidophiids, and the extinct madtsoiidae) (Rage 2008). According to both fossil records and molecular dating, it was only during the Miocene (~23 Ma) that the Neotropical snake assemblages began to look modern, with the appearance of vipers, colubrids, and elapids (coral snakes) (Albino and Montalvo 2006, Kelly et al. 2009). These clades probably dispersed to the American continent from Eurasia during the Oligocene/Miocene periods (Vanzolini and Heyer 1985). Therefore, different from most lizards and amphibians, the high temporal turnover of snake lineages during the Paleogene (66 - 23 Ma) is relevant to explain the current diversity of this group.

Although most of the aforementioned clades of snakes have been present from North America to Patagonia at some point since the Miocene, the current distribution of their species indicates different centres of diversity for each clade (e.g., Cadle and Greene 1993). In this way, distant sites with similar levels of species richness or endemism may present high phylogenetic dissimilarity and possibly uneven concentrations of ancient and recently diverged clades (Chapter IV).

Measuring biodiversity

Unveiling the biogeographical origins of distinct taxa is just the first step toward understanding current biodiversity patterns across geographical scales. First, on a local scale, biological communities are structured according to different factors, including habitat-use, dispersal limitation, and niche filtering (Wiens and Graham 2005, Chase and Myers 2011). For example, in sites with sandy-soils in the Cerrado savannas, the community of reptiles is composed of several species adapted to sand-diving or borrowing (e.g., featuring blunt snout and smooth scales) (Recoder et al. 2011). Several of these species are range-restricted to these sites in northern Cerrado, resulting in patterns of endemism. Nearby sites in the region may present very distinct reptile communities (e.g., flat lizards on rock outcrop sites) (Werneck et al. 2015). Species turnover between these sites may indicate the effects of niche filtering (if no intervening geographical barrier is present). On a regional scale, the increase in geographical or environmental distances among localities generally leads to increasing species turnover. Finally, on a continental-scale, the turnover of species may result in regionalization patterns (Moura et al. 2017a). Therefore, endemism and turnover not only synthesize how biodiversity is distributed across several scales but also provide clues on the processes generating it.

Phylogenetic information

A deeper understanding of biodiversity and the historical processes that shaped it to the current patterns can be obtained when including phylogenetic relationships in the picture. (Rosauer et al. 2009). First, different species concepts and schools of taxonomy (splitters versus lumpers) may introduce undesirable noise when mapping biodiversity patterns (Faurby et al. 2016). Species from different taxonomic groups may not be not directly comparable due to different degrees of separation of their evolutionary history, resulting in varying amounts of genetic diversity and morphological variation (De Queiroz 2007, Hughes et al. 2008). As a very well-known consequence, regions containing similar numbers of species may contain very different amounts of phylogenetic information (Rosauer et al. 2014). Thus, sites with the same levels of species endemism or species turnover may have very distinct levels of diversity if measured in terms of phylogenetic branches or genetic distances.

Patterns of turnover and regionalization

By measuring the turnover of phylogenetic branches among regions, it is possible to infer the degree of connectivity and isolation between two biotas through the evolutionary history (Antonelli 2017). For example, the fauna of snakes of Cuba, the Sierra Madre Occidental in Mexico, Patagonia in Argentina, and the Chilean Matorral do not share a single common species of snake. However, with phylogenetic turnover, it is possible to infer that Argentinian and Chilean faunas are just a few million years apart, whereas the Cuban fauna is more phylogenetically distinct from the Mexican fauna than the latter is from the Argentinian fauna, despite the greater geographic separation between them.

The increasing phylogenetic differences among sites at continental scales lead to phylogenetic regionalization (Daru et al. 2017). This phylogenetic differentiation is described as the amount of phylogenetic diversity (PD) shared among sites (Graham and Fine 2008). PD can be measured as the sum of the branch lengths within a minimum spanning path in a phylogeny among all taxa present in a site (Faith 1992). The dissimilarity in PD among sites or phylogenetic beta diversity is analogous to species beta diversity, and it is divided into two components: turnover and nestedness. For demonstration, the equation 1 (Leprieur et al. 2012), shows a simplified version of phylogenetic beta diversity between sites a and b (contained in a set of sites – *total*). **Phylogenetic turnover** is a component of beta diversity that accounts for differences in the phylogenetic branches replaced among sites (Leprieur et al. 2012). On the other hand, the nestedness component of beta diversity accounts for differences in phylogenetic diversity related to losses of phylogenetic branches among different sites (instead of replacement). Thus, phylogenetic turnover is useful for delimiting phylogenetic breaks among regions without the effects of differences in species richness.

Phylogenetic beta diversity ($PBDiv$ - PhyloSor):

$$PBDiv = \frac{2PD_{total} - PD_{site\ a} - PD_{site\ b}}{PD_{site\ a} + PD_{site\ b}}$$

(Eq. 1)

Where PBDiv ranges from 0 (sites a and b share all phylogenetic branches) to 1 (no phylogenetic branches are shared).

Patterns of endemism

The geographical restriction of taxa to a particular area – endemism – is one of the key concepts in biogeography, providing unique information about mechanisms behind the evolution of biotas and insights on the geological history (Harrison 2013). For instance, one of the reasons why small island systems such as the Galápagos Islands and Hawaii are such notable natural laboratories for observing evolution is related to their high endemism and isolation (Lomolino et al. 2006). Hence, a simple comparison of which taxa are endemic to a specific island or not can be used to infer to dispersal abilities of distinct organisms and even provides clues on the geological history of the islands (Inger and Voris 2001). The simplified biotas of islands and their limited geographical extent (and sometimes age) also facilitate the study of speciation and adaptation of organisms. Furthermore, the greater diversity of continental biotas makes the understanding of evolution much more difficult (Wallace, 1902). Therefore, the study of a set of range-restricted taxa to a small geographic area can be expected to reflect processes observed in isolated organisms on islands, and yet provide a tentative simplification of the continental biota (Nelson and Platnick 1981).

The concept of endemism has developed over the years from a descriptive, specialist-based definition to less arbitrary measurements based on the compilation of species distribution data. By mathematically defining endemism in a site as the species richness weighted by the range sizes of the respective species (i.e., weighted endemism), the confusion with the concepts of endemism is partially solved (e.g., any taxon is by definition restricted to a particular area). As follows, **Phylogenetic Endemism** (PE) is a measure of the sum of the phylogenetic branch lengths of taxa occurring in a particular area, weighted by the geographical ranges of each branch (Rosauer et al. 2009). This metric is equivalent to calculate the proportion of range-restricted phylogenetic diversity to a region (PD as in Faith 1992).

Phylogenetic endemism formula:

$$PE = \sum_{\{l \in L\}} \frac{Branch\ lenght_l}{Range_l}$$

(Eq. 2)

Where *l* corresponds to a phylogenetic branch and *L* refers to the branches included in the minimum spanning path among all taxa occurring in a site. This metric is equivalent to calculate the proportion of range-restricted phylogenetic diversity to a region (PD as in Faith 1992).

Information on phylogenetic relationships among taxa also enables inferring the age of endemism patterns. **Neo-endemics** or recently diverged, narrowly distributed taxa may indicate areas where recent speciation events have occurred (Lamichhaney et al., 2018). For example, the golden lancehead (*Bothrops insularis*) occurs on a small island off the Brazilian coast. This species probably diverged from the mainland *B. jararaca* during the Late Pleistocene (less than 126.000 years ago), and in such a short period developed new dietary preferences (birds instead of rodents), stronger venom, and different coloration (Wüster et al. 2005, Grazziotin et al. 2006). On the other hand, **paleo-endemics** or narrowly distributed taxa that are anciently diverged, provide clues onto past climatic and geological conditions (e.g., long-term geological isolation or climatic stability) or about lineages that have disappeared from most of their original ranges (Jones et al. 2009, Jordan et al. 2016). For example, the snakes from the Tropidophiidae family were widely distributed across the entire American continent and possibly even present in parts of Eurasia during the middle Eocene (~40 Ma, Head 2015). However, its current distribution now fragmented and relictual in South America (Fig 4).

Worldwide, the main patterns of endemism are coincident among several groups of terrestrial organisms such as plants, amphibians, reptiles, and birds (Kier et al. 2009), but not always coincident with the most diverse areas in terms of numbers of species (Orme et al. 2005). In general, primary productivity is associated with patterns of species richness, whereas environmental stability is associated with patterns of endemism (Jetz et al. 2004). Therefore, large scale comparative studies between patterns of species richness (or PD) and endemism (or PE) may also allow inferences on the potentially different evolutionary mechanisms implied in the formation of these patterns (Jetz and Rahbek 2002).

Figure 4 - Current geographical distribution of Tropidophiidae. This family of snakes was formerly distributed from Patagonia to North America and possibly even into Eurasia.

The current state of knowledge

Over the last two decades, the knowledge of the Neotropical herpetofauna has increased considerably. Significant gaps in the distribution of species have been filled, especially in South America. For example, biogeographical patterns were systemically studied for reptiles and amphibians in Cerrado (Nogueira et al. 2011, Valdujo et al. 2012, Azevedo et al. 2016), for snakes in Caatinga (Guedes et al. 2014), and for frogs and snakes in the Atlantic Forest (Moura et al. 2017b). Data-mining of several community-level studies led to the creation of a large species database for snakes in South America (Sawaya et al. 2008, Recoder et al. 2011, França and Braz 2013, Guedes et al. 2018). Lastly, in the case of snakes in Central America, the availability of data on online databases is particularly high (see Chapter IV, Guedes et al., 2018).

Despite these recent efforts in data collection, large-scale phylogenetic analyses that encompass a regional scale, leave alone the entire Neotropics, have yet to be performed. For example, large databases of specialist-derived polygons representing species ranges were used for mapping the worldwide distribution of all terrestrial vertebrates (Roll et al. 2017). However, levels of phylogenetic turnover and endemism are mostly unknown, especially for reptiles. Besides, on a regional scale, distribution data has to be even more accurate (e.g., presence records), and this is still far from being accomplished for most ecoregions of the Neotropics (e.g., for sampling gaps in snake distribution see Guedes et al., 2018).

OBJECTIVES

The main goal of this thesis is to use well-curated species distribution data integrated with phylogenetic information to analyse patterns of endemism and biological dissimilarity in the Neotropical herpetofauna. Additionally, this thesis provides a review of the origins of a relevant evolutionary arena for the Neotropical herpetofauna (the tropical savannas), and a study case on how to produce detailed information of taxonomy and distribution of species. Finally, in a related context, the use of species distribution data is applied in conservation prioritization.

More specifically, this thesis addresses the following questions:

1. How old are Neotropical savannas?
2. Are the biogeographical patterns of reptiles and amphibians in the Neotropical savannas congruent?
3. How to integrate morphology and environmental niche information to delimit species boundaries?
4. What are the portions of the Neotropics with highest concentrations of narrowly distributed snake lineages?
5. How can we better select key areas for conservation of biodiversity?

METHODS

Study groups and data sources

In this thesis, questions on the biodiversity patterns of distinct clades of reptiles and amphibians are tackled, in particular for snakes. **Chapter I** includes information on multiple organisms from the Neotropical savannas, such as vascular plants, arthropods, reptiles, mammals, and also from the fossil record (e.g., fossilised plant silica). This chapter is based on phylogenetic reconstructions and geographical information retrieved from the literature. **Chapter II** includes data on the geographical distribution and phylogenetic relationships of squamate reptiles and anuran amphibians in the Cerrado savannas of South America. **Chapter III** includes data on morphological characters and geographical distribution of the ground-dweller, secretive snakes of the genus *Xenopholis*. This data was obtained from specimens examined in zoological collections complemented by data retrieved from the literature. **Chapter IV** includes data on geographical distribution and phylogenetic relationships of all Neotropical snakes. **Chapter V** includes data on Afrotropical reptiles and amphibians based on original fieldwork in Mozambique.

Occurrence records

Occurrence records used in the analyses of **Chapters II, III,** and **IV** were partially compiled as part of this thesis. In **Chapter II**, presence records for species of reptiles and amphibians described after 2012 in the Cerrado savannas were aggregated to the already existing databases (Nogueira et al. 2011, Valdujo et al. 2012). The new data was published in Azevedo et al. (2016) (publication not included in this thesis). For **Chapter III**, the distribution data include mostly records examined from specimens in museums, with a minor contribution of records retrieved from the taxonomic literature. For **Chapter IV**, data was compiled by several different authors participating in the Atlas of Brazilian snakes (https://cnbiogeo.wixsite.com/cristiano-nogueira/atlas), complemented with literature records, additional voucher-verified specimens, and online databases (www.gbif.org/) for the remaining Neotropics published in Guedes et al. (2018). As part of this thesis, additional distribution data not included in Guedes et al. (2018) was generated from literature searches and careful cleaning of online databases (www.gbif.org/). Finally, for **Chapter V**, presence records were downloaded from online databases (www.gbif.org/) and used to complete records derived from fieldwork. All data will be made available through publications of the chapters in this thesis.

Phylogenetic information

Phylogenetic information was retrieved from fully sampled phylogenies for reptiles (Tonini et al. 2016) and amphibians (Jetz and Pyron 2018) in the **Chapters II**, **IV** and **V**. These phylogenies were based on time-calibrated backbone-trees estimated from molecular data. The remaining species without any molecular data available were randomly added within the genus to which they are taxonomically assigned (Thomas et al. 2013). Such random taxonomic assignments result in sets of alternative phylogenies that represent the many possible combinations of species relationships. One hundred of these phylogenies were sampled, and the resulting median values of each phylogenetic metric (e.g., phylogenetic diversity) were used in the subsequent analyses.

Data analysis

Most data preparation, analyses, and graphics were performed in R (R Core Team, 2019), an open software for statistics and graphics. Within R, several different packages were used for (1) preparing **spatial data**: raster (Hijmans 2019), sf (Pebesma 2018), rangeBuilder (Rabosky et al. 2016); (2) in various data estimations and **statistical tests**: rgeos (Bivand and Rundel 2019), betapart (Baselga et al. 2018), vegan (Oksanen et al. 2019), ecodist (Goslee and Urban 2007), gdm (Manion et al. 2018); (3) For handling **phylogenetic data**: treeman (Bennett et al. 2017), picante (Kembel et al. 2010), geiger (Harmon et al. 2007), and ape (Paradis et al. 2004); (4) for **graphics** ggplot2 (Wickham 2016); and (5) for **parallel processing**: pbapply (Solymos and Zawadzki 2019), and doParallel (Weston and Calaway 2019). The only exceptions were the analyses performed in R wrapping scripts to run Biodiverse, a Perl based set of software for spatial biodiversity analyses (Laffan et al. 2010). Analyses in Biodiverse included phylogenetic endemism, phylogenetic diversity, and several null distributions based on these two metrics in Chapter IV.

Mapping species ranges

Three different mapping strategies were used in this thesis. The simplest one was used in **Chapters II**, **III, and V,** consisting of the direct use of presence records of individual species or their assignment to a gridded area. For **Chapter IV**, species ranges were mapped as alpha-hulls, a geometrical representation of the outmost area encompassing a set of points (Fig 5). The alpha-parameter gives the degree of concavity allowed in the alpha-hull calculation. Depending on the distribution of the points (e.g., high or low densities), low alpha-values may produce more than one polygon, whereas increasingly higher values will converge to a minimum convex hull.

For the distribution of snakes, alpha-hulls were produced by sequentially increasing the alpha-parameter value until only one contiguous range per species was obtained (Rabosky et al. 2016). In the case of species with naturally disjunct ranges, for example, species occurring disjunctly in Amazonia and the Atlantic Forest, but not in the ecoregions in between, more than one polygon was allowed. For **Chapter III**, species distribution models were built to represent the range of suitable habitats. Models were produced using an ensembling of several different algorithms to represent the relations of presence records with climate and soil variables (Naimi and Araújo 2016).

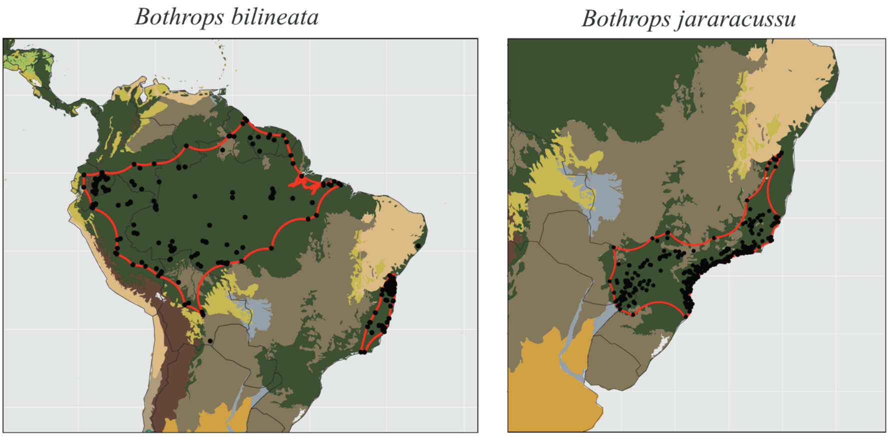


Figure 5 - Representation of alpha-hulls (red lines) around presence records (black circles) over a map of biomes. In green, tropical forests; the remaining colours represent different seasonally-dry biomes.

RESULTS AND DISCUSSION

Chapter I

The distribution of savanna ecosystems over ancient geological landscapes (e.g., in the Cerrado region, Fig. 6) contrasts greatly with the recent evolution of their woody vegetation, as inferred from molecular phylogenies. Unveiling the origins of the evolutionary arenas where the Neotropical herpetofauna evolved may also provide insights into the current biodiversity patterns of this group. This chapter presents a multidisciplinary overview of the origin, assembly, and expansion of Neotropical savannas.

From the fossil record, current evidence suggests that Angiosperm dominated tropical forests appeared a few million years after the extinction of the dinosaurs (~60 Ma). It was during this period and in this context that high CO2 concentrations and warm equatorial temperatures enabled the appearance of the largest snake ever, *Titanoboa cerrejonensis* (Boidae). On the other hand, the first recorded representatives of open ecosystems appeared much later in the fossil record (Eocene, ~38 Ma). A decrease in CO_2 concentrations occurred later, preceding the Miocene global cooling. Even then, the fossil record indicates that organisms typical of tropical environments (e.g., monkeys) were present in the high latitudes of Patagonia during the Middle Miocene (~15 Ma), suggesting a warm, forest-dominated South America.

Although the origins of savanna ecosystems are unclear, their expansion occurred only by the late Miocene (plant-silica microfossils and pollen data from Patagonia). The fossil record suggests the onset of savannas in northern South America only during the Pliocene (5–2 Ma), a period in which most woody plants of the Cerrado diversified. Interestingly, slightly older origins of clades characteristic of open ecosystems are indicated for lizard lineages in the Cerrado. Although evidence from multiple sources suggests that savanna ecosystems are more recent than forests, there are still substantial knowledge gaps concerning the diversification of organisms associated with the Neotropical savannas (e.g., legless lizards - Amphisbaena). Understanding the phylogenetic relationships of such key groups and the discovery of additional fossils are necessary for tracking the origins of the Neotropical savannas.

Figure 6 - The Cerrado: the savanna ecosystems and the ancient plateaus. Chapada dos Veadeiros, Brazil. Photo: Cristiano Nogueira.

Chapter II

Traditionally, reptiles and amphibians are studied together. As a consequence, naturalists have a unique opportunity to compare terrestrial vertebrates with entirely different biology evolving separately at least since the Carboniferous Period (> 300 Ma). In the Cerrado, most reptile species inhabit open savannas on well-drained plateaus, whereas amphibians are associated with vegetation close to river drainages. Every river drainage in the Cerrado is surrounded by a strip of gallery forest and associated wet grasslands. Additionally, in this region, the open vegetation surrounding river headwaters of plateaus and mountains are the main habitat for many amphibian species. Are these local habitat preferences reflected at the broader scale distributions of these organisms? This chapter seeks clues to this question using the phylogenetic turnover among sites for reptiles and amphibians.

Phylogenetic turnover in reptiles and amphibians is mostly not congruent, indicating that each group responds differently to geography and environment. Environmental filtering affects primarily the distribution of amphibians, whereas geographical distance is more important for reptiles. In particular, differences in the rate of turnover over geographical distance may be the result of the uneven distribution of habitats (Fig. 7). This lack of congruence is probably the combined result of distinct niche-filtering, dispersal limitation, and extinction rates, ultimately shaping large scale biogeographic regionalization patterns. In short, biodiversity patterns of a single group of organisms are poor surrogates for the entire biota.

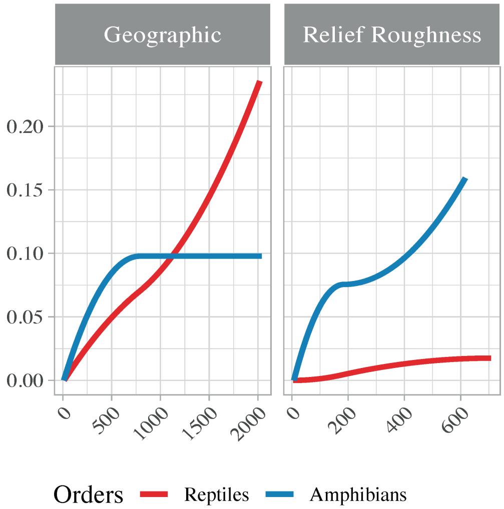


Figure 7. Phylogenetic turnover along geographical distance and elevational range (relief roughness). Open savannas are well distributed in the Cerrado region, thus the linear increase in turnover rates with geographical distance for reptiles. Headwater plateaus are mostly located in unevenly distributed areas with high relief roughness; hence, the nonlinear relation between turnover and distance for amphibians.

Chapter III

Despite the increasing amount of available data on species distribution in online databases, the accurate identification of voucher specimens in biological collections is still the gold standard in taxonomy. This procedure is especially critical for rare organisms for which tissue samples and consequently, molecular data are not readily available. In these cases, the detailed study of morphological characters to delimit species is essential. Additionally, the degree of ecological divergence among taxa (e.g., climatic niche overlap) and the mapping of suitable habitats in relation to potential geographical barriers may provide additional support for species delimitation. In this chapter, species distribution modelling and niche overlap analyses were used to complement traditional morphological approaches of species delimitation in the snake genus *Xenopholis* of South America.

The names currently allocated in the genus *Xenopholis* (*X. scalaris*, *X. undulatus* and *X. werdingorum*) were recognized as valid species based on the concordance between quantitative (meristic and morphometric) and qualitative (hemipenial and skull morphologies) characters with the ecological niche modelling. Each species has significantly distinct ecological niches from each other, corroborating the phenotypic evidence. All three species occur in the leaf litter habitats of different forests (i.e., Amazonia, the Atlantic Forest, and gallery forests in savannas; Fig. 8). Although the perceived rarity of *X. undulatus* is mostly based on fieldwork notes, this pattern could be explained by the shrinkage of suitable areas for this species since the Last Glacial Maximum. In summary, this chapter provides a case study of how to produce the detailed species databases used in this thesis.

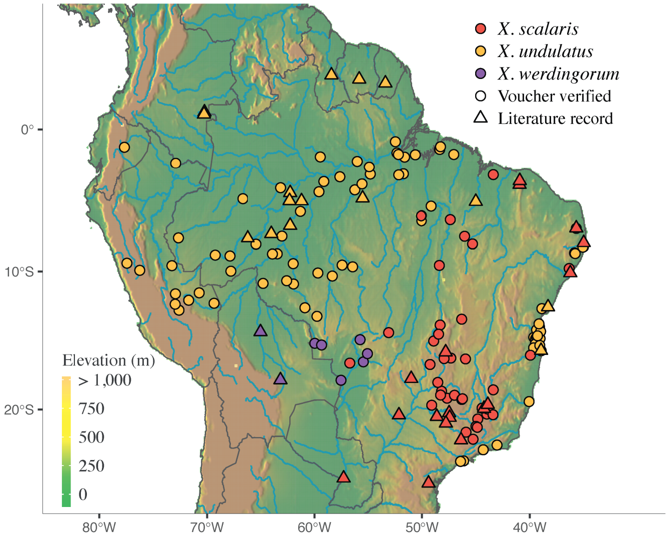


Figure 8 – Geographical distribution of the three species of the genus *Xenopholis* in South America.

Chapter IV

Few biodiversity patterns are as relevant in biogeography as endemism. Snakes are in general among the terrestrial vertebrates with the most elusive habits. To adequately sample localities for this group in tropical areas as well as producing sufficient species lists takes several years. As a consequence, it is only after centuries of biological collection and taxonomic work that it is now possible to provide continental-scale descriptions of biodiversity patterns for snakes. In this chapter, phylogenetic endemism of the Neotropical snakes is mapped. Additionally, the types of endemism (neo- or paleo-endemism) are determined as well as the main environmental correlates of geographic rarity.

We found that most phylogenetic branches that are geographically rare in Neotropical snakes are associated with the highlands of Central America, the Andean mountains, and the Atlantic Forest (Fig. 9). This pattern highlights the importance of variables such as relief roughness, climate buffering, and climate rarity in driving endemism patterns. Additionally, most of the areas with high phylogenetic endemism consist of both anciently diverged (paleo-endemism) and recently diverged phylogenetic branches (neo-endemism). Therefore, mountains are both museums *and* cradles of snake diversity in the Neotropics.

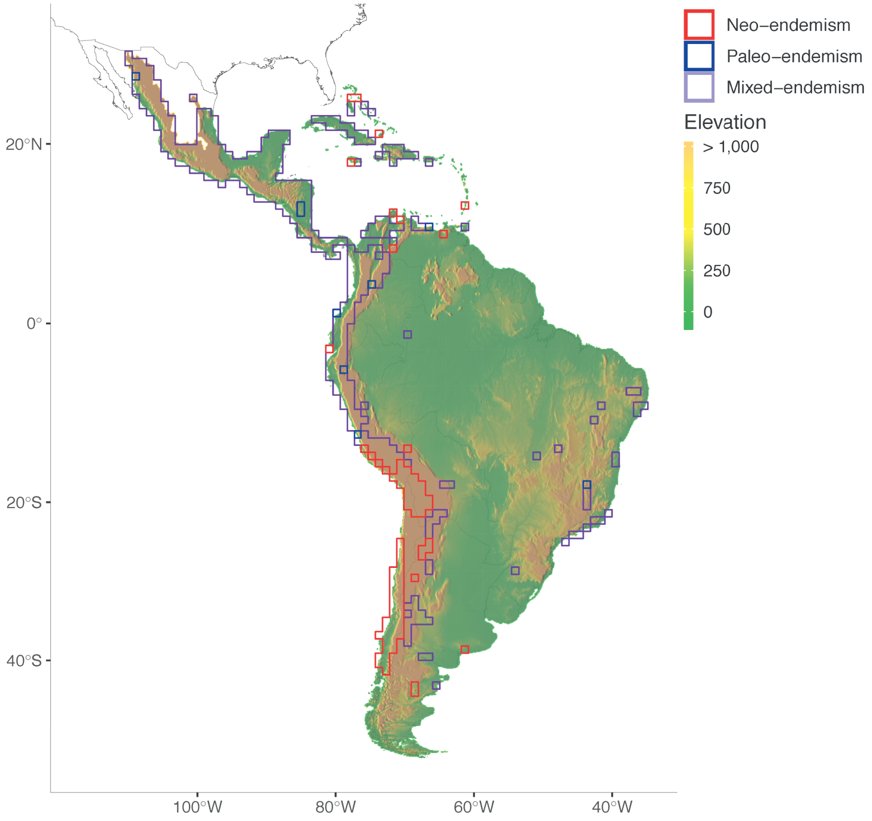


Figure 9. Phylogenetic endemism in Neotropical snakes. Mixed endemism denote areas with high paleo- and neo-endemism. Details in Chapter IV.

Chapter V

It is widely known that different biodiversity indices (BIs) provide conflicting outputs for conservation prioritization (Brooks et al. 2006). Although relevant, these indices do not incorporate important information on extinction risks used by conservation practitioners. In this chapter, different BIs are compared to indicate priority sites for conservation, including species richness, phylogenetic diversity, weighted endemism, and phylogenetic endemism. A new methodology for ranking key biodiversity areas (KBA) according to the International Union for Conservation of Nature (IUCN) criteria is suggested and compared to the aforementioned BIs.

For this study, a fieldwork survey was conducted in eight small inselbergs (under 1,200 m high) with similar areas (1-2 km^2) in Northern Mozambique. Inselbergs are relict relief units representing distinct habitats from their environmental context. In the case of the study sites of this chapter, they consisted of exposed bedrock and patchy vegetation. Amphibians (15 species) and reptiles (29 species) were sampled. A species that was formerly known from one locality (*Cordylus meculae*) and a potentially new species of Gecko were recorded.

None of the traditional BIs are able to provide an objective prioritization of the surveyed sites. Instead, the new index introduced in this chapter, “WEGE” (Weighted Endemism including Global Endangerment index), ranks sites according to IUCN criteria on a continuous scale, providing a transparent way for the decision-making process in conservation.

CONCLUSIONS

Almost two centuries have passed since Alfred Russel Wallace and other naturalists explored the biodiversity of Neotropical region. Since then, a massive amount of basic knowledge about species distributions enabled researchers to test several hypotheses concerning the evolution of entire clades, from local to continental scales. However, taxonomic work is still necessary for filling gaps in our knowledge about diversity and distribution, especially among reptiles and amphibians when compared to mammals and birds, which are considerably better studied. One of the contributions of this thesis is the careful taxonomic work the provides well-curated descriptions of the geographical distribution of snakes. Together with environmental data, modelling the potential distribution of species is a useful way of tackling the long-standing question of which physical features determine the ranges of species.

Our understanding of biodiversity patterns would not be complete without the use of phylogenetic methods, complementing the traditional species-based methods. In this line, endemism should be regarded as ‘the geographic rarity of that portion of a phylogenetic tree found in a given area’ as proposed by Rosauer et al. (2009). In this thesis, this concept was applied and revealed the importance of mountains and surrounding areas in the maintenance of anciently diverged as well as recently evolved Neotropical snakes. Furthermore, the comparison of phylogenetic turnover between reptiles and amphibians provided insights into how different responses to geography and environment are relevant to shaping large scale diversity patterns.

Biodiversity patterns are uneven in many different ways. Within the same groups of organisms, patterns of phylogenetic diversity and endemism may highlight geographically separated biological hotspots, as in the case of Neotropical snakes in Chapter IV. If compared between distinct groups of organisms, even the same biodiversity indices will highlight very distinctive biogeographical patterns, as in the case of reptiles and amphibians in the Cerrado. Therefore, different biodiversity levels and distinct sets of organisms should be considered whenever possible – whether the ultimate goal is to conserve based on the highest number, rarity, age, evolutionary divergence or spatial uniqueness of species, lineages, and communities.

CHAPTER CONTRIBUTIONS

Chapter I – In: Neotropical Diversification: *On the young savannas in the land of ancient forests*. JA conceived the idea, wrote the introduction and the outline of the manuscript. All other authors contributed writing about the topics outlined. JA wrote mostly about the diversity patterns in terrestrial vertebrates. JA wrote the final version connecting all topics.

Chapter II – *Contrasting responses to environment and geography drives phylogenetic turnover in amphibians and reptiles in Neotropical savannas*. JA conceived this study, provided empirical data, conducted all analyses, produced all graphics and wrote the manuscript.

Chapter III – *Taxonomic review of the genus Xenopholis Peters, 1869 (Serpentes: Dipsadidae), integrating morphology with ecological niche*. JA conducted only the analyses concerning species distribution modelling and niche overlap, produced all related graphics and wrote the corresponding parts of the manuscript (methods, results and discussion).

Chapter IV – *Museums and cradles of diversity are geographically coincident for narrowly distributed Neotropical snakes*. JA conceived this study, provided empirical data, conducted all analyses, produced all graphics and wrote the manuscript.

Chapter V – *WEGE: a new metric for ranking locations for biodiversity conservation*. JA participated in the fieldwork, contributed to the discussion about the traditional biodiversity indices (phylogenetic diversity, weighted endemism, phylogenetic endemism), and contributed revising the manuscript.

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I

In: Rull, V., Carnaval, A. Neotropical Diversification

On the young savannas in the land of ancient forests

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Abstract

Covering an ancient geomorphological landscape, and surrounded by some of the most diverse forests on Earth, the Neotropical savannas were once perceived by naturalists as ancient environments. However, current evidence suggests that tropical forests have existed in the Neotropics since the Paleocene and that most plant lineages present in South American savannas are recently derived from clades from the surrounding forested biomes. This chapter provides a multidisciplinary overview of the origin, assembly and expansion of Neotropical savannas, with focus on South America. For this, we consider available evidence from the fossil record, paleoenvironmental proxies (phytoliths), and phylogenetic information for both plants and animals. Paleoenvironmental reconstructions indicate suitable climates for central South American savannas since the middle Miocene, which is also when molecular phylogenies are used to infer the origin of some vertebrate groups typical of savannas. Fossil data indicate the ecological expansion of both C_3 and C_4 grasses in southern South America by the late Miocene. Fossil information indicates the onset of savannas in northern South America during the Pliocene, a period in which most woody plants of the largest extension of Neotropical savannas (the Cerrado) are thought to have diversified, as inferred by dated phylogenies. Although the combined lines of evidence indicate that Neotropical savannas in South America are indeed younger than their surrounding forests, the precise timing and factors that influenced the origin, assembly and expansion of Neotropical savannas remain contentious. Future research should aim at i) increasing and integrating knowledge about the diversification of important taxa characteristic to Neotropical savannas, ii) establishing continuous sequences of fossils, and iii) building accurate paleoenvironmental reconstructions for the entire Neogene.

Keywords: Biome origin, biome assembly, C_4 grasses, Cerrado, Llanos, phytoliths, molecular divergence times

Introduction

The tropical savannas of South America were once considered to be among the oldest ecosystems on the continent – a notion mostly based on their geographical distribution, especially in the Cerrado domain (Figure 1), which largely occurs on ancient geomorphological surfaces (Cole 1986; Ratter *et al.* 1997; Oliveira-Filho and Ratter 2002). The high species richness of the Neotropical savannas has also been interpreted as evidence of their antiquity, reflecting long periods for biological diversification over the Cenozoic (Cole 1986; Oliveira-Filho and Ratter 2002). However, this view has been challenged by molecular phylogenies inferred for vascular plants, which indicate a more recent origin of the lineages that currently inhabit the Neotropical savannas. According to those results, the diversification of Neotropical savanna lineages started only in the late Miocene and early Pliocene, *ca.* 10–4 million of years ago, mya (Pennington *et al.* 2006a, 2009).

In contrast, molecular evidence provides support for a much more ancient origin of Neotropical rainforest taxa, as exemplified by the radiation of major lineages of Malpighiales in the mid-Cretaceous, *ca.* 112–94 mya, and the origin of palms – characteristic of most current tropical rainforests – by *ca.* 100 mya (Couvreur *et al.* 2011). Although such lineages originated mainly in the Cretaceous, the establishment of rainforest ecosystems occurred only by the early Paleogene, as indicated by the fossil record (Davis *et al.* 2005; Wing *et al.* 2009). Consequently, South America may be considered a land of ancient forests co-occurring with relatively young savannas. However, the precise timing and factors that influenced the origin, assembly, and expansion of tropical savannas remain debated. For that, as exemplified by studies of the tropical forests, data should be sought from multiple sources (Antonelli *et al.* 2018b).

The term savanna has a wide and diverse use in the literature. Here we consider tropical savannas the ecosystems characterized by vegetation with continuous grassy ground cover, regardless of tree cover density, and with an ecological prominence of C_4 grasses as in the Cerrado *sensu lato* in central Brazil (the terms C_3 and C_4 refers to the different pathways distinct groups of plants use to capture CO_2 for photosynthesis with the last one being generally more related to plants found in warmer, drier and more seasonal climates) (Bourlière 1983; Sarmiento 1984; Lehmann *et al.* 2011). Globally, biomes in which tropical savannas predominate are also referred to as "savannas", with additional vegetation types occurring within the savanna biome (*e.g.,* gallery forests; Olson *et al.* 2001; Moncrieff *et al.* 2016). Here we use the term "domain" to refer to each of the geographically separated core areas of the savanna biome (Ab'Sáber 2003). The savanna domains of the Neotropics are mainly coincident with the "terrestrial ecoregions" of Olson *et al*. (2001) and the "biogeographical provinces" of Morrone (2017), but the two former classifications treat parts of the Amazonian and the Atlantic forest domains as distinct ecoregions and provinces, which is less convenient for our discussion in this chapter.

In the Neotropics, there are three main domains of the savanna biome: the Llanos and the Guianan domains in northern South America, and the Cerrado domain in central South America (Olson *et al*. 2001; Ab'Sáber 2003; Figure 1). There are also several smaller areas of savanna scattered across the Neotropics, such as within lowland Amazonia, in the Atlantic Forest, in Central America, and the Caribbean islands (Pennington *et al*. 2006b). For simplicity, in this chapter, we consider forests as any year-around vegetated ecosystems without a grassy ground cover, and with varying degrees of closed canopy (Lehmann *et al*. 2011). We distinguish between two main categories of forests: seasonally-dry tropical forest or SDTFs (*sensu* Pennington *et al.* 2000) and tropical rain forests. We use these simplified definitions of forests and savannas to gather data across a wider geographical and temporal extent. Although in the dry season SDTFs have a similar physiognomy to savannas, a significant factor distinguishing them is that savannas burn regularly and naturally, while SDTFs do not. This difference in fire regime is also reflected by their vegetation: fire-intolerant cacti, for instance, are particularly conspicuous in SDTFs (Banda *et al*. 2016). As these ecosystems and biomes are within the Neotropical region, they share approximately the same pool of lineages, making such simplifications less problematic than when comparing biomes across different continents (Corlett and Primack 2006; Moncrieff *et al*. 2016).

In terms of its geographical extent, its species richness, endemism, and floristic composition, the Cerrado domain can be considered the most cohesive of the Neotropical savannas (Silva and Bates 2002). It is mainly distributed across ancient well-drained plateaus (500 – 1500 m elevation), which are covered primarily by tropical savannas (Eiten 1972). It also extends across more recently dissected, adjacent peripheral depressions and valleys, where tropical savannas are less widespread. In some areas, they become replaced by SDTFs (Ratter *et al*. 1978). In contrast, the Llanos are not as geographically extensive and are located atop of relatively recently formed geomorphological surfaces, which were subject to sporadic marine transgressions during the Neogene (Diaz de Gamero 1996; Jaramillo *et al*. 2017b).

Additionally, the establishment of the climatic conditions associated with savannas in northern South America seems to be more recent. This recent formation is probably reflected in the relatively low species richness, and endemism observed in the savanna of these northern domains (particularly for animals, but less so for plants) relative to the Cerrado savannas (Huber *et al*. 2006). Nevertheless, even young Neotropical systems, such as the high-elevation Páramos, may be extraordinarily diverse (Madriñán *et al*. 2013).

Most phylogenetic information regarding the ages of origination, assembly, and expansion of Neotropical savanna clades are derived from plant lineages of the Cerrado domain. However, phylogenetic information based on molecular data can at most only provide hypotheses for the ages of origination and diversification of extant lineages. Those dates may closely reflect the biomes they currently inhabit (*e.g.*, Bytebier *et al*. 2011) but may also be of much more recent origin, and not necessarily reflect the ecological expansion of those biomes. The fossil record represents direct evidence, but, in the area currently occupied by the Cerrado domain, the number of available records is overall scarce; most of the information about the expansion of savannas or its fundamental components (*e.g.,* C_4 grasses) relates to the northern and southern parts of South America. Given the pros and cons of each data source, to understand the evolution of tropical savannas in South America, it is necessary to integrate available data from all suitable sources and from across the whole continent.

Our goal in this chapter is to review and integrate different lines of evidence that can inform on the evolutionary history of Neotropical savannas in South America. Specifically, we combine information from dated molecular phylogenies, the fossil record, and paleoenvironmental reconstructions (Figure 2) to better understand the origins, assembly and expansion of South American tropical savannas.

Evolutionary patterns of extant clades

Here we present an account of phylogenetic and biogeographical patterns of extant organisms, with a focus on the patterns of organisms in the savannas of the Cerrado domain, where such data are more complete. We do not aim at providing a comprehensive review, but rather highlight some telling examples. Beyond the importance of phylogenetic and biogeographical information derived from plant clades, the presence of animals adapted to specific environmental conditions such as precipitation seasonality, fire regimes, and open vegetation structure can also give clues on the evolution of tropical savannas.

Plants. Most phylogenetic evidence for the recent origin of tropical savannas is based on woody legumes (Fabaceae), which dominate most Neotropical biomes. In the Cerrado, the colonization of savannas by legume clades has been influenced by adaptations to fire and drought that emerged independently in several clades (Simon *et al*. 2009; Batalha *et al*. 2011; Simon and Pennington 2012). Thus, habitat shifts may have been significant in determining the high level of plant species and morphological diversity in savannas (Souza-Neto *et al*. 2016). For instance, species of the genera *Andira*, *Bauhinia, Lupinus, Stryphnodendron* and *Mimosa* have adapted to drought and fire, diversifying in savannas only recently from lineages of rainforest and SDTFs, *ca.* 10–4 mya (Simon *et al*. 2009; Simon *et al*. 2016; Souza-Neto *et al*. 2016).

Besides such within-genus recent diversification, clades of woody legumes that occur both in the Cerrado and in the Caatinga domains are generally older than the *in situ* Cerrado radiations (*e.g., Cassia* clade, *ca.* 18–9 mya, Souza-Neto *et al.* 2016). The same is true for some Neotropical SDTFs legume clades, such as *Coursetia, Poissonia,* and *Ruprechtia,* which are older than savanna clades within these genera, ranging from *ca.* 20–8 mya (Pennington *et al*. 2004; de Queiroz and Lavin 2011). The estimation based on dated phylogenies is also congruent with the fossil record, which shows that SDTFs were already present around 13 to 12 mya (Burnham and Johnson 2004). Taken together, an increasing amount of evidence for woody legumes supports the important role of probably more ancient ecosystems of the surrounding biomes in the assembly of Neotropical savannas.

Other plant clades corroborate the results found within the Fabaceae, such as the Bignonieae lineages Fridericia and Xylophragma, in the Cerrado (Lohmann *et al*. 2012). Within *Pradosia* (Sapotaceae), savanna lineages evolved even more recently, less than *ca.* 1 mya, and the adaptation to savanna dry conditions and fire involved significant morphological changes, such as the enlargement of woody structures underneath the soil (*geoxylic* habits, Terra-Araujo *et al*. 2015). Moreover, colonization of the Cerrado and other seasonal habitats has involved adaptations to drought and fire-tolerance in palm species such as *Sabal palmetto* and *Serenoa repens* (McPherson and Williams 1998; Abrahamson 2007). Further, while the palm lineages *Allagoptera* and *Attalea* (Arecaceae) diversified *ca.* 10 mya, the savanna lineages of this clade diversified only after *ca.* 1 mya (Freitas *et al.* 2016; Bacon *et al*. 2017). In addition, some species of *Allagoptera* show progressive shifts towards increasingly dry habitats through time, with origins in tropical rain forests, followed by colonization to SDTFs, then to the savannas of the Cerrado Domain, and, finally, to the sandy, coastal Restinga ecosystems (Bacon *et al*. 2017).

In summary, phylogenetic patterns of woody plants support the hypothesis of recent assembly of the savannas of the Cerrado domain, as proposed by Pennington *et al.* (2004; 2009). However, most plant diversity in savanna ecosystems is related to non-woody elements (more than 75% of all plant species; Filgueiras 2002). Therefore, we cannot reject the possibility that other plants in Neotropical savannas belong to lineages that are more ancient, or at least older than the woody elements of the Cerrado flora. For example, the typical open fields on rock outcrops in the higher elevation areas in the Cerrado domain ("*campos rupestres*") might include lineages potentially older and more diverse than the ones mentioned in this chapter (de Sousa *et al*, 2013). However, we do not cover in detail the evolution of the *campos rupestres* here, which might have involved adaptations more specific to the poor soils and relatively colder climates where this physiognomy occurs (Neves et al. 2018)

Arthropods. Most biogeographical studies of arthropods in South America examined diversification in the context of the eastern South American dry vegetation domains (Cerrado, Chaco and Caatinga), or the "South American Dry Diagonal" (Prado 1993; Werneck 2011). Most such studies rely on ancestral state reconstructions on molecular phylogenies, rather than the arthropod fossil record - which is scarce in the region. As such, they only provide indirect clues into the origins of the savannas in the region.

For example, phylogenetic analyses indicates that turtle ants (Myrmicinae: *Cephalotes*) had a burst in speciation beginning *ca.* 12 mya, followed by a significant increase in dispersal out of the Cerrado and Chaco (Price *et al*. 2014). Besides, around nine mya, the Cerrado and Chaco clades of *Zoniopoda* grasshoppers (Orthoptera: Romaleidae) diverged from each other (Pocco *et al*. 2018). Moreover, lineages adapted to wet environments of the eastern Andes and Atlantic Forest were apparently widespread until the appearance/expansion of Chaco and Cerrado vegetation. For instance, extant *Forsterinaria* Satyr butterflies (Nymphalidae: Satyrinae) from the Andes and southeastern Brazil diverged at *ca*. 7–11 mya, and ancestral state reconstruction suggests that dispersal across dry domains has become less likely towards the present than before 11 mya (Matos-Maraví *et al*. 2013). Similarly, the divergence of southeastern and northwestern South American Cayenne ticks (Acari: Ixodidae: *Amblyomma cajennense*) at *ca.* 10 mya may have been driven by the onset of drier climatic regimes in the Cerrado domain (Beati *et al*. 2013).

On the other hand, some arthropod clades of the Cerrado are derived from ancestral clades of the surrounding forested domains, instead of the "Dry Diagonal", as reported for butterflies and *Tropidopedia* bees (Brown and Gifford 2002; Aguiar and Melo 2007). Also, the Cerrado spiders of the genus *Oligoxystre* (Theraphosidae) and *Araneus venatrix* (Araneidae) have their closest relatives in the Atlantic Forest, the former diverging from its sister clade in the late Miocene (Guadanucci 2011; Peres *et al*. 2017). In general, studies that focused on extant arthropods indicate a late Miocene origin of taxa associated to savannas, with open domains acting as a strong geographical barrier for taxa that occur in the surrounding rain forest domains (Morrone 2006; Morrone 2014; Ferrari *et al*. 2015). However, the absence of data on extinctions, together with several gaps in our understanding of the origins of relevant ecological and functional groups (*e.g.* termites), prevent more conclusive answers about the origins of the South American savanna arthropods.

Tetrapods – Both mammals and birds show low levels of species endemism in the Cerrado (<10%; Macedo 2002; Marinho-Filho *et al.* 2002). In both groups, the oldest divergences of Cerrado lineages have been dated back to the late Miocene, with diversification inferred to the Pliocene and Pleistocene – thus temporally congruent with those reported for woody plants.

For birds, it has been hypothesized that the open ecosystems (both savannas and grasslands) of the Cerrado domain have been historically occupied by ancient endemic lineages, whereas younger endemic lineages have primarily occupied the forested areas (Silva 1997). The few published studies involving birds in the region indicate Pliocene (Passeriformes: *Neopelma*) or even Pleistocene (Passeriformes: *Paroaria capitate* and *P. gularis*) divergences between lineages from the Cerrado domain and lineages typical of Amazonia or the Atlantic Forest (Lopes and Gonzaga 2013; Capurucho *et al*. 2018).

Divergences between lineages of the open ecosystems of the Cerrado domain and those of other open domains in South America (mostly grasslands) occurred from the Miocene to the Pleistocene (Chaves *et al*. 2015), suggesting a more complex assembly of birds in the Cerrado domain than proposed by Silva (1997).

For mammals, one of the most ancient divergences for a Cerrado endemic has been estimated for the monotypic rodent genus *Calassomys* (Muroidea), *ca.* 7 mya (Pardiñas *et al*. 2014). The divergence time between the rodent genus *Podoxymys* (Muroidea) of the Tepuis and its closest relatives in the Cerrado has been used to suggest a connection between the central and northern savannas *ca.* 3 mya (Leite *et al*. 2015), although long-distance dispersal and extinction should not be disregarded to explain the same pattern (Fine and Lohmann 2018). It has also been proposed that still during the Pliocene, the Cerrado served as the center of diversification for the rodent genus *Calomys* (Muroidea; Almeida *et al*. 2007). For marsupials, a complex scenario has been inferred with groups colonizing the Cerrado domain from the Caatinga (*e.g.,* Didelphidae: *Thylamys karimii*), between the Miocene and Pliocene whereas lineages derived from Amazonia colonized the Cerrado during the Pleistocene (*e.g.,* Didelphidae: *Monodelphis domestica*; Pavan *et al*. 2016). In Neotropical primates, which are strongly associated with forests, there are cases of inferred diversification in savanna biomes during the Pleistocene, *ca.* 1–2 mya (Alfaro *et al*. 2015).

Amphibians and reptiles (collectively, the herpetofauna) are represented by more than 200 endemic species in the Cerrado domain, indicating their potential to unveil the history of South American savannas (Valdujo 2011; Werneck 2011; Nogueira *et al*. 2011; Azevedo *et al*. 2016; Guedes *et al*. 2018). It can be argued that due to their relatively low mobility, herpetofaunal lineages preserve signals of the first evolutionary events in the savannas of the Cerrado domain. The oldest divergence times of herpetofaunal lineages studied so far in the Cerrado date back to the early and middle Miocene. Available studies suggest, for example, that the anole lizard *Norops meridionalis* diverged from its Amazonian sister clade *ca.* 21 mya (15 mya lower bound of confidence interval; Guarnizo *et al*. (2016). Data also indicate that the savanna clade of the lizard genus *Kentropyx* also diverged from its Amazonian sister lineage during the middle to late Miocene (Werneck *et al.* 2009), similar to some of the leptodactylid frogs of the genus *Adenomera* colonized the Cerrado domain from Amazonia (Fouquet *et al*. 2014). The split of early diverging lineages in the region indicates a relatively early arrival of this group, before the origination and diversification of the woody plant species typical of today's savannas.

The topography of the region seems to have influenced the coincident patterns of geographical range restriction of herpetofaunal species in the highlands of Central Brazil (Azevedo *et al*. 2016). This is also suggested by phylogenetic studies of the lizard lineages *Ameiva, Kentropyx*, *Phyllopezus*, *Polychrus acutirostris*, and *Gymnodactylus amaralli*, as well as the frog genus *Rhinella* and snakes of the *Bothrops neuwedi* group (Werneck *et al*. 2009; Maciel *et al*. 2010; Thomé *et al*. 2010; Giugliano *et al*. 2013; Machado *et al*. 2013; Domingos *et al*. 2014; Fonseca *et al*. 2018). Although indicating relatively old initial divergences, geological and climatic events of the Pliocene and Pleistocene also appear to have affected the geographical distribution of lineages of the aforementioned clades, although we cannot disregard the influence of idiosyncratic colonization histories of individual clades (see discussion in Smith *et al*. 2014), nor the role of biotic interactions and other factors influencing the diversification of these taxa.

Inferences from the fossil record

While most information regarding extant groups of organisms of Neotropical savannas is derived from clades occurring in the Cerrado domain, the fossil record in this area is virtually absent for the proposed periods of origin and expansion of tropical savannas. For these reasons, we expand our focus to other regions of South America to find clues to the origin and expansion of environmental conditions and organisms associated with savannas.

Northern South America – In this region, the plant fossil record indicates that most of the areas currently covered by savannas were occupied by rainforest during the Paleogene (Jaramillo and Cárdenas 2013). The Neogene witnessed a massive expansion of several modern ecosystems, including savannas, which replaced areas formerly occupied by lowland forests. Several areas that nowadays are dominated by SDTFs and savannas were occupied by a different ecosystem during the early Neogene. For example, the upper Magdalena valley of Colombia was covered by humid forests *ca.* 13–11 mya (Kay *et al*. 1997), and northwestern Venezuela and northeastern Colombia were occupied by a humid forest up to at least the early Pliocene (*ca.* 3.5 mya) (Hambalek 1993; Hambalek *et al*. 1994; Jaramillo *et al*. 2015; Carrillo *et al*. 2018). The extensive savannas of the Llanos of

Colombia and Venezuela were occupied by a humid forest up to *ca.* 6 mya (Jaramillo *et al*. 2006; Jaramillo *et al*. 2017b), which, during glacial times (last *ca.* 2.7 mya), developed extensive sand dunes free of vegetation (Morales 1979; Khobzi 1981; Carr *et al*. 2015; Tripaldi and Zarate 2016). However, the magnitude of local dune fields, and their effect on modern savanna distribution are still poorly understood. Such northern South American sites indicate a recent expansion of dry climates and associated vegetation across a portion of the Neotropics.

Southern South America – The functional morphology of extinct endemic groups of native ungulates (meridiungulates) was traditionally used to reconstruct the ecological shift of forest to grasslands during the Cenozoic. Specifically, the evolution of meridiungulates with high-crowned teeth, a trait that characterizes herbivorous ungulates in grassland ecosystems today, was long interpreted as an indication of the expansion of grasslands or savannas in the middle Eocene of Patagonia (*e.g.,* Stebbins 1981; Jacobs *et al.* 1999). Analyses of middle and late Eocene fossil plant silica assemblages (phytoliths, see below), from fossilized soil horizons at Gran Barranca, Patagonia, Argentina, has supported this notion – pointing to the emergence of subtropical savannas already by *ca.* 40 mya (Mazzoni 1979; Zucol *et al.* 1999; Zucol *et al*. 2007; Zucol *et al*. 2010).

More recent and detailed studies have led to a very different interpretation of the paleosol biosilica record at Gran Barranca and nearby outcrops, namely that grasses were a relatively minor element on the landscape before the late Miocene (Strömberg *et al.* 2013; Strömberg *et al*. 2014; Selkin *et al*. 2015). This inference is consistent with the low relative abundance of grass phytoliths recorded in the outer shell of dung beetle ball trace fossils from Gran Barranca and other Patagonian sites (Strömberg and Stidham 2001; Sánchez *et al*. 2010; Strömberg 2011), as well as pollen data (Barreda and Palazzesi 2007; Palazzesi and Barreda 2012). However, these results contrast sharply with previous interpretation of high abundance of grasses in the early-middle Eocene (*e.g.,* Zucol *et al.* 2010). Dunn and co-authors (2015) further used epidermal phytolith shape to show that, despite being nearly grass-free, the vegetation in southern South America became increasingly open during the middle and late Eocene, culminating in non-analogue shrublands with abundant palms. This opening of landscapes may have been a consequence of the establishment of arid climates in southern South America by the middle Eocene, as inferred from stable isotopic-, sedimentological-, and paleosol climate proxy data (Bellosi

2010; Bellosi and Krause 2013; Kohn *et al*. 2015). Together, these lines of evidence are consistent with a hypothesis that the high-crowned cheek teeth of many meridiungulates may have evolved in response to eating plant tissues partially covered by dust or volcanic ash in the dry shrublands of Patagonia, rather than as an adaptation to feeding on grass (Dunn *et al*. 2015).

During the early Miocene, habitats became increasingly wet in Patagonia, a shift that seems to have preceded an expansion of more closed habitats by the middle Miocene, as shown by stable isotope data and biosilica records (Dunn *et al*. 2015; Kohn *et al*. 2015). A similar increase in forested areas in Patagonia can also be deducted from the fossil record of New World monkeys, which are commonly associated with closed canopies (Silvestro *et al*. 2018). Interestingly, palms decreased, and grasses became more abundant, albeit still not dominant, during approximately the same time (Strömberg *et al*. 2013; Strömberg *et al*. 2014), lending support to the hypothesis that these open-habitat grasses were more mesic. The exact timing for an expansion of grass-dominated habitats in Patagonia is not known from phytoliths, but pollen data from southern South America indicate that typical grassland species became abundant only in the last *ca.* 10 mya (Barreda and Palazzesi 2007; Palazzesi and Barreda 2012). The spread of C_4 grasses appears to have occurred relatively soon thereafter (*ca.* 5 mya), based on stable carbon isotope ratios from mammal tooth enamel, paleosol carbonates, and leaf waxes (Latorre *et al*. 1997; Kleinert and Strecker 2001; Bywater-Reyes *et al*. 2010; Hynek *et al*. 2012; Rohrmann *et al*. 2016). A study using phytoliths, combined with stable isotopes, further showed that high-latitude C_3 environments existed alongside the lowland C_4-dominated vegetation (Cotton *et al*. 2014).

In summary, fossil evidence from southern South America suggest that the appearance of open vegetation predated that inferred for the northern parts of the continent by over 30 myr, Open, shrubland habitats that were not analogous to savannas appeared first in the south, as early as the middle Eocene, likely influencing the evolution of large, herbivorous mammals. Grass-dominated ecosystems, however, started to dominate the landscape only over the last ten myr.

Savannas and the Great American Biotic Interchange – After the opening of environments in both northern and southern South America in the late Miocene and early

Pliocene (see above), a large number of mammalian herbivores from North America arrived in South America in the Pliocene and Pleistocene following an expansion of savannas in Central America (Bacon *et al.* 2016). Prior to the Great American Biotic Interchange (GABI), the South American mid-Miocene large herbivore fauna was relatively disparate, at least at the ordinal level, consisting of giant sloths (Pilosa), armadillos/glyptodonts (Cingulata), a few large rodents, and three closely related clades of endemic South American ungulates (part of the Meridiungulata mentioned earlier [*sensu* McKenna and Bell 1997], *i.e.*, Astrapotheria, Notoungulata, Litopterna; MacFadden 2006). After the GABI, the record indicates the arrival of a diverse array of North American herbivores, such as ungulates (Artiodactyla and Perissodactyla) and relatives of elephants (Proboscidea) in South America. This resulted in an extremely diverse megaherbivore fauna in the late Pleistocene, which may have been the most diverse of any area of the world at that time in both species and phylogenetic diversity (Faurby and Svenning 2015). Because there is ample evidence from contemporary ecosystems that reductions in megafauna can sometimes lead to drastic increases in tree cover (Daskin *et al.* 2016), we postulate a coupling between the arrival of the North American mammals and an increase in the South American savannas, a hypothesis that could be potentially tested with the fossil record.

Climate evolution and savannas

The global distribution of savannas is largely constrained by annual precipitation and precipitation seasonality (Lehmann *et al.* 2011). Nevertheless, extensive areas in the world in which tropical savannas currently occur are predicted to support forests instead (Bond 2005). Such areas are generally in more mesic environments and disturbances such as fires or herbivory help to keep the vegetation open (Bond and Midgley 2012). In less mesic environments, such as extensive areas of well-drained plateaus in central Brazil, precipitation seasonality can still be the main determinant of the predominance of savanna ecosystems, although soil composition is also an important predictor of the distribution of riparian forests, SDTFs and savannas (Ruggiero *et al.* 2002; Bueno *et al.* 2018).

Several factors interact to produce the precipitation seasonality in the area currently occupied by the Cerrado domain, including the geography of the whole continent and the latitudinal location, orientation and height of the Andean mountains – which according to global circulation models determine moisture transportation across the continent (Sepulchre *et al.* 2010). Seasonal and annual changes in the atmospheric circulation are in turn related to the thermal differences between the continental landmass and the surrounding oceans, characterized by a monsoonal system – the South American Convergence Zone (Gan *et al.* 2004; Liebmann and Mechoso 2011). This climatic system appeared first sometime during the Miocene, probably related to global-scale changes in ocean circulation, tectonic movements, and variation in $p\mathrm{CO}_2$ (Herbert *et al.* 2016). Due to its complexity, it is difficult to model more precisely when and where in South America a monsoonal system was first established, and how the climatic changes affected the origin and spread of savannas in central South America.

In contrast, in northern South America*,* a simple mechanism helps explains the origin of the climate seasonality determining the occurrence of savannas. In this region, precipitation is largely controlled by the amplitude and degree of migration of the intertropical convergence zone (ITCZ). During the austral summer, the ITCZ migrates to the south and positions itself over southern Colombia, Ecuador, and the Amazon basin (Poveda *et al.* 2006), leaving large portions of northern South America under dry conditions. Precipitation increases over northern South America when the ITCZ migrates north during the boreal summer (Poveda *et al.* 2006). This shift of the ITCZ produces a long dry season over the region occupied by savannas and xerophytic forests in northern South America. Therefore, we propose that the ITCZ has shifted at some point within the last six million years, yielding the current climate configuration. Two events have been proposed to affect the ITCZ during the late Neogene. The first one relates to the onset of the thermohaline circulation *ca.* 10–12 mya, as a consequence of the closure of the Central American Seaway, which pushed the ITCZ southward to its modern position (Sepulchre *et al.* 2014; Bacon *et al.* 2015; Montes *et al.* 2015; Jaramillo *et al.* 2017a; Jaramillo 2018). The second relates to the onset of extensive permanent ice in the Northern Hemisphere *ca.* 2.7 mya, which would have pushed the ITZC south to its current position (Flohn 1981; Shackleton *et al.* 1984; Chiang and Bitz 2005), generating conditions for the expansion of the savannas in northern South America. However, to date, the fossil record is not available for the period covering the late Pliocene to the early Pleistocene to fully support any of these two alternatives.

Modeling approaches – Paleomodeling can provide insight into the origin and expansion of environmental conditions correlated to the occurrence of savannas. Beyond atmosphere-ocean circulation models, paleosols and paleotopography can also be modeled (Bragg *et al*. 2012; Dowsett *et al*. 2016), allowing the prediction of palaeovegetation (Henrot *et al*. 2017). The potential distribution of vegetation since the Last Glacial Maximum (LGM) inferred through such models suggests a surprising contraction of savannas and expansion of tropical forests (Costa *et al*. 2017), although former studies indicated precisely the contrary (Braconnot *et al*. 2007). Reconstruction of the forest/savanna cover for the Pliocene (mid-Piacenzian, *ca.* 3 mya) indicates the potential presence of savannas in the eastern part of current Amazonia and central Brazil. During this time, forests were predicted to have occupied the southern part of the current Cerrado distribution, connecting the Atlantic Forest and Amazonia (as also predicted for the LGM; Dowsett *et al*. 2016). It is also suggested that for the late Miocene biomes indicates the potential presence of savannas in vast areas of the eastern Amazon basin and the central and southern areas of the current Cerrado domain (Pound *et al*. 2011). Simulations for the predominance of trees, shrubs, or grasses (instead of modelling whole domains), for the late Miocene, predict that grasslands occurred in the eastern and northern Amazon basin but not coincident with the current distribution of the Cerrado (Bradshaw *et al*. 2015). A model for the middle Miocene (17–15 mya) still indicates the potential occurrence of savannas, but with a predominance of forests for most areas of South America (Henrot *et al*. 2017). However, paleontological sites and data are even rarer in South America; in fact, middle Miocene fossil localities are almost all located in the temperate zone of the Northern Hemisphere (Henrot *et al*. 2017), preventing model validation. There is also considerable uncertainty in inferring modern vegetation analogues. Eocene-Oligocene (*ca.* 33.9–33.5 mya) biome classifications from fossilized plant communities do not point to the presence of any grass-dominated biomes in South America, but instead different types of forests and shrublands (Palazzesi and Barreda 2012; Strömberg *et al*. 2013; Pound and Salzmann 2017). In summary, despite the caveats and the difficulties involved in modeling biomes in deep geological time, the existing analyses suggest that environmental conditions favorable to savannas have existed since the middle Miocene, whereas environmental conditions suitable for forests and non-analog open ecosystems dominated the continent in earlier times.

Origin, assembly, and expansion of Neotropical savannas

Origins – The relatively recent emergence inferred from current evidence for Neotropical savannas provides a great opportunity to understand the origins, taxonomic assembly, and expansion of modern ecosystems and biomes.

Within the general framework of biome shifts and niche evolution (Donoghue and Edwards 2014), there are two main scenarios for the origin of savanna ecosystems. One envisions that multiple forest species gradually responded to a changing climate and independently developed adaptations to fire and seasonal drought. The competing alternative is that, once precipitation seasonality and a fire regime developed and created an open environment, species in surrounding environments and which were pre-adapted to those conditions succeeded in colonizing and diversifying the new environment. This latter model has been suggested for fire-prone habitats such as the South African *fynbos*, the *chaparral* in California and the *kwongan* in southwest Australia (see discussion in Bytebier *et al.* 2011).

In the Neotropics, information derived from molecular phylogenies and the biogeography of extant taxa appears to support the second scenario: early diversification of some vertebrate clades (e.g. herpetofauna) indicate the presence of open ecosystems in the early and middle Miocene, preceding the diversification of woody plants in the late Miocene and early Pliocene. Besides, the diversification of some lineages typical of the high elevation open grasslands in central South America (*campo rupestre*) pre-dates the diversification of the Cerrado woody-flora (Silveira *et al*. 2016), a pattern that may indicate that grassland ecosystems arose first in a region that remains under-sampled for fossil data.

Assembly– Over evolutionary time, it has been suggested that lineages rarely cross the boundaries of major biomes across continents (Crisp *et al*. 2009). This does not seem to be the case of the savannas in Cerrado since many congeners are found in Amazonia, the Atlantic Forest, and SDTFs. This indicates that habitat shifts have been an important process in the generation of savanna diversity (Souza-Neto *et al*. 2016). A recent study examining connectivity amongst all Neotropical biomes found that the Cerrado and Chaco have been colonized primarily by Amazonian species (Antonelli *et al*. 2018). The telling cases revised earlier in this chapter suggest that the assembly of plants, arthropods and tetrapods typical of savannas in the Cerrado domain occurred mainly during the late Miocene to the Pleistocene, with lineages colonizing mainly from surrounding biomes, although, generally, very little is known on the degree of niche conservatism of such lineages.

Expansion of Savannas –At a global scale, savanna expansion appears to differ in time among continents. Although the fossil record underlying this conclusion is still scarce (Edwards *et al*. 2010), most empirical data indicate that savannas had not yet expanded *ca.* 15 mya (Jacobs *et al*. 1999; Edwards *et al*. 2010; Strömberg 2011). What factors could have induced the expansion of Neotropical savannas in South America? Beyond changes in precipitation and seasonality (Lehmann *et al*. 2011), levels of CO_2 may also have played a critical role, as grasses cannot compete with trees under high levels of CO_2 or reduced water stress (*e.g.,* Higgins and Scheiter 2012). Levels of CO_2 are thought to have been high (>500 parts per million, *ppm*) during most of the Paleogene (Royer *et al*. 2011), reaching relatively low levels by the mid-late Oligocene (*ca.* 34 mya). Although proxy data suggest that atmospheric CO_2 temporarily rose during the middle Miocene, to levels above 400 ppm (*e.g*., Kürschner *et al*. 2008, Zhang *et al*. 2013), the issue is far from settled. It is believed that CO_2 levels decreased drastically to reach <200 ppm during glacial times at the beginning of the Pleistocene (Royer 2006; de Boer *et al*. 2010; Royer 2010; Royer *et al*. 2011). During the glacial/interglacial cycles of the Pleistocene (starting at 2.6 mya), CO_2 oscillated in tandem with global temperature, ranging from *ca.* 280 ppm during interglacial periods to 180 ppm during glacial periods (Monnin *et al*. 2001; Siegenthaler *et al*. 2005; Lüthi *et al*. 2008; Tripati *et al*. 2009). Neogene global climate change leading to less precipitation and higher temperatures, coupled with lower atmospheric CO_2 concentrations favoring C_4 grasses has therefore been suggested to have driven vegetation changes in Neotropical savannas (Beerling and Osborne 2006; Osborne and Beerling 2006).

Although a coupling between CO_2 levels and the expansion of savannas is suggested, this is not coincident with the origins and diversification of C_4 grasses. Different groups of C_4 grasses have originated since at least *ca.* 30 mya, with most origins occurring since the early Miocene (*ca.* 20 mya; Spriggs *et al*. 2014). The diversification of C_4 grasses that dominate in South America (*e.g.,* Andropogonae*, Paspalum*) occurred since the middle Miocene (*ca.* 15 mya; Spriggs *et al*. 2014). This predates, by a few million years, the commonly cited ages for when C_4 grasses become ecologically dominant on other continents (<10 mya). Given that CO_2 had reached relatively low levels already by the mid-late Oligocene, additional factors are also linked to the more recent rise and dominance of C_4 grasses (summarized in, *e.g.,* Edwards *et al.* 2010). Therefore, the causes of the late expansion of C_4-dominated ecosystems are still under debate. The fossil record revised in this chapter agrees with a late Miocene (Patagonia) to early Pliocene (north South America) increase in dominance and geographical extension of open ecosystems in South America.

Advancing knowledge on the origin and evolution of savannas

Phylogenies and the age of savannas. Phylogenies of extant organisms can provide important information on the age of biomes, but the evidence is circumstantial. Survival in savanna ecosystems likely requires drought adaptations for lineages initially from forested ecosystems, and physiological and morphological adaptations are proposed to be linked to the transitions among biomes (Donoghue and Edwards, 2014). However, a phylogeny cannot easily tell us if such adaptations originated long after the biome transitions (Zanne *et al*. 2014). Therefore the lack of old lineages of plants or animals that are currently characteristic of the savannas in the Cerrado domain cannot be seen as definitive proof against an older origin of this ecosystem (Wang 1994; Wang *et al*. 1999). If dispersal between biomes is predominantly from forested to open biomes, as recent data for the Neotropics suggest (Antonelli *et al*. 2018), we would not expect to observe more than a few old endemic lineages in the savannas of the Cerrado domain, especially if newly arising lineages regularly outcompete the existing ones. Distinct lineages of organisms can have an old and continuous history, but without continuous and reliable fossil data, all estimates of the ages of organisms in a certain area may be biased towards younger ages (Nagalingum *et al*. 2011; Matzke and Wright 2016). Phylogenetic estimation and the current distribution of taxa need to be complemented by integrating information from the fossil record and about Earth's climatic conditions through time (Fritz *et al*. 2013), as reviewed in this chapter.

Modeling savanna/forest transitions. Tropical forests and savannas seem to represent alternative states in certain areas, and their transition may be associated with thresholds or tipping points in environmental variables, especially precipitation (Archibald *et al*. 2011; Hirota *et al*. 2011; Lehmann *et al*. 2014). Savannas can potentially shift to SDTFs in drier areas with high soil fertility, or shift to a semideciduous or evergreen forest in areas with higher water availability (Souza-Neto *et al*. 2016; Bueno *et al*. 2018).

Therefore, modeling the past distribution of biomes without considering the different kinds of environmental thresholds from their diverse composing ecosystems can be imprecise. Modelling biomes or ecosystems can also be misleading due to the various assumptions made, such as the concordance between the distribution of linages and the distribution of a particular biome (Särkinen *et al*. 2011; Collevatti *et al*. 2013). Finally, although climatic conditions probably varied considerably in the past, recent evidence shows that stable biome states are possible for some combinations of climatic drivers, challenging the climatic determinism needed for biome reconstructions through time (Moncrieff *et al*. 2016). Despite these uncertainties, paleoclimatic reconstructions would benefit from the integration of specific knowledge on savanna thresholds and tipping points, as well as the validation and refinement of models based on fossil and phytolith data.

Filling gaps in the fossil record. There are fossil sites in northern South America that could offer a poorly explored window to study the origination and evolution of tropical savannas, such as the fossil faunas of the Falcón basin in north-western Venezuela and in the Cocinetas basin in northern Colombia (Sánchez-Villagra *et al*. 2010; Jaramillo *et al*. 2015; Carrillo *et al*. 2018). The Pliocene-Pleistocene terrestrial mammal fauna of this region includes a high diversity of herbivores with a wide range of body sizes, which suggests there was enough vegetation cover to sustain a complex community of herbivores (Amson *et al*. 2016; Pérez *et al*. 2017; Carrillo *et al*. 2018). Quantifying the relation between ecomorphological traits of the local fossil community (e.g., tooth structure, limb proportions) and the expected environmental properties (e.g., temperature, precipitation) could help characterize the replacement of forests by savannas in the region.

Fossil plant silica assemblages – phytoliths – have not been employed yet to study specifically the evolutionary history of the region currently occupied by the Cerrado biome. Such data could be a valuable addition to the scarce pollen and macrofossil information presently available. Studies described above using phytoliths from other parts of South America point to the potential of this record to uncover the non-analogue vegetation types and conditions that shaped the assembly of all grassland ecosystems on the continent. As amply demonstrated in recent work on modern grassy ecosystems (Hirota *et al*. 2011; Staver *et al*. 2011; Lehmann *et al*. 2014), such historical contingencies matter for predicting future vegetation responses to ongoing environmental change and therefore have direct conservation implications (Griffith *et al*. 2017).

Conclusions

Current knowledge indicates that the striking diversity found today in South American Neotropical savannas is most probably not the result of a long geological history or stability of this ecosystem, at least not relative to Neotropical rainforests. Environmental conditions for the occurrence of savannas have existed since the middle Miocene, and molecular phylogenies of some vertebrate groups indicate the presence of open ecosystems in central South America around then. This timing coincides broadly with the initial diversification of C_4 grasses inferred from phylogenies. However, the fossil data indicates a time lag for the expansion and ecological dominance of both C_3 and C_4 grasses a few million years later, in the late Miocene. The period of expansion and ecological dominance of C_4 grasses is coincident with the timing in which the woody flora of the savannas in the Cerrado started to diversify, as suggested by molecular phylogenies. This time lag between the origin and expansion of savannas in central South America could potentially suggest that lineages of trees that are adapted to the open fire-prone savannas diversified and became ecologically dominant after open habitats, presumably grasslands (e.g. "*campos rupestres*") and associated fauna had emerged.

Further research on tropical savannas should focus on poorly known but potentially ecologically dominant or/and ancient taxa in the tropical savannas, which might include many herbs, small shrubs, termites, ants, and fossorial lizards (Filgueiras 2002; Constantino 2005; Costa *et al*. 2010; Colli *et al*. 2016). Unfortunately, areas in which the tropical savannas occur are also particularly favorable to agriculture and farming, undergoing an alarming rate of deforestation. Protecting those species-rich and fragile ecosystems will be crucial for our ability to answer many of the remaining questions about the origins and evolution of Neotropical savannas and their biotas.

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Figures

Figure 1 – Current distribution of the Cerrado Domain and other open vegetation domains discussed in this review (Llanos, Caatinga, Chaco) in relation to broadly defined rain forests (Amazonia, Atlantic Forest). See text for details and definitions.

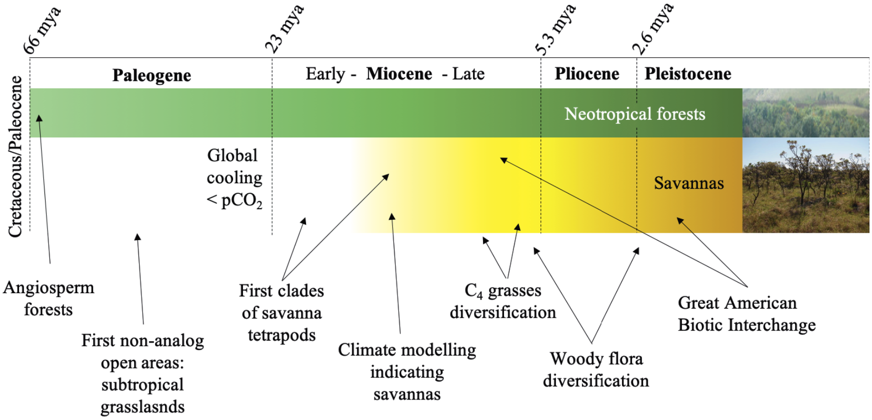

Figure 2 – Approximate timeline (not to scale) summarising the origin and expansion of South American tropical savannas. Rain forest ecosystems appeared in the early Paleocene (indicated by macrofossils and pollen). The first non-analog open biomes (shrublands) appeared in the middle Eocene (indicated by macrofossils, phytoliths, and pollen data). A decline in the concentration of CO_2 started already during the end of the Oligocene, preceding the Miocene global cooling. First divergences in clades of typical savanna vertebrates occurred during the middle Miocene (indicated by molecular phylogenies), the same time in which climatic modeling indicates the presence of appropriate conditions for the occurrence of savannas. C_4 grasses and the woody flora diversified from the late Miocene to the Pleistocene (indicated by molecular phylogenies). Savanna expansion occurred after the late Miocene (indicated by macrofossils, phytoliths, and pollen data). Finally, the Great American Biotic Interchange may have been increased by the expansion of savannas in South America (indicated by molecular phylogenies and fossil data).

II

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Research Paper

Contrasting patterns of phylogenetic turnover in amphibians and reptiles are driven by environment and geography in Neotropical savannahs

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ABSTRACT

Aim To map patterns of phylogenetic turnover and disentangle the geographical and environmental factors that drive the phylogenetic composition of distinct taxa. Cross-taxonomic congruence in biodiversity patterns is key to understanding the main drivers of community structure, for biogeographical regionalization and to guide conservation.

Location The Cerrado savannahs of South America.

Taxa Reptiles and Amphibians.

Methods To measure the proportion of phylogenetic branches shared among sites (i.e., phylogenetic turnover), we built presence-absence matrices, including only well-sampled localities from previously compiled inventories. We then tested whether phylogenetic turnover is different from null hypothesis expectations based on species turnover, geographical distance, and elevation. We used generalized dissimilarity modelling (GDM) to test whether topography, soil, or climate best explain phylogenetic turnover. Finally, we mapped the observed and the GDM-predicted phylogenetic regions to assess geographical congruences between reptiles and amphibians.

Results There is no significant correlation between the phylogenetic turnover of reptiles and amphibians, as evidenced by non-congruent regionalization patterns and by different responses to geographical and environmental gradients. For reptiles, geographical distance is the most important factor explaining phylogenetic turnover, whereas, for amphibians, a set of climatic variables and relief roughness are more important.

Main conclusions

Different responses of phylogenetic turnover between reptiles and amphibians to geographical and environmental distances have ultimately shaped the phylogenetic regionalization of these two groups. This incongruence is a first indication of the differential importance of niche-filtering, dispersal limitation and extinction rates in each herpetofaunal group. In general terms, the diversity patterns of one group cannot be used as a surrogate to map general patterns and to understand the drivers of diversity of other groups.

Keywords Cerrado, regionalization, beta diversity, Neotropics, connectivity

INTRODUCTION

The discussion on the relative influence of local habitat use and dispersal limitation of distinct organisms on large scale biodiversity patterns is old and long-standing (de Candolle, 1820; Nelson, 1978). However, with the current knowledge of the geographical distribution of multiple species and their respective evolutionary relationships, this question can be more precisely tackled (Saladin et al., 2019). By measuring the number of phylogenetic branches that are shared among sites to give an indication of the evolutionary distance between them (i.e., phylogenetic turnover), it is possible to compare the responses of distinct organisms to geographical barriers and environmental gradients across different scales (Pennington et al., 2006; Weinstein et al., 2014). Therefore, phylogenetic turnover can be used to disentangle the effects of dispersal limitation and environmental filtering (e.g., due to limited niche-evolution) in the biodiversity pattern of distinct organisms (Eiserhardt et al., 2013).

The degree to which geographical and environmental factors affect phylogenetic turnover may vary over the evolutionary history of distinct taxa. A way to infer the intensity of these factors in the evolutionary history of organisms is by comparing how much phylogenetic turnover departs from species turnover (Weinstein et al., 2014). For example, the rate of phylogenetic turnover is expected to be lower than the rate of species turnover when most taxa above species level are present in the majority of sites. This low phylogenetic turnover indicates high dispersal rates and weak historical isolation (Guevara et al., 2016). Conversely, high phylogenetic turnover is thought to be related to ancient geographical barriers or dispersal limitation (although high extinction rates can lead to the same pattern) (Graham & Fine, 2008). In addition, higher phylogenetic turnover is expected with increasing elevation due to greater isolation and greater habitat heterogeneity in highlands (Bryant et al., 2008). If both the degree of phylogenetic turnover over the evolutionary history and the responses to geography and environment are similar among taxa, the result is the phylogenetic regionalization of the entire biota (Daru et al., 2017).

Cross-taxonomic patterns of endemism also suggest that general evolutionary processes (e.g., vicariance) are shaping biodiversity patterns (Hausdorf & Hennig, 2004). In the Cerrado savannahs of South America, almost two hundred species of squamate reptiles (i.e., lizards and snakes) and anuran amphibians (i.e., frogs and toads) have narrowly distributed ranges coincident with several relief units (Fig. S1 – Azevedo et al., 2016). These relief units – generally plateaus above 500 m – are also known for harbouring several narrowly distributed species of plants, fish and, to a smaller extent, mammals and birds (Silva, 1997; Simon & Proença, 2000; Marinho-filho et al., 2002; Nogueira et al., 2010a). Most of these Cerrado endemic species are often highly adapted to the open environments of savannah ecosystems (Nogueira et al., 2011; Valdujo et al., 2012). Cerrado highlands and plateaus are the most climatically stable areas for savannah ecosystems, potentially explaining the large number of Cerrado endemics in these areas (Werneck et al., 2012). Although endemism patterns may influence phylogenetic turnover (Daru et al., 2017), these patterns are not necessarily related, as several areas of high endemism may harbour only closely related species (e.g., within the same phylogenetic region). To date, patterns of phylogenetic turnover in the Cerrado savannas have not been mapped, and the comparison of these patterns between reptiles and amphibians may provide clues on the evolution of the Cerrado biota and the general factors shaping turnover patterns.

Squamate reptiles and anuran amphibians generally present very distinctive habitat requirements (Vitt & Caldwell, 2013). Most amphibian species tend to be restricted to riparian habitats whereas reptiles are also present in the interfluves. Consequently, for amphibians, water-related variables can be more critical to driving connectivity among sites than for most reptiles, since species of several amphibian clades rely on standing water for breeding (Wells, 2007). For reptiles, ground characteristics can be more critical to driving connectivity among sites due to habitat preferences of distinct taxa, from fossorial organisms to rock-outcrops specialists (Vitt, 1993; Lema, 2001). Thus, if local habitat use influences connectivity and isolation among regions for long periods, this may result in the formation of phylogenetically distinct regions between reptiles and amphibians.

In this study, we estimate phylogenetic turnover for amphibians and reptiles in the Cerrado, testing the effects of geographical and environmental distances. More specifically, we address the following questions: *(1) Does the phylogenetic turnover among sites result in phylogenetically similar regions for reptiles and amphibians?* As in the case of endemism patterns, we expect to find similar patterns of phylogenetic regionalization between both groups. *(2) How much does phylogenetic turnover differ from species turnover?* We expect to find a higher decoupling of phylogenetic turnover in amphibians than in reptiles due to the generally higher dispersal limitations in amphibians (Chen et al., 2011). *(3) What are the correlates of phylogenetic turnover?* Habitat-use differences between reptiles and amphibians may influence the importance of the rate of turnover along environmental gradients (Ferrier et al., 2007). We expect to find a greater importance of soil-related variables for reptiles and precipitation-related variables for amphibians, due to their habitat-preferences mentioned above. In addition, we expect to find higher phylogenetic turnover among sites in highlands for both groups as a result of the plateaus/highlands patterns of endemism for both groups in the Cerrado (Azevedo et al., 2016).

MATERIAL AND METHODS

Study area

The Cerrado is a vast, savannah-dominated region occupying about 1.2 million square kilometres in central-eastern South America (Ratter et al., 1997; Silva & Bates, 2002). The topography is characterized by plateaus, generally above 500 m, and depressions eroded by major drainage systems (Silva, 1997; Ab'Sáber, 1998; Fig. S1). Gallery forests are ubiquitous at most scales, along with typical interfluvial grasslands and savannahs (Silva & Bates, 2002). This high water availability differentiates the Cerrado savannahs from most savannahs worldwide (Cole, 1986) and is possibly the reason for the high species richness and endemism of amphibians in the region (Valdujo et al., 2012b).

Distributional data

To address all three questions, we produced presence–absence matrices required to calculate both species and phylogenetic turnover. For these matrices, we used a previously compiled dataset of occurrence records for 265 species of squamate reptiles and 193 species of anuran amphibians occurring in the Cerrado (Nogueira et al., 2011; Valdujo et al., 2012b; Azevedo et al., 2016). We crossed the species records with a grid of 0.1 degrees of resolution adjusted to a cylindrical equal-area Behrman projection. This choice reflects the finest resolution for a substantial part of our data (Valdujo et al., 2012a). To define minimally sampled sites, we only included in the subsequent analyses cells containing: (1) at least 20 recorded species of reptiles, and 15 species of amphibians – these numbers reflect a conservative choice based on the minimal number of species of each group ever found in well-sampled sites in the Cerrado (Nogueira et al., 2005; Sawaya et al., 2008; Costa-Campos & Freire, 2019); (2) sites with at least two species endemic to the Cerrado savannahs; and (3) in the case of reptiles, sites with at least one species of lizard and one species of snake endemic to the Cerrado, as these two groups differ in detectability levels (Vitt & Caldwell, 2013). For comparison, we also produced two subsets of the presence–absence matrices including only species endemic to the Cerrado (100 reptiles and 92 amphibians; Azevedo et al., 2016). Species of reptiles and amphibians endemic to the

Cerrado are generally more associated with open savannah ecosystems, thus better reflecting the history of the typical Cerrado biota than the non-endemic, either more habitat-generalist or forest dwelling species (Nogueira et al., 2011).

Phylogenetic data

To calculate phylogenetic turnover, we followed the phylogenetic hypotheses proposed by Tonini et al. (2016) and Jetz & Pyron (2018) for reptiles and amphibians respectively. As species without molecular data were randomly assigned to genus-level (Thomas et al., 2013), we repeated all phylogeny-dependent analyses for a sample of 100 of these phylogenies. We then used the median values in all subsequent analyses. In the resulting phylogenies, around 60% of all species were included through randomly assignments to genus-level. Therefore, we recognize that our results should be carefully interpreted at the species level.

Dissimilarity values

We measured the phylogenetic turnover between sites with the Simpson derived pair-wise phylogenetic dissimilarity index (Leprieur et al., 2012), which ranges from 0 (all branches are shared between sites) to 1 (no branches are shared). Phylogenetic turnover is one of the two phylogenetic beta diversity components and accounts for the number of phylogenetic branches that are replaced from one site to another. The second component is called nestedness and accounts for losses without replacement in phylogenetic branches from one site to another (Baselga, 2010). For control, we estimated the total multiple site phylogenetic beta diversity related to each of these components (Baselga, 2013). However, for the subsequent analyses, we only considered the turnover component to separate the potentially complex effects of differences in species richness among sites (Soininen et al., 2018). For the tests against the null distributions (see below), we also measured the species turnover as the Simpson pair-wise dissimilarity (Koleff et al., 2003).

To test whether phylogenetic turnover is decoupled from species turnover (*Question 2*), we regressed their scores against each other. We then compared this result to a null distribution of phylogenetic turnover. We generated this null distribution by calculating the resulting phylogenetic turnover after shuffling the species composition of each site while keeping the species turnover and richness constant in 1,000 randomizations (trial-swap algorithm; Miklós and Podani 2004). We considered phylogenetic turnover to be decoupled from the species turnover if the resulting slope was outside the interval between the 2.5 and 97.5% of the slopes resulting from the null distribution (Fig. S2).

To address *Question 3*, we followed the same procedure described above to test whether there is a significant increase in phylogenetic turnover with distance. In the case of elevation, to calculate the overall turnover in lowlands and highlands, we calculated the multiple site phylogenetic turnover (Baselga, 2013) for three different elevation classes: 0–250 m, 251–500 m, 501–750 m, and 751 m or more. Multiple-site phylogenetic turnover is only comparable among categories with equal numbers of sites. Therefore, we randomly selected ten sites from each class in 1,000 resampling rounds. To test if turnover was significantly different between the different elevation classes, we compared turnover values from each elevation category with the values derived from randomly selected sites at 1,000 resampling rounds.

Environmental characterization

To characterize the climate of each site (*Question 3*), we downloaded 19 bioclimatic variables from the CHELSA project (http://chelsa-climate.org/; Karger et al., 2016). We also characterized soils in each site from layers of predicted values of the proportion of sand, clay and coarse fragments (median values for the first 15 cm of the soil surface) downloaded from soilgrids.org (Hengl et al., 2014). Additionally, we estimated the elevation, relief roughness, and slope of each site. We measured relief roughness as the largest difference in elevation of each site with its eight closest neighbours. We then retained only variables with no multicollinearity problems after calculating their Variance Inflation Factor (VIF), using a threshold of 10 (Naimi et al., 2014). This selection resulted in five layers for precipitation and five for temperature representing most of the climatic variation in the region. Among the non-climatic variables, the slope was excluded due to high collinearity with relief roughness. Although we automatically removed variables with high VIF, we discussed all highly colinear variables as groups (e.g., relief roughness and slope; Fig. S3). Biological expectations of how the geographical distribution of reptiles and amphibians is affected by each set of environmental variables are available in the supplementary material, Table S1.

Modelling phylogenetic turnover

To test which set of environmental variables best explain phylogenetic turnover (*Question 3*), we used Generalised Dissimilarity Modelling (GDM), a modelling technique based on regressions of non-linear distance matrices (Ferrier et al., 2007). First, we produced GDM-models with all possible combinations of variables with low VIF-scores described above (five temperature, five precipitation, three soil, relief roughness, elevation, and geographical distance). Besides testing elevation and distance separately (described above), we also included them in the GDM-models to test if their relationship with phylogenetic turnover shift by including more direct niche-related variables such as temperature. Then, starting from the full model, we successively eliminated the variable with the smallest contribution each time. In each round, we calculated the relative variable importance through matrix permutations for each variable (n = 1,000). Variable importance was then calculated as the proportion of change in the explained model deviance when comparing models with and without the respective variable. Variable significance was also estimated from the mentioned permutations (P-value < 0.05). Model selection using Akaike's Information Criterium (AIC) does not apply to techniques of regression matrices such as GMD (Wagner & Fortin, 2015). Therefore, we used an equivalent strategy by selecting the model with the highest value of explained deviance which retained only variables that were important in more than 160 rounds of permutation (Heinze et al., 2018). We then visualized the most important variables using I-splines, which describe non-linear monotonic relationships among variables and turnover (i.e., partial ecological distance). All GDM-models were produced with the R-package *gdm* (Ferrier et al., 2007).

To take into consideration the variation in the phylogenies used here, we weighted the contribution of each site to the GDM-models by the standard deviation in the phylogenetic diversity of a site divided by the respective number of species at each site (PD – Faith, 1992). In this way, sites with considerable PD variation per species due to the random taxonomic assignment will have a lower contribution to the final results.

To address *Question 1*, we compared the observed values of phylogenetic turnover between reptiles and amphibians as well as the GDM-model predictions projected onto geographical space. We classified sites that are phylogenetically more similar to each other by clustering both the observed and the predicted turnover using UPGMA, a widely applied method for clustering dissimilarity matrices in bioregionalization analyses (Kreft & Jetz, 2010). As our intention is not to provide a definitive bioregionalization scheme, but rather assess whether breaks in the phylogenetic turnover are congruent between reptiles and amphibians, we mapped the 12 first clusters for both groups in different colours.

RESULTS

Phylogenetic beta diversity – Nearly all phylogenetic beta diversity for reptiles (turnover = 0.94, nestedness = 0.03) and amphibians (turnover = 0.95, nestedness = 0.02) is explained by the turnover component. This shows that the vast majority of differences in phylogenetic branches shared among sites are not related to losses in phylogenetic branches without replacement.

Species turnover and geographical distance – For all datasets (both reptiles and amphibians with all species or only Cerrado endemics), phylogenetic turnover increases linearly with species turnover (Table 1, Fig. S4 a-b). For distance, there is a linear increase in the phylogenetic turnover for all reptiles (Fig. S5a) which is not higher than the null distribution (Table 1). For amphibians, phylogenetic turnover decreases after 1,300 km (Table 1, Fig S5b, and S5d). For both groups, the highest values of phylogenetic turnover are concentrated within the first 1,000 km, indicating that very distant sites on the opposite borders of the Cerrado share more branches in the phylogeny than some sites that are close to each other.

Phylogenetic turnover and elevation – Phylogenetic turnover increases with elevation for amphibians, being lower than the randomized resampling below 500 m (Fig. 1). This result is similar for the Cerrado endemic amphibians, but with turnover higher than random above 750 m. For reptiles, there are no differences in the turnover rates among the elevational classes, except for the lower turnover for endemic reptiles below 250 m (Fig. 1).

Correlates of dissimilarity – Geographical distance is the most important variable driving phylogenetic turnover for reptiles, whereas precipitation seasonality is the most important for amphibians (Table 2). There are also considerable differences in variable importance between the datasets of all reptile species and endemic reptiles only (Table 2). Amphibians and reptiles present very distinct changes in turnover rates along gradients of relief roughness (linear increase only for amphibians) and geographical distance (linear increase only for reptiles) (Fig. 2). In contrast, turnover rates present the same responses to precipitation seasonality for both groups (Fig. 2).

Observed versus predicted turnover – The clustering of phylogenetic turnover indicates spatial mismatches between the direction of turnover in reptiles and amphibians. For reptiles, there is a north–south or latitudinal differentiation in the phylogenetic composition of sites (Fig 3a).

However, this differentiation is not abrupt, with several communities classified in different clusters occurring side by side. For amphibians, the larger cluster (sites in red, Fig. 3b) indicates a phylogenetically similar composition of amphibian communities along most of the Cerrado, with significant breaks mostly in the east (Fig. 3b). The predicted turnover values also indicate a more phylogenetically uniform biota for amphibians (Fig. 3d) when compared to reptiles (Fig. 3c). In addition, there is a higher spatial mismatch between observed and predicted clusters for reptiles, indicating a lower tracking of environmental conditions for this group.

DISCUSSION

We found that phylogenetic turnover is largely incongruent between reptiles and amphibians in the Cerrado savannahs of South America. Our results contrast with the geographical congruence in patterns of species endemism among species of both groups (Azevedo et al., 2016). This may indicate that the relief units that delimit areas of endemism influence phylogenetic similarity in different ways for each group. These differences are likely to be the result of very distinct responses of each group to geography and environmental variables, indicating that small scale habitat preferences and ecophysiological differences may lead to detectable imprints in the biogeographical distribution of species.

Species composition, geographical distance and elevation

In general, amphibians and reptiles present higher species turnover rates when compared to other vertebrates (Buckley & Jetz, 2008; Chen et al., 2011). However, we found that rates of phylogenetic turnover do not depart from the null expectation based on species turnover (*Question 2*). This coupling between both turnover estimations and species richness could be related to the still incomplete molecular data in the large-scale phylogenies used here, which is known to decrease the power of analyses depending on branch length estimations (Title & Rabosky, 2016). However, the reasonably complete genus-level phylogenetic resolution in the same phylogenies allowed us to infer that phylogenetic turnover is not driven mostly by species from the same genus (e.g., as in Amazonian white-sand forests; Guevara et al., 2016b). Also, turnover in both groups is not predominantly driven by species from distantly related lineages, which is more common when comparing sites from two distinct biogeographical regions (e.g., Neotropics *vs.* Nearctic; Antonelli, 2017). Finally, the slightly higher decoupling of phylogenetic turnover with the species turnover for amphibians may indicate either higher dispersal limitations or higher extinction rates for this group (Myers et al., 2013; Weinstein et al., 2014).

The increase in the phylogenetic turnover with distance is parallel to the distance decay in similarity, the most expected outcome of biological turnover when dispersal limitation is the main process structuring biodiversity (Nekola & White, 1999). For amphibians in the Cerrado this pattern does not hold, as sites far apart tend to be phylogenetically more similar than sites at intermediate distances (*Question 3*). This pattern may be related to the presence of species typical of forested biomes in the sites close to the Cerrado ecotones. Alternatively, this non-linear relationship of phylogenetic turnover with distance may be related to the fact that in the Cerrado, small streams in open savannahs or grasslands are the primary habitat for most endemic amphibians (Valdujo et al., 2012b). This environment is mostly restricted to plateaus and highlands, which are not uniformly distributed in the Cerrado. Consequently, turnover in amphibians does not increase with distance after 1,000 km (Fig. 2b). Also, since riparian habitats are locally and regionally rare, amphibians may also have experienced higher extinction rates for during past climate changes (i.e., lower stability, Araújo et al., 2008). In contrast, open savannahs are the primary habitat for most endemic reptiles, regardless of the presence of streams (Vitt, 1991). Such open savannahs are spread over the entire biome (Eiten, 1972) thus, partially explaining the linear increase of turnover with distance for reptiles.

In parallel with the distance decay in similarity, biological turnover also tends to increase with elevation (McKnight et al., 2007; Peixoto et al., 2017). Here we found that elevation influences the phylogenetic composition of amphibian communities but not of reptiles (*Question 3*). This result is in contrast with the congruent patterns of species endemism for both groups in the Cerrado highlands (Azevedo et al., 2016). However, when analysed in conjunction with other environmental variables, site elevation was not selected as an important variable not even for amphibians (Table 1, Table S1). This lower importance is probably related to the fact that elevation is a more indirect correlate of species niches as compared to relief roughness, temperature and precipitation (Guisan et al., 2017). For example, a site in an area with higher relief roughness will probably contain a higher number of narrow streams and rock outcrops (the habitat of several anuran species) than a site on a flat area at the same elevation (Valdujo et al., 2012a). Additionally, relief roughness is also related to higher environmental heterogeneity, potentially leading to lower extinction rates (Ruggiero & Hawkins, 2008). Therefore, the combination of niche availability and climatic stability may explain the more heterogeneous phylogenetic composition of anuran communities in these sites.

Environmental correlates of phylogenetic turnover

The assembly and diversification of reptiles and amphibians in the Cerrado region occurred mostly from the Early Miocene to the Early Pleistocene (Nogueira et al., 2011; Valdujo et al., 2012b). Given this time scale, most typical Cerrado lineages would have had enough time to disperse to favourable environments within the region, in cases where there was enough habitat connectivity. Still, dispersal limitation seems to drive most phylogenetic turnover in reptiles, resulting in a linear, larger increase of turnover with distance for this group (Fargione et al., 2003).

Our study indicates that environmental filtering is more important in explaining turnover in amphibians, whereas geographical distance is more important for reptiles (*Question 3*). This result is in line with previous studies showing that species composition of amphibian communities in the region is highly structured according to the proximity to different biomes (Valdujo et al., 2012a). In this way, for amphibians, niche-filtering processes related to the presence of breeding-sites (e.g., relief roughness) and thermoregulation (warmer temperatures and high precipitation) drive phylogenetic differentiation. This combination of climatic variables is related to the fact that in amphibians, the permeability of the skin constrains thermoregulation (Wells, 2007). Therefore, extremes of temperature in highly seasonal areas may demand additional physiological and behavioural adaptations in amphibians, as most Cerrado species spend most of their time outside water as adults (Cunningham et al., 2016).

Contrary to our expectations, soil variables were not important in explaining turnover in reptiles (*Question 3*). One possible explanation is that soil variables, such as the proportion of sand and clay, vary and drive turnover in an even finer geographical scale than the available data (Figueiredo et al., 2018). It is also possible that these variables only drive turnover at shallow phylogenetic levels, which could be masked when analysing phylogenetic turnover in groups with such a deep evolutionary history as reptiles and amphibians (Rosauer et al., 2014). For example, several of the lizard and snake species adapted to sand soils (e.g., in the Jalapão region), have closely related species occurring in other Cerrado environments (e.g., *Kentropyx, Cnemidophorus, Apostolepis, Bachia*) (Werneck et al., 2009; Nogueira et al., 2010b). This low level of niche conservatism and high lability of traits related to the use of soil in reptiles is a pattern observed worldwide, with convergent adaptations of lizards and snakes to specific sandy or rocky substrates (Rodrigues, 1993; Arnold, 1995).

Phylogenetic regionalization

In broader geographical scales, biological turnover is consistently congruent for most terrestrial vertebrates, especially in areas with steep relief or in biome transitions (McKnight et al., 2007; Buckley & Jetz, 2008). In contrast to the Atlantic Forest or Amazonia, proposals of regionalization for the Cerrado biota are rare, and the Cerrado is generally classified as one continuous terrestrial unit (Olson et al., 2001; Morrone, 2017), or subdivided along the Espinhaço range (Colli-Silva et al., 2019). A few existing studies show that phytogeographical regions in the Cerrado are more structured in the east, especially close to the Atlantic Forest (Ratter et al., 2003; Neves et al., 2018). For amphibians, a regionalization scheme based on species turnover indicates six equally distributed regions (Valdujo et al., 2012a), in contrast to our results based on phylogenetic turnover. Instead, our results indicate a homogenous phylogenetic composition of amphibian communities with few breaks in the phylogenetic composition. In the case of the reptiles, our results indicate sites from different clusters occur side by side, without sharp breaks in the observed phylogenetic turnover.

Phylogenetic differentiation among biological communities is driven by the history of speciation and extinction of species and by intrinsic clade characteristics related to dispersal limitation and niche-conservatism (Daru et al., 2017). In contrast to predictions for species endemism (Azevedo et al., 2016), our results indicate that diversity is unevenly distributed for different groups of organisms when considering their phylogenetic relationships. These differences hamper cross-taxonomic generalizations on how biodiversity evolved in the region and highlight the phylogenetic uniqueness of different portions of the Cerrado savannahs.

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BIOSKETCH

Josué A. R. Azevedo is broadly interested in the biogeography and evolution of terrestrial vertebrates. This work is the continuation of his Masters project on the Cerrado herpetofauna and part of his Ph.D. thesis in Gothenburg, Sweden. He collaborates in the Antonelli lab (https://antonelli-lab.net/) and on the Atlas of Brazilian snakes (https://cnbiogeo.wixsite.com/ cristiano-nogueira/atlas).

Author contributions: JARA, PHV, and CCN provided with species distribution data. JARA and SF planned the project. JARA conducted the analyses, produced the figures, tables, and led the writing. All authors contributed to the interpretation and discussion of the results and approved the final version of this manuscript.

TABLES

Table 1 – Rate of increase in phylogenetic turnover with species turnover and with geographical distance. The numbers indicate the rate of turnover (coefficient) estimated by linear regressions. Numbers in brackets indicate the proportion of times phylogenetic turnover rates were higher than the null distribution. Rates of phylogenetic turnover departed more from species turnover for amphibians than for reptiles (numbers in brackets and Fig. S4). There was a linear increase in phylogenetic turnover with distance only for reptiles (Fig. S5).

Dataset	Species turnover	Geographical distance
Reptiles	1.39 (0.56)	1.39 (0.08)
Endemic reptiles	0.64 (0.70)	Non-linear
Amphibians	1.05 (0.04)	Non-linear
Endemic amphibians	0.48 (0.08)	Non-linear

Table 2 – Generalized Dissimilarity Modelling – GDM – best model for reptiles and amphibians and the respective subsets composed only of the species endemic (End-) to the Cerrado. All four models explain significantly more deviation than expected by random ($P < 0.001$). Asterisks indicate: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. With the exception of geographical distance and precipitation seasonality, significant variables for reptiles are not the same as for amphibians.

	Reptiles	End-Reptiles	Amphibia	End-Amphibia
Best model				
Model deviation	206.3	405.7	154.3	457.5
Percentage of deviation explained	12.8	18.0	34.6	29.1
Variable importance				
Geographical distance	34.5***	9.0***	5.5***	7.0***
Relief roughness	-	-	5.3*	6.4**
Percentage of sand	-	-	1.8	3.1*
Temperature seasonality	-	-	2.8	-
Max. temp. (warmest month[†])	12.1	-	4.1*	5.8**
Precipitation (wettest month)	-	7.3	5.8**	2.9
Precipitation seasonality	19.2*	6.8	18***	24.4***
Precipitation (warmest quarter[††])	-	18.2**	-	-

†Highly correlated with Elevation. [††] Highly correlated with Minimum Winter Temperatures.

FIGURES

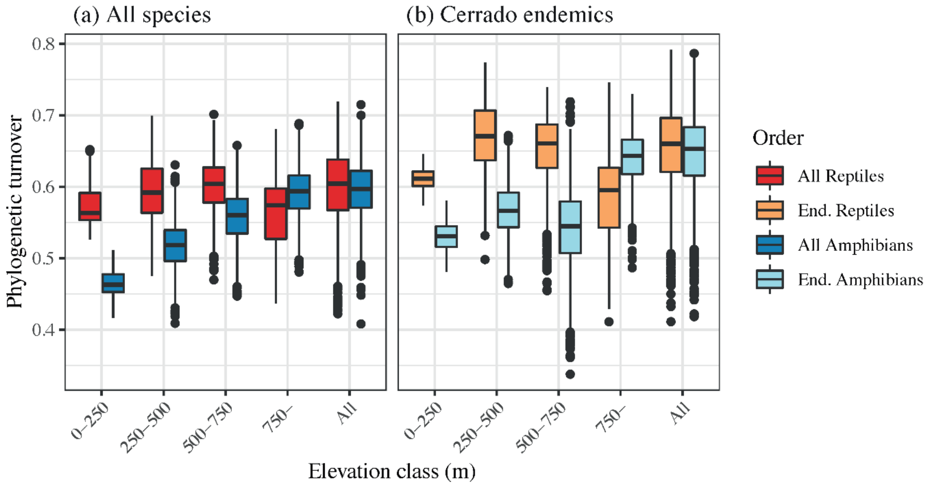

Figure 1 – Overall phylogenetic turnover in relation to elevation in the Cerrado (binned into four elevation classes). Boxplots represent the distribution of values from multiple rounds of resampling of 10 sites at each elevation class (n = 1,000). For reptiles, phylogenetic turnover in each elevational class is not different from the turnover in randomly selected sites (All). For amphibians, phylogenetic turnover is significantly lower than random at low altitudes (all amphibians < 500 m, endemic < 750 m).

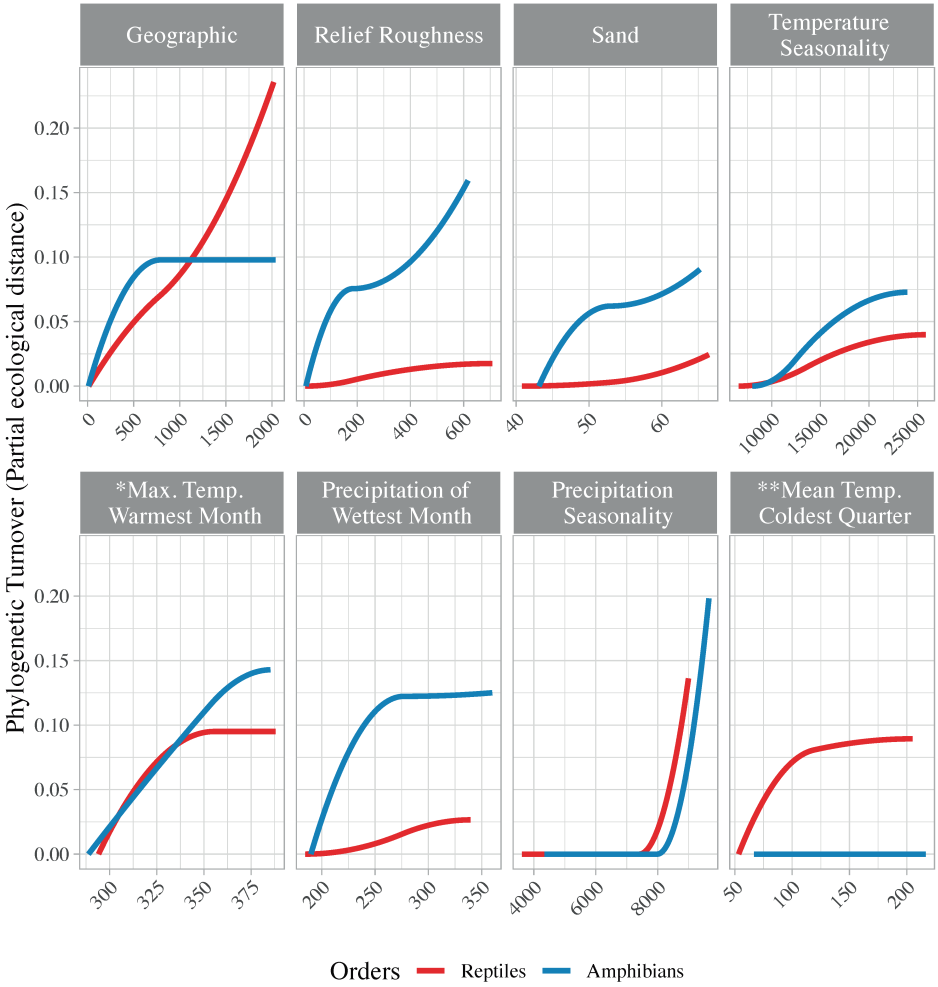

Figure 2 – Geographical and environmental predictors of phylogenetic turnover. I-splines were estimated from generalized dissimilarity models. The height of each I-spline indicates the relative contribution of each variable to the phylogenetic turnover observed along each environmental gradient (i.e., the partial ecological distance). Asterisks highlight variables with variation in additional climatic/environmental classes: *Highly colinear with elevation; **Highly colinear with minimal winter temperatures (see Table S1 and Fig S3). Precipitation seasonality is a key variable for both reptiles and amphibians, whereas the relationship of phylogenetic turnover to all other variables differs considerably between each group.

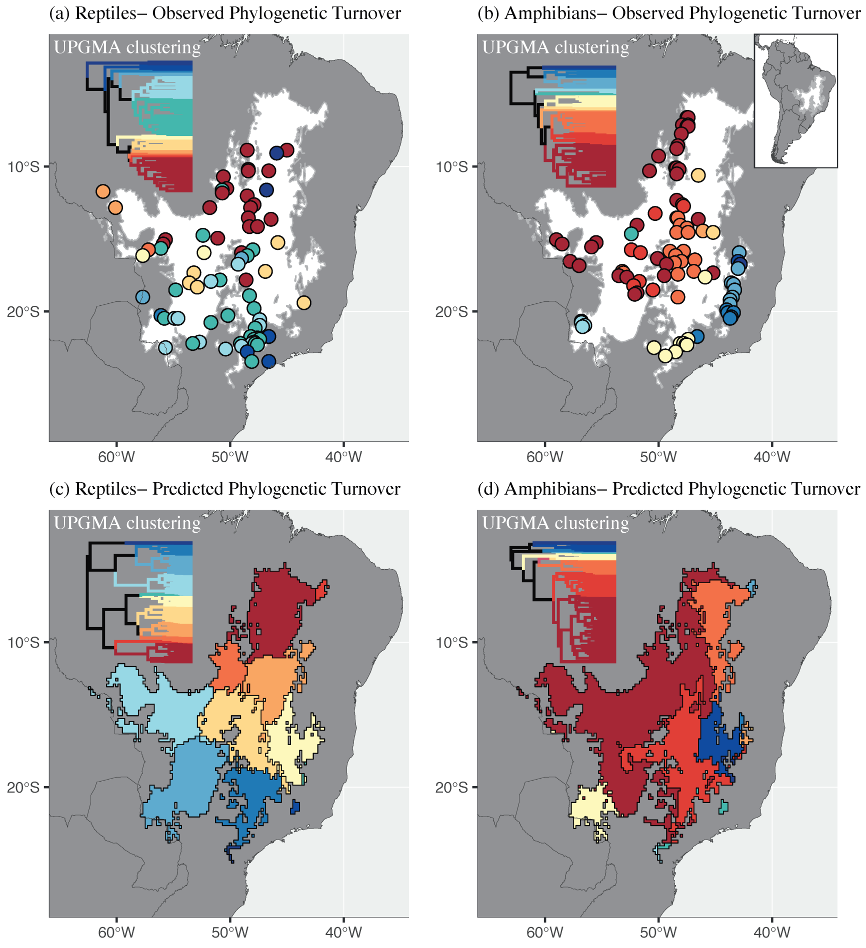

Figure 3 – Clustering of phylogenetic turnover for reptiles and amphibians of the Cerrado savannahs. Sites with the same colour share more phylogenetic branches (= less turnover). Each terminal of the UPGMA dendrograms indicates a distinct site. **(a-b)** Clustering of the observed pairwise phylogenetic turnover for well-sampled sites (at least 15 amphibian spp. and 20 reptile spp., and two Cerrado endemics). **(c-d)** Clustering of the generalized dissimilarity modelling predictions of phylogenetic turnover with geographical distance, precipitation, and temperature variables for reptiles (Table 1), plus soil and elevation variables for amphibians. This figure demonstrates strikingly contrasting patterns of phylogenetic turnover between reptiles and amphibians in both the observed and in the predicted patterns.

Supplementary table

Table S1 – Environmental correlates of turnover for reptiles and amphibians selected in the best GDM-model (Generalized Dissimilarity Modelling) and the respective biological interpretation. In parenthesis, highly correlated variables (> 0.9) not included in the model selection but representing equivalent environmental gradients in the region (See also Fig. S2). Asterisks denote a negative correlation with the selected variables.

Selected variable (correlated variables)	Biological interpretation
Relief roughness (Slope)	Geographical barriers, environmental complexity.
Percentage of Sand (Percentage of clay*)	Soil characteristics. Influence vegetation structure, locomotion strategies for reptiles, breeding sites for amphibians.
Temperature Seasonality (Precipitation of Driest Month)	Climatic seasonality/stability in general. One of the main differences between Cerrado and the surrounding forested biomes.
Max. Temp. of the Warmest Month (Elevation*, Mean Temp. Wettest Quarter, Mean Temp. Driest Quarter, Mean Temp. Warmest Quarter, Mean Temp. Coldest Quarter.)	Encompasses a broad range of temperature characteristics inversely correlated to elevation in the Cerrado region, with the highest temperatures occurring in lower elevation areas.
Precipitation of the Wettest Month (Precipitation of Wettest Quarter)	The amount of precipitation in the rainy season influences booms of food availability and the reproduction season for both groups.
Precipitation Seasonality (Precipitation Driest Quarter*)	Highly seasonal areas in the Cerrado demands ecophysiological and behavioural adaptations of organisms, influencing species ranges.
Precipitation of the Warmest Quarter (Min Temp. Coldest Month*, Mean Temp. of Coldest Quarter*)	These variables represent a climatic latitudinal gradient (within Cerrado only), with coldest temperatures in the southern parts of the Cerrado. Minimum temperatures limit ranges of ectothermic organisms.

Supplementary figures

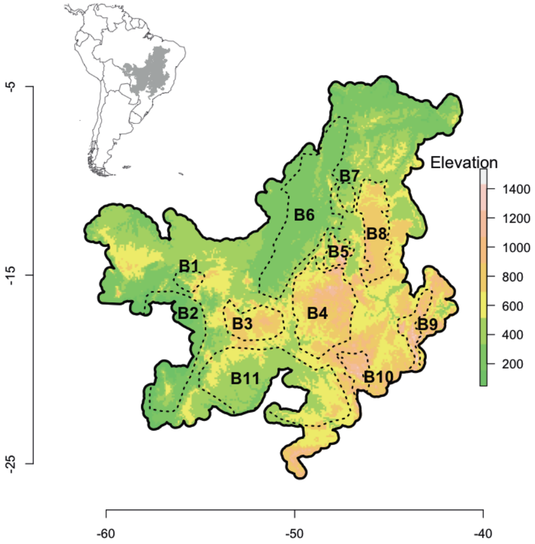

Figure S1 – Topography of the Cerrado and highlighted biogeographical units delimited from the congruent distribution of narrowly distributed reptiles and amphibians (Azevedo et al., 2011). Borders of the Cerrado were smoothed to improve visualization of the areas (from Olson et al., 2001). Biogeographical units are coincident with some of the main geomorphological surfaces in Brazil. Besides heterogeneous in terms of elevation, such surfaces represent units of the relief linked by similar geological origins and similar geophysical composition.

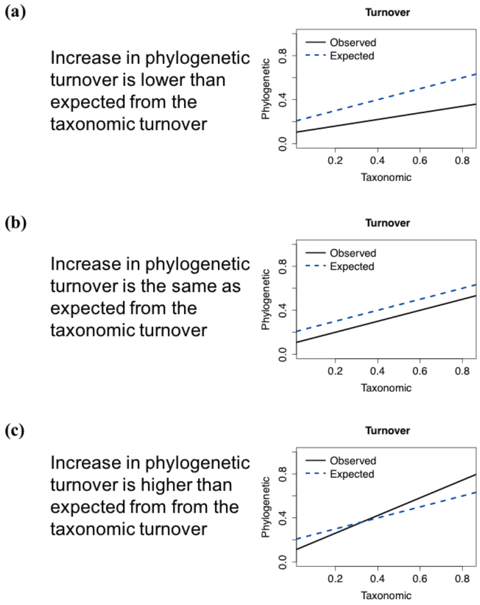

Figure S2 - Predictions of the relationships between phylogenetic and taxonomic turnover estimated by a least-squares regression. **(a)** Lower than expected rates indicate that most sites are composed by closely related species. **(b)** Rates not different than random indicate lack of phylogenetic structuring. **(c)** High rates indicate that sites are composed by more distantly related species than expected from changes in species composition.

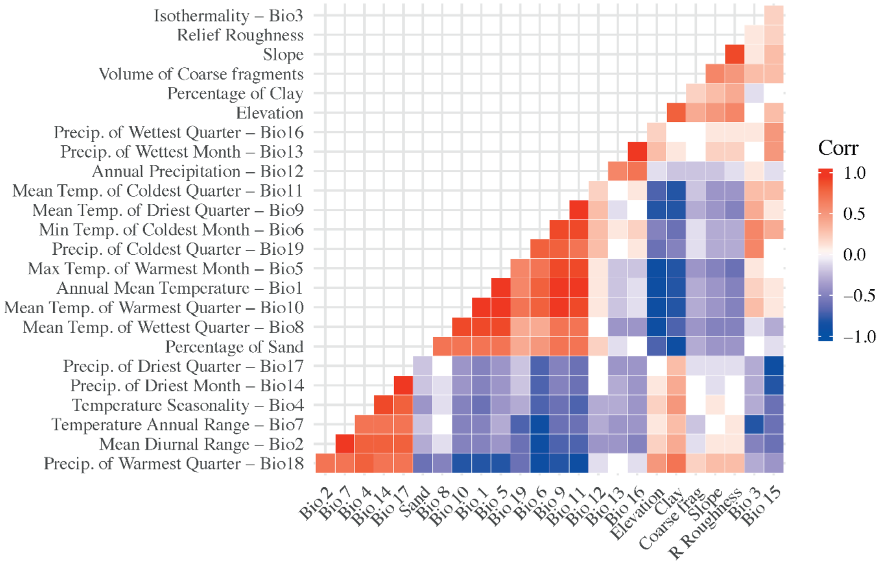

Figure S3 – Correlations among all variables environmental variables. Variables are grouped by their similarity (complete linkage). Variables selected for the final models fall mostly into different groups (see table S1).

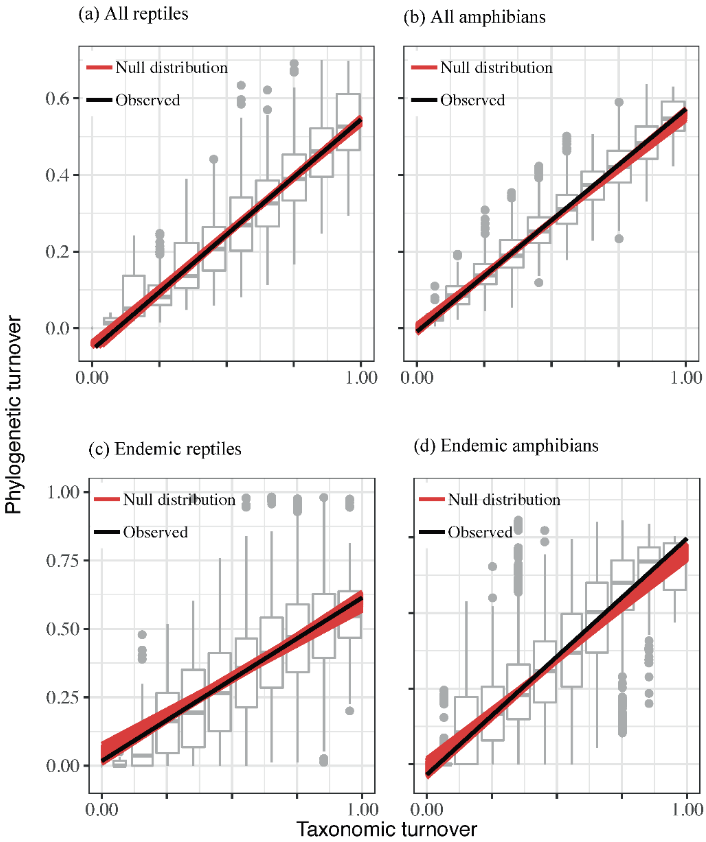

Figure S4 - Relationships between phylogenetic turnover and species turnover estimated by linear regressions. Boxplots indicate the distribution of pairwise phylogenetic turnover values for each binned class. **(a-b)** There is a linear increase in phylogenetic turnover for reptiles and amphibians, which is not different from the null expectation. **(c-d)** Similar results were found for the species endemic to the Cerrado. In general, there is a tendency for higher increase of phylogenetic turnover with species turnover in amphibians.

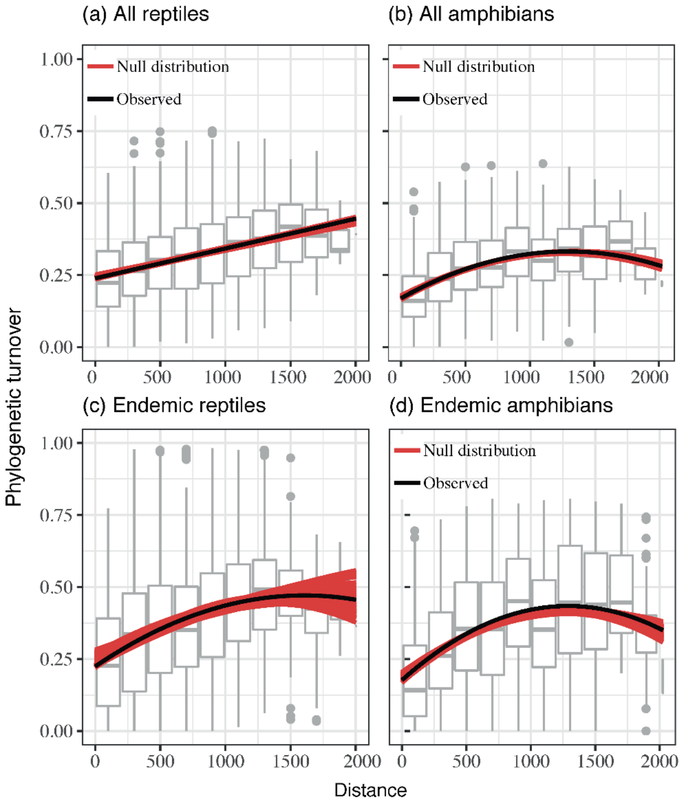

Figure S5 – Relationships between phylogenetic turnover and geographical distance (a) There is a linear increase in turnover rates with distance for reptiles. **(b)** For amphibians, turnover rates are not linear, as well as for **(c)** for endemic reptiles, and **(d)** endemic amphibians. For both reptiles and amphibians, the increase in turnover with distance is not different from the null distribution. Boxplots indicate the observed values. Notice that for both groups, the highest values of turnover occur within the first 1,000 km.

III

Manuscript submission to *PLOS ONE*

Taxonomic review of the genus *Xenopholis* Peters, 1869 (Serpentes: Dipsadidae), integrating morphology with ecological niche

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ABSTRACT

We evaluated the taxonomic status of species currently allocated in the genus *Xenopholis* (*X. sca*laris, *X. undulatus*, and *X. werdingorum*) on the basis of concordance between quantitative (meristic and morphometric) and qualitative (color pattern, hemipenes and skull features) analyses of morphological characters, in combination with ecological niche modeling. We recognize all three taxa as valid species and improve their respective diagnosis, including new data on color in life, pholidosis, bones, and male genitalia. We found low overlap among the niches of each species, entirely corroborating the independent source of phenotypic evidence. The allopatric distribution of species in the genus *Xenopholis* is also well supported by the non-overlapping model predictions. Even though all three species occurred in the leaf litter of distinct forested habitats, *Xenopholis undulatus* is found in the elevated areas from Brazilian Shield (Caatinga, Cerrado and Chaco), whereas *X. scalaris* occurs in the Amazon and Atlantic forest rainforest, and *X. werdingorum* in the Pantanal wetlands.

Keywords: Ecological niche modeling**,** linear morphometry, skull osteology. *Xenopholis scalaris, Xenopholis undulatus, Xenopholis werdingorum.*

INTRODUCTION

The dipsadid snake genus *Xenopholis* Peters 1869 constitutes a monophyletic group that comprises three species: *Xenopholis scalaris* (Wucherer, 1861), *X. undulatus* (Jensen, 1900) and *X. werdingorum* Jansen, Álvarez and Köhler, 2009 (Grazziontin *et al.,* 2012; Zaher *et al*., 2009). It includes small to moderate-sized snakes (300–450 mm), with cryptozoic lifestyle (i.e., underneath soil surface and high humidity habitats) (Cunha and Nascimento, 1978; Carvalho and Nogueira, 1998; Martins and Oliveira, 1998; Jansen *et al.,* 2009; Costa *et al.,* 2013), feeding on anurans (Cunha and Nascimento, 1993; Martins and Oliveira, 1998; Argôlo 2004; Bernarde and Abe, 2010) and, eventually, lizards (Costa *et al.,* 2013). Members of the genus are widely distributed in the Neotropics: *Xenopholis scalaris* ranges from the cis-Andean portion of South America along the ombrophilous forests of Colombia, Guianas, Ecuador, Peru, Bolivia and Brazil, with disjunct populations along the Atlantic Forest (Wucherer, 1861; Cunha and Nascimento, 1993; Martins and Oliveira, 1998; Argôlo, 2004; Silva *et al.,* 2006; Bernarde and Abe, 2010; Prudente *et al.,* 2010; Zaher *et al.,* 2011; Silva *et al.,* 2012; Wallach *et al.,* 2014; Hamdan *et al.,* 2015; Roberto *et al.,* 2017; França *et al.,* 2019); *Xenopholis undulatus* is distributed in Paraguay and also in open areas of the Brazilian Shield, from Maranhão south to Paraná (Wallach *et al.,* 2014); and *Xenopholis werdingorum* occurs at the Chiquitanos Forests of Bolivia and at border with Pantanal wetlands (Powell *et al.,* 2016; Jansen *et al.,* 2009; Wallach *et al.,* 2014). Due to their small body sizes and secretive lifestyle, species of this genus are rarely found (Carvalho and Nogueira, 1998; Cunha and Nascimento, 1978), resulting in poor representation in herpetological collections and scarce literature regarding their biology and morphological variation (Jansen *et al.,* 2009; Vieira *et al.,* 2012).

Herein, we evaluated the taxonomic status of species currently allocated in the genus *Xenopholis* (*X. scalaris*, *X. undulatus*, and *X. werdingorum*) on the basis of concordance between quantitative (meristic and morphometric) and qualitative (hemipenial and skull features) analyses of morphological characters, in combination with niche overlap analyses. From the resulting refined locality records, we produced environmental niche modeling for gaining insights on the determinants of species distributions in this genus under current and past environmental conditions.

Taxonomic résumé

Wucherer (1861) described *Elapomorphus scalaris* based on two specimens from Canavieiras (15º39'S 38º57'W; 5m above sea level, hereafter asl) and Mata de São João (12º32'S 38°18'W; 31m asl), state of Bahia, Brazil. Peters (1869) erected the genus *Xenopholis* in order to accommodate *Elapomorphus scalaris*. Boulenger (1896) synonymized *Xenopholis braconnieri* Peters 1869 and *Gerrhosteus prosopis* Cope 1874 with *Xenopholis scalaris* based on a specimen from "brasilien" (= Brazil) and two specimens from Nauta in the Peruvian Amazonia*,* respectively. Werner (1925) named *Sympeltophis ungalioides* based on an individual from central Brazil. Peters and Orejas-Miranda (1970) placed this last taxon in the synonymy of *Xenopholis scalaris.* Jensen (1900) described *Oxyrhopus undulatus* based on a specimen from Lagoa Santa (19º38'S 43º53'W; 835m asl), Minas Gerais, Brazil. Schenkel (1900) named *Paroxyrhopus reticulatus* based on a specimen from Bernalcue (= Bernal Cué, 25°15'S 57°17'W; 218m asl), Paraguay. Werner (1913) described *Oxyrhopus latifrontalis* based on a specimen collected in the east of Minas Gerais State, Brazil. Amaral (1923) described *Paroxyrhopus atropurpureus* based on an individual collected at Nova Baden (19°58'S 44°6'W; 848m asl), state of Minas Gerais, Brazil. Amaral (1929) placed *O. latifrontalis* and *P. atropurpureus* in the synonymy of *P. undulatus*. Peters and Orejas-Miranda (1970) recognized *P. undulatus* and *P. reticulatus* as valid species. Hoge and Federsoni (1975) proposed the synonymy of later two names and transferred *Oxyrhopus undulatus* to the genus *Xenopholis* due to unique vertebral morphology with neural spines expanded, forming rugose shields. Jansen *et al.,* (2009) described *Xenopholis werdingorum* based on three specimens from Santa Cruz de la Sierra, department of Santa Cruz, Bolivia. The author diagnosed *X. werdingorum* from *X. undulatus* mainly based on differences in dorsal coloration.

MATERIAL AND METHODS

Material and techniques for phenotypic characters

We examined 349 *Xenopholis* specimens including: 261 *Xenopholis scalaris*, 76 *X. undulatus*, and 12 *X. werdingorum* housed at 20 herpetological collections. The institutional abbreviations are as detailed in Sabaj (2016), except for CORBIDI; MUSM; MZUESC; PUC-MG; PUC-RS and URCA (detailed in Appendix 1).

The terminology used for the cephalic scales follows Peters (1964), while the counting of ventral and subcaudal scales are based on Dowling (1951). We measured most variables with an analogical caliper DIGIMESS® to the nearest 0.01 mm, except for snout-vent and tail lengths, which were taken with a millimetric ruler to the nearest 1.0 mm. We examined maxilla in situ under stereoscope through a narrow lateromedial incision between the supralabials and the maxillary arch. After removing tissues covering the maxillary bone, we counted the teeth and the empty sockets of specimens preserved in alcohol. We determined the sex of individuals by checking for the presence of the hemipenes through a ventral incision at the base of the ventral surface of the tail. We defined mature individuals through inspection of convoluted deferent ducts in males, and the occurrence of vitellogenic follicles (at least 5 mm in length; Resende and Nascimento, 2014), eggs or pleated glandular uterus in females (Shine, 1988a, 1994). We prepared hemipenes according to the method for the preparation of preserved hemipenes modified from Pesantes (1994), by replacing KOH for distilled water (see Passos *et al.,* 2016). Prior to the inflation with petroleum jelly, the organs remained 15 minutes in alcohol solution of Alizarin red to stain the ornamented calcareous structures according to an adaptation from the original procedure used by Uzzell (1973) and modified by Harvey and Embert (2009). Terminology for hemipenial descriptions follows Dowling and Savage (1960) and Zaher (1999). We examined osteological features through µCT Scan high-resolution images (*Xenopholis scalaris*, MNRJ 17070 and UMMZ 245078; *Xenopholis undulatus* UMMZ 108820; *Xenopholis werdingorum*, UFMT-R 12051) and dried skulls (*Xenopholis undulatu*s, MNRJ 18728). We scanned specimens with a high energy µCT Scan Skyscan 1176/Bruker system at COPPE, Instituto Alberto Cruz Coimbra de Pós-Graduação e Pesquisa de Engenharia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. We reconstructed the images using the FDK algorithm (Feldkamp, Davis and Kress, 1985) with the software InstaRecon version 1.3.9.2, and analyzed the results with the software CTVox version 2.7.0. We accessed µCT from UMMZ specimens through MorphSource Project <morphosource.org>. We prepared dried skulls following the techniques modified from Hangay and Dingley (1985). We followed Cundall and Irish (2008) for osteological terminology.

Geographical data

Coordinates of localities were acquired by consulting the original data available in museum catalogs, digital databases, or geographical gazetteers (e.g., IBGE 2011). We refined, whenever possible, the origin of records obtained from the literature or in museum databases without specific field coordinates using Google Earth Pro 7.1.2 (Google, 2005). We include literature data only when the information was sufficient to ensure the unequivocal identification of the species.

Species concept

In this study, we followed the "general lineage" species concept from de Queiroz (1998, 1999). We considered the presence of one or more exclusive apparently fixed diagnostic characters (either morphological or ecological), which distinguishes a given taxon from the others as a species delimitation criteria. Nonetheless, as the sample sizes assessed here were in some cases too small for statistical tests of qualitative characters (Wiens and Servedio 2000), we looked for concordance between discrete and continuous characters, as well as corroboration from environmental niche modeling. The correspondence between these kinds of data might represent independent evidence for species delimitation. However, in the cases of discrete characters, we explicitly searched for congruence with additional lines of evidence to increase the confidence for diagnosing (see Passos and Fernandes 2009; Passos *et al.,* 2010a).

Operational analytical units

We divided the available sample into four groups based on the current taxonomy and considering the disjunct pattern of distribution of *Xenopholis scalaris*. The operational analytical are: Group 1 = *Xenopholis scalaris* from the Atlantic Forest; Group 2 = *X. scalaris* from the Amazon Basin; Group 3 = *X. undulatus*, and Group 4 = *X. werdingorum.* From strictly exploratory analytical purposes, we further divided the populations of *Xenopholis scalaris* to investigate if there is any additional level of phenotypic differentiation. We considered Atlantic Forest populations into a single group due to the relatively small sample available and divided the Amazonian samples in two operation unities: a Guiana Shield group with locations to the north of the Amazon River and to the west of the Negro River; and south of the Amazon River within the Amazon Basin (see Henderson *et al.,* 2009 for a similar analytical approach).

Quantitative analyses

To reduce the ontogenetic bias in the morphometric analyses, we selected only adult individuals to compose the dataset. As we did not find information about sexual maturity for the genus in the literature, we performed a small incision above the cloaca of the specimens to delimit the smallest mature specimen (details below).

We evaluated the assumptions of univariate normality and homoscedasticity with Kolmogorov-Smirnov and Levene tests, respectively (Zar, 1999). In cases where the distributions of the characters violated such assumptions, we performed non-parametric tests or excluded such variables from the analyses (Zar, 1999). We performed analyses of univariate (ANOVA) or multivariate (MANOVA) variance in order to test for the presence or absence of sexual dimorphism within each group (Gotelli and Ellison, 2011). We found evidence of sexual dimorphism in some groups; therefore, we performed parametric tests separately for males and females. We also performed discriminant function analyses (DFA) with 95% confidence from an exploratory perspective to evaluate the quantitative discrimination between currently recognized species. Individuals and variables with missing data above 30% were discarded from the statistical analyses (see Passos and Fernandes, 2009). The remaining missing data was substituted by the ingroup mean for each variable with the function "replace missing data" (StatSoft, 1998). All the statistical tests were performed in Statistica 5.1 (StatSoft, 1998).

Qualitative analyses

We selected the following variables for the population frequency analyses: (1) dorsal color with spots forming discontinuous lateral bands along the body (e.g., *X. scalaris*), (2) irregular spots forming winding vertebral stripe (e.g., *X. undulatus*), (3) absence of spots with a nearly uniform dorsal coloration (e.g., *X. werdingorum*); (4) presence of well-defined or inconspicuous vertebral line (e.g., *X. scalaris*).

Hemipenis were analyzed as follows: hemipenial body regarding the general shape of capitulum (elongated = capitulum shorter than the hemipenial body vs. cylindrical = capitulum approximately of similar length than the hemipenial body); sulcus spermaticus bifurcation (outside capitulum vs. within the capitulum); level of lateral expansion of the sulcus spermaticus; capitulum ornamented with spinulate or papillate calyces; and arrangement (serial [linearly or transversally] vs. irregular) of hooked spines on the sulcate, lateral and sulcate sides of hemipenial body; and ornamentation of medium-distal portion of hemipenial body on the asulcate side (nude, vs. ornamented with papillae or spines).

Niche modeling and niche overlap

We generated model predictions for *Xenopholis scalaris* and *X. undulatus* with ensemble forecasting modeling (Araújo *et al.,* 2007). Ensemble modeling integrates properties of algorithms of different complexities, generally yielding higher prediction accuracy (Le Lay *et al.,* 2010). We obtained the final ensemble models for each species by applying an AUC-weighting (Area Under the Curve) to the results of 15 different modeling algorithms available in the *sdm* R-package (Table S1 – Naimi and Araújo 2016). The performance of the models were assessed using 5-fold cross-validation (10 replications), totaling 150 models per species. The predictive performance of the final ensembled models was measured using True Skill Statistics – TSS (Alloche *et al.,* 2006). Due to the small number of known localities for *X. werdingorum* (N = 8), we used an ensembling of small models technique (Breiner *et al.,* 2015). For this, models were produced with all possible combinations of only two environmental variables each time and then weighted by AUC. For the small models, we used only four different modeling algorithms (Table S1), as modeling performance in this technique does not increase with the use of additional algorithms (Breiner *et al.,* 2015). We recognize that the low number of presence records makes the model for *X. werdingorum* less reliable than the others; therefore, we carefully interpret the results for this species.

To produce the models, we randomly generated 148 pseudo-absences, which is equivalent to the total number of presence records for all *Xenopholis* species at the chosen resolution (see below). We chose the random selection of pseudo-absences due to the low detectability of the *Xenopholis*, which precludes the use target-group bias corrections – e.g., by selecting pseudo-absences only in well-sampled localities for all snakes species in South America, but in which *Xenopholis* species are absent – (Ranc *et al.,* 2017). As the species of the genus *Xenopholis* are mostly found in forested habitats, we delimited the extent area for sampling the pseudo-absences to the region to east of the Andean mountains corresponding to the maximum latitudinal range of tropical forests since the Last Maximum Glacial (Costa *et al.,* 2018), which encompasses all known records of *Xenopholis*. This was done to incorporate environmental characteristics of areas in which species of this genus would probably be able to disperse (e.g., no clear geographical barriers such as the Andean mountains).

We obtained ten temperature and nine precipitation bioclimatic layers from the CHELSA project (Karger *et al.,* 2017). The scarcity of climatic stations in the Neotropical region is known to decrease modeling performance (Soria-Auza *et al.,* 2010). However, CHELSA variables are estimated from both climatic stations and from models of atmospheric circulation, which improves climate predictions for isolated areas. We also downloaded soil variables from soilgrids.org (Hengl *et al.,* 2014), including the percentage of clay and sand – median values for the first 15 cm of the soil surface – and elevation data from GMTED2010 (Danielson and Gesch, 2011). We aggregated by median and projected all layers to an equal-area projection with a resolution equivalent to 0.2 × 0.2° at the 30th degree of latitude.

As very little is known about specific habitat requirements in *Xenopholis*, to select the most relevant environmental variables for each species, we run a first round of models using the Random Forests algorithm in the *sdm* R-package (Breiman, 2001, Naimi and Araújo, 2016). We ran models with all possible combinations of three variables each time (N = 1,540), calculating the most important variables using an AUC-based permutation – median values per variable (Janitza *et al.,* 2013). From this result, we chose the first six more important variables with no multicollinearity problems (Variance Inflation Factor < 10) (Naimi *et al.,* 2012), except for elevation. Elevation is an indirect predictor of species niche, and therefore, high response values for this variable may indicate that additional environmental determinants are missing for the targeted species (Guisan *et al.,* 2017). Due to the small number of records, for *X. werdingorum*, we performed the Radom Forests variable selection by modeling all combinations with two variables each time. Finally, we also projected the distribution of the species to the Last Glacial Maximum (Community Climate System Model 4, LGM – 22,000 years ago).

We tested the degree of niche overlap in the environmental space for each pair of species using the Schoener's D metric (Schoener, 1970), which goes from zero (no overlap) to one (total overlap). For this, we used the PCA-env approach to produce a reduced two-dimensional linear representation of all 22 variables described above (Broennimann *et al.,* 2012). To test the significance of the overlap, we used two different randomizations. In the first one, occurrence records of each pair of species are shuffled 100 times (niche equivalence). In the second, the whole density of occurrence records of one of the species pairs (calculated as part of the PCA-env approach) is randomly reallocated within the available climatic space 100 times. The significance is accessed by comparing the observed D metric with the distribution of the mentioned randomizations. All niche overlap tests were performed in R using the scripts provided by Broennimann *et al.* (2012). We additionally tested niche overlap for the disjunct records of *X. scalaris* in Amazonia and the Atlantic Forest, to verify whether these populations are isolated not only in the geographical but also in the environmental space.

RESULTS

Quantitative analyses

The analysis of the gonad maturation indicated a SVL of 207 mm for the smaller adult male in *Xenopholis*. Therefore, we considered individuals (males and females) ≥ 207 mm as adults for all subsequent statistical approaches. We found sexual dimorphism for *Xenopholis scalaris* and *Xenopholis undulatus* in the number of ventral scales, with females presenting higher values ($F_{220.2}$ = 18.4; $p < 0.001$); in the number of subcaudal scales, with male presenting higher values ($F_{220.2}$= 95.95, $p < 0.001$); and in the number of pre-ocular scales ($F_{220.2}$= 4.50, $p = 0.03$), with males presenting greater number. All other meristic and morphometric variables did not exhibit apparent secondary dimorphism. Due to the low sample size for *Xenopholis werdingoru*m ($n < 30$), we performed a non-parametric Kolmogorov-Smirnov test that indicated dimorphism for the tail length in this species ($p < 0.05$).

The discriminant analyses performed for males (17 variables, $n = 124$) and females (17 variables, $n = 159$) showed that the disjunct set of populations of *Xenopholis scalaris* from Amazonia and the Atlantic Forest entirely overlap in the 95% confidence intervals. In contrast, *X. undulatus* and *X. werdingorum* were completely discriminated between themselves and to the populations from *X. scalaris* (Figs. 1–2). In DFA for males, the first DF was responsible for 47.21% of discrimination considering the predefined groups, while the second DF was responsible for 12.55% (Fig. 1). In DFA for females, the first DF was responsible for 51.45% discrimination and the second for 11.92% (Fig. 2). The additional discriminante analyzes aiming to segregate Amazonian populations of *Xenopholis scalaris* resulted in a high degree of overlap considering the 95% confidence intervals for each group in both analyzes (males and females).

There is a high level of overlapping among the DFA confidence ellipses for Amazonian populations of *X. scalaris* subdivided into three groups (Figs. 3–4). In synthesis, the Atlantic Forest and Amazonian populations of *Xenopholis scalaris* are not distinguishable from each other nor between north and south groups (considering the Amazon River as a putative barrier for north/south dispersion). In contrast, *X. scalaris* as a single evolutionary unity is distinguished from *X. undulatus* and *X. werdingorum*, differing from each other by number of dorsal spots, ventral and subcaudal scales, number of anterior dorsal scales rows (from cervical region to midbody), and number of prefrontals (Table 1).

Qualitative analyses

Color pattern

We divided *Xenopholis scalaris* into Atlantic Forest (*n* = 109) and Amazonian (*n* = 152) sets of populations. All specimens examined had discontinuous bands on the lateral region of the body, with ventral and supralabial scales uniformly white. The vertebral line was well defined (Atlantic Forest 59.6%, Amazonia 56.6%) or barely distinct (Atlantic Forest 40.4%, Amazonia 43.4%); dorsal ground color light brown (Atlantic Forest 29.4%, Amazonia 27.6%), brown (Atlantic Forest 50.5%, Amazonia 46.7%) or dark brown (Atlantic Forest 21.1%, Amazonia 25.6%); dorsum of head is light brown (Atlantic Forest 29.4%, Amazonia 27.6%), brown (Atlantic Forest 49.5%, Amazonia 46.7%) or dark brown (Atlantic Forest 21.1%, Amazonia 25.6%). As there are no obvious geographical barriers separating subpopulations of *Xenopholis undulatus* and *X. werdingorum*, and both species present relatively conspicuous coloration through its entire distribution, we do not perform additional population frequency analyses for these species.

Hemipenial morphology

The analysis of hemipenial variation (Fig. 5) revealed some unique characteristics to each previously recognized species. For *Xenopholis scalaris*, all hemipenes for both the Atlantic Forest (*n* = 7) and Amazonia (*n* = 10) specimens are as follow: unilobed with centrolinear sulcus spermaticus bifurcation within capitulum; capitulum and hemipenial body with similar length; capitular grooves are well defined on the asulcate and lateral sides of the organs, and barely defined at sulcate face of hemipenis; no ornamentation on the proximal region of the organ; hemipenial body ornamented with nearly 10 hooked spines on both faces of the organ. Among the Atlantic Forest specimens, 71.43% of the organs are elongated, and 28.57% have a cylindrical shape. All Amazonian specimens present cylindrical organs (Fig. 6).

There is no clear variation on the hemipenis of *Xenopholis undulatus* (*n* = 2) and *Xenopholis werdingorum* (*n* = 3). However, the scarce sample available for both species may have influenced such result.

Niche modeling and overlapping

The six most important environmental used in the models were mostly not coincident among all species (Table S2). All models used in the ensembling forecast presented consistent performance, with AUC values ranging from 0.76 to 0.89 (scale from 0 to 1) and TSS ranging from 0.42 to 0.68 (scale from -1 to +1), which indicates low rates of false positive and false negative predictions. The final model for *Xenopholis scalaris* under the current climate predicts a vast region with highly suitable environments in Amazonia and a narrow zone with suitable environments along the Brazilian Atlantic Forest (Fig 7a). The projection for *X. scalaris* for the LGM climate indicates that the distribution of suitable environments for this species might have been more limited back then (Fig 7b). For *X. undulatus,* areas with higher suitability are distributed mostly on the highlands of the Cerrado, and in intermediate values in the remaining lowlands and valleys of Cerrado and on the highlands the Caatinga (Fig 7c). Different from *X. scalaris*, areas with highly suitable conditions might have been more widely distributed in the LGM for *X. undulatus* (Fig 7d). None of the current projections for both species indicate highly suitable areas coincident with the distribution of *X. werdingorum*. The ensemble of small models for *X. werdingorum* (AUC = 0.66-0.88, TSS = 0.09-0.15), indicate highly suitable areas were predicted mostly in the Pantanal, Chaco and additional seasonally-dry tropical formations in Bolivia and Brazil (Fig. 8), but not in the highlands inhabited by *X. undulatus*.

The niche overlap tests between *X. undulatus* and *X. werdingorum* (Table 2) indicates no significant overlap in the niches occupied by the ranges of these two species (i.e., niche similarity), although this is not true for the presence records (niche equivalency). All remaining results indicate that the overlap in the niches of pairs of species is not different than random, including the test for the disjunct populations of *X. scalaris* in Amazonia and the Atlantic Forest.

Taxonomic decision

Based on the results obtained through the congruence of our quantitative and qualitative phenotypic analyses evaluated here in combination with niche modeling and niche overlapping, it was not possible to separate the predefined groups into new taxonomic units. In this way, we chose to maintain the current taxonomic arrangement for the genus *Xenopholis*, increasing the diagnosis of each previously recognized species.

Xenopholis scalaris Wucherer, 1861

Elapomorphus scalaris Wucherer, Proc. Zool. Soc. of London 1861:325. (two syntype from municipalities of Canavieiras 15º39'1"S 38º57'42"W and Mata de São João 12º31'50"S 38°17'59"W, state of Bahia, Brazil).

Xenopholis braconnieri Peters, Monatsberichte der Koniglichen Preussische Akademie des Wissenschaften zu Berlin 1869:441. (unknown provenance).

Gerrhosteus prosopis Cope, Proceedings of the Academy of Natural Sciences of Philadelphia 1874:71. (two syntype collected by Professor James Orton at Nauta on the Peruvian Amazon).

Sympeltophis ungalioides Werner, 1925:52 Sitzb. Nath. Naturwiss. Akad. Wiss. Wien 1, 134:52. (from Central Brazil).

Comparative diagnosis. *Xenopholis scalaris* can be distinguished from all congeners by the following unique characters: (1) dorsum of head from red to reddish-brown in life, and light brown or pale brown after preservation (vs. black in *X. undulatus* and *X. werdingorum* in life and after preservation); (2) dorsal ground color of body red, reddish-brown to orange in life and light or pale brown after preservation, with black alternated paravertebral blotches, sometimes connected forming conspicuous cross-bands (vs. dorsal ground color covered with conspicuous black, with a broad and irregular vertebral stripe in *X. undulatus*, and dorsum black with three paraventral scale rows, orange in life and pale brown after preservation); (3) dorsal scales rows 17/17/17 (vs. 19/19/17 in *X. undulatus* and *X. werdingorum*); (4) ventral scales in males 126–169, 128–175 in females (vs. 160–190 in males of *X. undulatus* and 181–195 of *X. werdingorum*, 168–196 in females of *X. undulatus* and 180–196 *X. werdingorum*); (5) subcaudal scales 28–45 in males, 27–42 females (vs. 36–55 in males of *X. undulatus* and 46–54 of *X. werdingorum* and 33–60 in females of *X. undulatus* and 38–48 *X. werdingorum*); (6) postocular scale single (vs. two postoculars in *X. undulatus* and *X. werdingorum*); (7) hemipenis unilobed with bifurcated sulcus spermaticus (vs. unilobed usually with undivided sulcus spermaticus in *X. undulatus* and bilobed organ in *X. werdingorum*); (8) hemipenis strongly capitulated on the sulcate side (vs. little capitulated in *X. undulatus* and no capitulated in *X. werdingorum*); (9) capitulum ornamented with spinulate calyces (vs. papillate on distal portion of capitulum in *X. undulatus* and entirely papillate in *X. werdingorum*); (10) hemipenial body ornamented with hooked spines and longitudinal plicae (vs. hemipenial body ornamented with hooked spines and dispersed papillae); (11) pupil red (vs. brown in *X.*

undulatus and *X. werdingorum*); (12) neural spine of vertebrae without septum perpendicular to longitudinal axis of body (vs. presence of a narrow longitudinal septum in *X. undulatus* and *X. werdingorum*).

Color pattern in life (Fig. 9). Dorsum of head and body reddish-brown; dorsal ground color of the body reddish-brown along 6th to 12th scale rows, with black alternated paravertebral blotches, sometimes connected and forming conspicuous cross-bands (one to two scales long); paravertebral blotches or bands generally extending three or four scale rows in the vertebral region on each side of body; first five scale rows usually uniformly orange; sometimes paraventral rows covered with few black marks (dots or spots) on the limit of lighter paraventral region along fifth scale row; supralabials mostly creamish white with little invasion of red pigmentation on its dorsal edges; ventral surface of body uniformly creamish white to creamish yellow. Iris red.

Color pattern in alcohol 70% (Fig. S1). The color pattern after preservation is very similar to coloration in life, only changing to fading red, orange, and yellow pigments. The orange and red pigments become pale brown and brown, respectively; while yellow and creamish yellow become cream.

Quantitative variability for secondarily dimorphic characters. Number of ventral scales 126–169 (mean = 135.25, SD = 6.11, N = 115) in males, 128–175 (mean = 139.33, SD = 7.33, N = 131) in females; number of subcaudal scales 28–45 (mean = 35.90, SD = 3.73, N = 115) in males, 27–42, (mean = 32.09, SD = 2.46, N = 131); and number of preoculars in males (1-2, mean = 1.09, SD = 0.28, N = 104), number of preoculars in females (1-2, mean = 1.04, SD = 0.17, N = 128). Refers to Table 3 for variables with no sexual dimorphism.

Hemipenial morphology (Fig. 10). Fully everted and maximally expanded hemipenes rendered a unilobed, unicalyculate and semicapitate organ; capitulum similar or barely slender than hemipenial body; capitular crotch strongly developed on the asulcate side and nearly indistinct at sulcate face of hemipenis; capitulum clavate or almost attenuated and similar or shorter than hemipenial body; capitulum uniformly covered by spinulate calyces; basal region of capitulum on the asulcate and lateral faces with hooked spines entering hemipenial body through capitular crotch; hemipenial body elliptical and scattered with large hooked spines; hemipenial body usually covered with three rows hooked spines (5/5/2), almost transversally arranged from the capitular groove to proximal region of hemipenial body; sulcate and lateral faces of hemipenis with six/seven hooked spine on each side of the sulcus spermaticus; hemipenial body with longitudinal plicae among hooked spines; larger spines generally located laterally below sulcus spermaticus bifurcation; sulcus bifurcates for about half of organ within capitulum, with each branch centrolinearlly oriented and running to the distal region of capitulum, but not reaching its apex; sulcus spermaticus margins expanded after sulcus bifurcation and not bordered by spinules; basal naked pocket absent or indistinct; most basal region of hemipenis without spinules and with longitudinal plicae.

Skull morphology (Figs. 11-14). SNOUT COMPLEX: *Premaxilla:* delimits the skull anteriorly, contacting nasals dorsoposteriorly (Fig. 11A) and septomaxillae in its posteromesial portion (Fig. 11C); narrow and ventrally inclined transverse processes (Fig. 12A), posteriorly oblique, not contacting maxillae (Fig. 11); ascendant process with a pair of lateral projections on its base (Fig. 12A); base of ascendant process wider than its tapered dorsal edge, which is inserted between pair of nasals (Fig. 12A); vomerian processes short and divergent, contacting anteromedial portion of vomers (Fig. 11B); nasal process present (Fig 11C). *Septomaxillae:* located dorsally to the vomers, ventral to the nasals and posteriorly to the premaxilla (Fig. 11); together with the vomers, forms the vomeronasal organs capsule (voc – Figs. 13-14); Conchal process with tapered edge posteriorly turned, not overlapping with the anterior portion of the maxilla or with transverse process of the premaxilla (Fig. 11); conchal process in contact with the mesolateral portion of nasals (Fig. 12A); reduced contact with the nasals, forming a large orifice bordered by the premaxilla, nasals and the septomaxillae (Fig. 11C); posterior portion in contact with the septomaxilar process of the frontal bone (Fig. 13C). *Vomers:* located in the anteroventral portion of the skull, posteroventrally to the premaxilla (Fig. 11B); anterior process laterally to the vomerian process of premaxilla, contacting it (Fig. 11B); mesolateral projection not overlapped by the palatines; posterior process with small foramen in its ventral portion and vertical lamina concave. *Nasals:* located in the dorsal surface of the skull, posteriorly to the premaxilla and anteriorly to the frontals, not contacting it (Fig. 11A); large in dorsal view, about the same extension as the frontals; mesial portion with wide lateral process that curves ventrally, with straight edge contacting conchal process of septomaxilla (Fig. 11C); ascendant process of premaxilla inserted between the pair of nasals, in its anterior portion (Fig. 11A); short frontal process, not contacting the frontal; in lateral view, recess on the anteroventral portion, forming an orifice bordered by the premaxilla, septomaxillae and the nasals (Fig. 11C).

BRAINCASE: *Frontals:* located in the dorsal surface of the skull, posteriorly to the nasals and anteriorly to the parietal (Fig. 11); contacts the prefrontals in the anterolateral region; inter-olfactory pillar located on its anterior portion (Fig. 12B), visible in frontal view, with small septomaxillar processes ventrally contacting the septomaxilla; frontal with about the same extension of nasals and half the extension of the parietal in dorsal view (Fig. 11A), does not contact the nasals; anterior margins straight and obliques with respect to lateromedial axis; posterior margins slightly concave and obliques regarding the lateromedial axis (Fig. 11A); lateral margins straight, with anterior and posterior portions about the same width; prefrontal process absent; suture with prefrontal oblique; in dorsal view, pair of frontals wider than longer, not contacting postorbital; in lateral view, small posteroventral process, delimiting ventral surface of the optical foramen, which is small, with less than the interorbital sept height, totally inserted in the frontals, with parietal only bordering its posterior margin (Fig. 11C); in frontal view, frontal subolfactory process enclosing the optic nerve canal (sub – Fig. 12C); frontal supraorbital shelf present (sos – Fig. 12B and C). *Parietal:* located posteriorly to frontals, contacting it anteriorly (Fig. 11A); contacts supraoccipital posteriorly, prootic posterolaterally (Fig. 11A), and parabasisphenoid ventrolaterally (Figs. 13C, 14A-B); anterolaterally portion contacts postorbital in dorsal view (Fig. 11A); parietal does not contact supratemporals or close the braincase cavity ventrally, which is enclosed by parabasisphenoid (Fig. 11B); subtriangular shape in dorsal view; sutures with frontal convex, given anterior margin a convex aspect with a small projection on the mesial portion (where the pair of frontals meet each other) (Fig. 11A); small postorbital process; posterior margin convergent with parietal-exoccipital suture convex; dorsolateral crests slightly developed, convergent, not contacting each other, originating in the most anterolateral point of parietal and converging to suture with exoccipital at its medial region; in frontal view, two small processes (one on each side) on the ventromedial portion, which form the border of orbital foramina, and a pair of postorbital processes (Fig. 12B). *Supraoccipital:* located in dorsal surface of skull, contacting parietal anteriorly, exoccipitals posteriorly, prootic anterolaterally, and laterally supratemporals (Fig. 11A); anterior margin convex in dorsal view; dorsolateral crests of parietal continue over supraoccipital, becoming a transversal crests on the posterior region of the bone, which corresponds to dorsal surface of semicircular canal; longitudinal crest originates in the medial portion of transversal crests (Fig 11A); transversal crests slightly oblique relative to lateromedial axis; both lateral portions of supraoccipital form cavities inside, which begin at about the middle line of the bone; two dorsal canals correspond to anterior and posterior vertical semicircular canals (avsc and pvsc, Fig. 13) and a ventral canal belongs to cavum vestibuli (cv, Fig. 13), connecting to prootic and exoccipitals – semicircular canals and cavum vestibuli forms the ear capsule; its dorsal surface bears two pairs of small foramina on the mesolateral portion. *Exoccipitals:* irregular in shape, delimiting dorsoposterior portion of the skull (Fig. 11); contacts supraoccipital anteriorly and atlas posteriorly (Fig. 11A); its posteroventral portion forms, with basioccipital, the occipital condyle (oc, Fig. 13C), located on the ventral margin of foramen Magnum; contacts prootic anterolaterally and basioccipital ventrally (Fig. 11); foramen oval located in its anteromesial portion, in lateral view – a cavity where the columella auris is inserted (Fig. 11C); in the suture between exoccipitals and prootic there is a continuity of the foramen; other foramina are present ventral to the foramen oval; bears part of the posterior vertical semicircular canal and horizontal semicircular canal (Fig. 13B), which have continuity in the supraoccipital and prootic; posterior margin straight, slightly oblique to lateromedial axis (divergent) (Fig. 11A); transversal crests of supraoccipital continues at lateral portion of exoccipitals, where supratemporal relies (Fig. 11); sutures with basioccipital straight. *Basioccipital:* located in the ventral portion of the skull, delimiting it ventroposteriorlly (Fig. 11B); anteriorly delimited by parabasisphenoid, with suture straight, and posteriorly delimited by atlas; posterior edge forming the main portion of occipital condyle, on the margin of foramen Magnum (oc, Fig 13C); anterolateral portions contact prootic and posterolateral portions contact exoccipitals, with both sutures straights and obliques to the anteroposterior axis; shape nearly pentagonal; two small dentigerous processes, forming a slightly developed crest on its mesial portion (Fig. 11B); mesolateral processes absent. *Parabasisphenoid:* composed by fusion of basisphenoid with parasphenoid; elongated triangular bone in ventral view, with anterior tip tapered (Fig. 11B); located ventrally on the braincase, contacts medial portions of frontals anteriorly, basioccipital posteriorly, prootics posterolaterally, and parietal laterally; in ventral view, a pair of small foramina pierce the bone close to its posterolateral margin, corresponding to posterior opening of the Vidian canal; in dorsal view (from the inside of the endocast) there is a well delimited crest forming a cavity in which the posterior foramina of the Vidian channel opens (cVc, Fig. 13B); anterior openings of the Vidian canal located on the suture with parietal, in its mesial portion (apVc, Fig. 14B); in dorsal view, anterior portion of bone, the parasphenoid rostrum (pr, Fig. 14A), overlapped by frontals (Fig. 13B); in ventral view, edges of the parasphenoid rostrum and subolfactory processes of frontals borders the optic nerve canal (onc, Fig. 14A), which is totally enclosed by the parabasisphenoid after the parasphenoid rostrum. *Prootics:* located lateroposteriorlly in the braincase (Fig. 11); contacts parietal anteriorly and anterodorsally, supraoccipital posterodorsally, exoccipital posteriorly, basioccipital posteroventrally, and parabasisphenoid anteroventrally; most of the dorsal face overlapped by supratemporal (Fig. 11A); in lateral view, two large foramina present, being the foramen for maxillary branch of trigeminal and the foramen for mandibular branch of trigeminal (Fig. 11C); both foramina open in the interior of the braincase, and they are apart from each other by the laterosphenoid; there is a foramen, connected to foramen oval, situated in its posterior margin (foramen oval). However, the columella auris restricted to above the exoccipital; in dorsal view, longitudinal crest absent; there are other small foramina ventral to the maxillary and mandibular branches of trigeminal foramina. *Prefrontals:* irregular and located anterolaterally to frontals, forming anterior limit of the orbit (Fig. 11); ventrally, contacts palatine process of maxilla and maxillary process of palatine; in lateral view, anterior portion with a convex projection and posterior portion concave; ventral portion narrow and dorsal portion broader (Fig. 11C); lateral foramen absent; prefrontal-frontal suture oblique; lacrimal foramen visible in frontal view (lf, Figs. 12A, 13C), on its ventral region, with well-developed lacrimal process (lp, Fig. 13C); mesomedial process well developed (mp, Fig. 13C) and posteroventral process slightly developed. *Postorbitals:* located anterolaterally to parietal, contacting only this bone (Fig. 11); forms posterior limit of orbit; subtriangular shaped, with dorsal edge straight and ventral edge tapered; anterior margin slightly concave and posterior straight (Fig. 11C).

PALATOMAXILLARY ARCH: *Maxillae:* located on the anterolateral portion of the skull (Fig. 11); contacts ventral region of prefrontal in its mesomedial portion, through the palatine process, and the ectopterygoid in its posterior portion; does not contact premaxilla, postorbital and palatine; arched shaped, with lateral lamina convex and medial lamina concave; bears 15 posteriorly curved prediastemal teeth of about the same size, and two postdiastemal grooved tooth, about the same size of prediastemal ones; diastema with size equivalent to one teeth socket; palatine process located on the medial face of the bone, from teeth 9-12, with tapered edge posteriorly curved (pp, Fig 14A); posterior portion of maxilla wider. *Palatines:* located on the medial portion of the palatomaxilar arch, in the ventral face of the braincase (Fig. 11B); contacts pterygoid on its posterior portion and prefrontal through the maxillary process; bears seven teeth; broad and elongated shape with two processes: laterally, there is maxillary process, with wide basis and tapered end posteriorly curved and displaying 5-7 teeth (mp, Fig. 14A), and medially the choanal process, broad and ventrally concave, situated after the last tooth to the end of the bone, not contacting parabasisphenoid (cp, Fig. 14A); posterior edge single. *Ectopterygoids:* located on the mesolateral portion of the skull (Fig. 11B); contacts maxilla on its anterior portion and pterygoid at posterior portion; elongated shape with anterior edge with expanded bifurcation and posterior edge unique; expanded portion corresponds to about one third of its total extension; in dorsal view, lateral branch of bifurcation has a small lateral process (Fig. 13); first third of the bone, from its posterior end, contacts pterygoid, displaying less than half of the extension of pterygoid. *Pterygoids:* elongated, located on the posterior portion of palatomaxillary apparatus at ventral face of the braincase (Fig. 11B); contacts palatines on its anterior portion and ectopterygoid at mesolateral portion; bears 28 posteriorly curved teeth, being the anterior tooth larger than the posterior ones; in ventral view, anterior portion tapered, getting wider abruptly on the level of the 13º tooth, where it contacts ectopterygoid (Fig. 11B); gets broad again after the end of the teeth row, curving laterally (Fig. 11B); small lateral process on the pterygoid-ectopterygoid joint; anterior edge simple; pterygoid extension corresponding to more than half of the whole skull extension; in dorsal view bears a lateral depression, from the articulation with ectopterygoid to edge of the bone.

SUSPENSORIUM AND MANDIBLE: *Supratemporals:* located on the dorsoposterior portion of the skull (Fig. 11); overlaps much of the dorsal surface of the prootic and anterolateral portion of exoccipitals, contacting the most lateral part of supraoccipital; elongated shaped and dorsoventral compressed; posterior boundary beyond the posterior limit of the braincase. *Quadrates:* articulating with supratemporals anterodorsally and with the glenoid cavity of the compound bone posteroventrally (Fig. 11A, C); bears a small mesomedial process in posterior view, the articulatory process of quadrate, which articulates with the *columella auris*; approximately triangular in lateral view, with dorsal portion straight and ventral portion tapered; about the same width in all its extension on posterior view; small anterodorsal process in contact with supratemporal. *Columella auris:* small bones, located on the lateroposterior portion of the braincase (Fig. 11C); articulates with exoccipital through the foramen oval; formed by an anterior portion, round and expanded, which is inserted in the foramen oval, and an elongated and tapered region, extending towards quadrate. *Mandible:* composed of two sets of bones, the hemimandibles, that are arch-shaped and compound bone. *Dentaries:* located in the anterior tip of the hemimandibles, posteriorly contacting angular and splenial in medial view and compound bone in lateral view; medially arched and elongated shaped; in lateral view bifurcated in its posterior edge, forming the dorsal and ventral processes of dentary; dorsal process longer than ventral; dorsal surface with 24 posteriorly curved teeth, being the anterior teeth longer than the posterior; dorsal process extending from tooth 14-24; ventral process in the level of tooth 14-21; in medial view, splenial overlaps ventral process, being visible only anterior portion of the bone and its dorsal process; meckel canal located between the ventral surface of the dorsal process and splenial; mental foramen on the level of 10º-11º teeth. *Splenials:* located in the posteroventral portion of dentary in lateral view; contacts angular posteriorly; triangular-shaped, with anterior edge tapered and posterior straight; bears the anterior milohiode foramen close to the joint with angular; about same extension of angular, but broader; small tapered process on the contact with dorsal portion of angular-splenial joint; posterior limit on the level of dentary-compound bone suture. *Angulars:* located in the posterior portion of splenial in medial view; contacts splenial anteriorly, dentary anteriorly (dorsally and ventrally) and compound bone along all its extension; bears the posterior milohioide foramen on its mesoanterior portion; triangular shaped, with anterior edge straight and posterior tapered; anterior limit on the level of dentary-compound bone suture; posterior boundary surpasses the posterior limit of dorsal process of dentary; small tapered dorsoanterior process, on the suture with splenial; angular-splenial suture visible in ventral view. *Compound bones:* represent the fusion between prearticular, articular, and surangular bones; largest bone of the mandible, located on its posterior portion, with elongated shape; contacts dentary and angular anteriorly, and articulates with quadrate posteriorly, through the glenoid cavity, a saddle-shaped cavity; in lateral view, anterior region projects between dorsal and ventral processes of dentary; in medial view, anterior edge projects dorsally to angular and ventrally to dorsal process of dentary; anteriorly to glenoid cavity, two crests are present: the prearticular and the surangular crests; in lateral view, prearticular crest slightly higher than surangular; between those crests there is a cavity that ends in its anterior portion with a foramen, the posterior orifice of the inferior dentary canal, which possesses a way out to the lateral face of the bone thought a foramen situated slightly posterior to the end of dorsal process of dentary (anterior surangular foramen); retroarticular process present.

Distribution (Fig. 15). Based on available records obtained by examination of preserved samples, literature data, and environmental niche models, *Xenopholis scalaris* is restricted to lowland ombrophilous forests to the east to the Andes. This species occurs in the Amazonia domain in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, and Suriname, with disjunct populations along the Brazilian Atlantic Forest from Pernambuco to Rio de Janeiro States (Fig. 12). Mumaw *et al.,* (2016) included this species in the snake fauna of Venezuela based on the AMNH-R 4443 specimen. However, such a record is doubtful since the location for this specimen is Brazil-Venezuela, with no additional information. Even though the occurrence of this species in the Amazonian portion of Venezuela is very likely, as far we know, there is no voucher of *X. scalaris* with precise provenance came from Venezuela. Therefore, considering only the accurate data available, we exclude *X. scalaris* from the Venezuelan snake fauna.

Xenopholis undulatus **Jensen, 1900**

Oxyrhopus undulatus Jensen, 1900. Videnskabelige meddelelser fraden Naturhistoriske forening i Kjobenhavn, 1900:106. (two syntypes collected by Prof. Reinhardt and Prof. E. Warming from Lagoa Santa 19º38'S 43º53'W, Minas Gerais, Brazil).

Paroxyrhopus reticulatus Schenckel, 1901. Verh. Naturforsh. Ges. Basel 13:169. (holotype female from Bernal Cue 25°15'S 57°17'W, Paraguay).

Oxyrhopus latifrontalis Werner, 1913. Mitt. Naturhist. Mus. Hamburg 30:30. (holotype male collected at the eastern portion of the state of Minas Gerais, Brazil).

Paroxyrhopus atropurpureus Amaral, 1923. Proc. New England Zool. Club. Vol.8:90. (holotype adult male IBSP 3003 from Nova Baden 19°58'S 44°6'W, state of Minas Gerais, Brazil; paratype adult male MZUSP 1499 from a locality near Mariana, state of Minas Gerais, Brazil).

Paroxyrhopus latifrontalis - Amaral, 1930. Mem. Inst. Butantan 4:208. (holotype, HMZ 4811, from the west of Minas Gerais).

Paroxyrhopus undulatus Bailey in Peters & Orejas Miranda, 1970. Cat. Neotrop. Squamata Snake:238.

Paroxyrhopus reticulatus Bailey in Peters & Orejas Miranda, 1970. Cat. Neotrop. Squamata Snake:238.

Comparative diagnosis. *Xenopholis undulatus* can be distinguished from all congeners by the following unique characters: (1) dorsum of head black in life and after preservation (vs. dorsum of head red to reddish-brown in life and brown or pale brown in preservative in *X. scalaris*); (2) dorsal ground color covered with a conspicuous black, broad and irregular vertebral stripe (vs. dorsal ground color of body red, reddish brown to orange in life, and light or pale brown after preservation, with black alternated paravertebral blotches, sometimes connected forming conspicuous cross-bands in *X. scalaris.* Dorsum black with three paraventral scale rows orange in life and pale brown after preservation); (3) dorsal scales rows 19/19/17 (vs. 17/17/17 in *X. scalaris*); (4) ventral scales in males 160–190, 168–196 in females (vs. 126–169 in males of *X. scalaris* and 181–195 of *X. werdingorum*, 128–175 in females of *X. scalaris* and 180–196 *X. werdingorum*); (5) subcaudal scales 36–55 in males, 33–60 in females (vs. subcaudal scales 28–45 in males of *X. scalaris* and 46–54 of *X. werdingorum*, 27–42 females of *X. scalaris* and 38–48 *X. werdingorum*); (6) two postocular (postocular single in *X. scalaris*); (7) hemipenis unilobed usually with undivided sulcus spermaticus (vs. hemipenis unilobed with bifurcated sulcus spermaticus in *X. scalaris* and bilobed in *X. werdingorum*); (8) hemipenis little capitulated (vs. hemipenis strongly capitulated on the sulcate side in *X. scalaris* and not capitulated in *X. werdingorum*); (9) capitulum ornamented with papillate on the distal portion of capitulum (vs. capitulum ornamented with spinulate calyces in *X. scalaris* and entirely papillate in *X. werdingorum*); (10) hemipenial body ornamented with hooked spines and dispersed papillae (vs. hemipenial body ornamented with hooked spines and longitudinal plicae in *X. scalaris* and hemipenial body ornamented with lateral spines and dispersed papillae in *X. werdingorum*); (11) pupil brown (vs. pupil red in *X. scalaris*); (12) neural spine of vertebrae with a narrow septum perpendicular to longitudinal axis of body (vs. absent in *X. scalaris*).

Color pattern in life (Fig. 16). Dorsal part of the head almost entirely black, except for irregular red spot(s) or blotch(es) covering the parietal and/or the occipital region; lateral surface of the head black on the dorsal edges of the supralabials; supralabials usually uniformly creamish white, sometimes with invasion of black pigment; dorsal ground color of body red to reddish-orange, except for the first one or two scale rows, which are creamish white or cremish red; dorsum with a conspicuous and winding vertebral stripe, extending from the cephalic-cap to the tip of the tail; vertebral stripe with lateral projections in zig-zag or symmetrical expansion to the paravertebral region; lateral expansion reaching seventh or eighth scales rows toward to the vent; between third to sixth or seventh dorsal scale rows there are black spots or blotches (half-scale to two scales long) on the interspaces among the lateral expansion of vertebral stripe; sometimes those black marks connected to a lateral expansion along the body extension, giving impression of an irregular dorsal pattern; ventral surface of body uniformly creamish white to creamish red. Iris brown.

Color pattern in alcohol 70% (fig. S2). The color pattern after preservation is very similar to coloration in life, only changing to fading red, orange, and reddish-orange pigments. The orange and reddish-orange or red pigments become pale brown and brown, respectively.

Quantitative variability for secondarily dimorphic characters. Number of ventral scales 160–190 (mean = 178.86, SD = 7.3, N = 22) in males, 168–196 (mean = 181.02, SD = 6.35, N = 49) in females, number of subcaudal scales 36–55 (mean = 44.59, SD = 5.52, N = 22) in males, 33–60 (mean = 41.46, SD = 5.22, N = 49) in females and number of preoculars 1–1 (mean = 1, SD = 0, N = 22) in males, 1–2 (mean = 1.04, SD = 0.20, N = 48) in females. Refers to table 4 for variables those did not present sexual dimorphism.

Hemipenial morphology (Fig. 17). Fully everted and maximally expanded hemipenes rendered an unilobed, unicalyculate, and weakly semicapitate organ; capitulum slender than hemipenial body; capitular crotch barely distinct on the asulcate side and nearly indistinct at sulcate face of hemipenis; capitulum attenuated and shorter than hemipenial body; capitulum uniformly covered by papillate calyces; on the sulcate face of organ there two rows of lateral hooked spines inside capitulum area; basal region of capitulum on the sulcate and lateral faces with hooked spines arranged approximately in traversal rows; hemipenial body on the sulcate side with about six to seven lateral hooked spines, three distal rows following shallow transversal grooves; hemipenial body elliptical and scattered with large hooked spines and disperse papillae; asulcate side of hemipenis with four rows of hooked spines, transversally arranged into three shallow grooves; lateral hooked spines decreasing in size on both side of organ from the capitulum toward the basal region of hemipenial body; hemipenial body with papillae among hooked spines on both sides of organ; larger spines located laterally below capitulum; sulcus spermaticus usually undivided and running to distal region of capitulum, but not reaching it apex; sometimes sulcus spermaticus bifurcates on distal region of capitulum (cf. Zaher, 1999); sulcus spermaticus margins expanded inside capitulum, and not bordered by some papillae along hemipenial body; proximal region of hemipenial body with high concentration of papillae; basal naked pocket absent or indistinct.

Skull morphology. The cranium of *Xenopholis undulatus* is very similar to the cranium of *X. scalaris*, differing from this in the following aspects:
SNOUT COMPLEX: *Premaxilla:* ascendant process with no pair of lateral projection on its base, the same width in all its extension, and presenting a blunt edge; vomerian processes with lateral lamina convergent and medial lamina parallel to the anteroposterior axis, overlapping vomer; nasal process absent. *Septomaxillae:* conchal process with a tapered, but blunt edge, posteriorly turned. *Vomers:* anterior process under overlapped by vomerian process of premaxilla; posterior process with a medium-size foramen in its ventral portion. *Nasals:* small in dorsal view, smaller than frontals. *Frontals:* Largen than nasals and about 2/3 of parietal extension; contacts postorbital on its posterolateral portion; optical foramen inserted equally in the frontals and parietal. *Parietal:* sutures with each frontal almost straight and oblique to the lateromedial axis, given the anterior margin of parietal a concave aspect. *Supraoccipital:* Lateral portion does not contact supratemporals; no visible foramina in dorsal view. *Exoccipitals:* large foramen oval located on its anteromesial portion, in the suture with prootic, in lateral view; no visible foramina ventral to the foramen oval; suture with basioccipital straight and presenting a small process. *Basioccipital:* suture with parabasisphenoid slightly concave; sutures with exoccipitals bear mesolateral processes slightly developed; three short dentigerous processes, forming

a slightly developed crest on its mesial portion that continues until the mesolateral processes. *Prootics:* foramen oval situated in the suture with exoccipital; three other small foramina ventral to the maxillary and mandibular branches of trigeminal foramina are present. *Prefrontals:* in lateral view, the anterior portion with a convex projection in the mesial region and concave lamina dorsal to it.

Postorbitals: contacting parietal and most lateroposterior portion of frontal in dorsal view; nearly "C" shaped, with anterior border concave and posterior convex, and a small posterior process on the most ventral point. *Maxillae:* Bears 16 prediastemal teeth; grooved tooth larger than the prediastemal teeth; palatine process located between 8-12 tooth. *Palatines:* bears ten teeth; maxillary process from teeth 7-10. *Pterygoids:* bears 14 teeth; no lateral process in the pterygoid-ectopterygoid joint; in dorsal view, bears a crest from the articulation with ectopterygoid to the edge of the bone. *Supratemporals:* does not contact supraoccipital; posterior boundary does not surpass the posterior limit of the braincase. *Quadrates:* approximately triangular in the posterior view, with ventral edge large and dorsal edge tapered; anterodorsal process absent. *Dentaries:* bears 23 teeth; dorsal process extending from tooth 16-23; ventral process on the level of tooth 16-21; mental foramen on the level of 12-13º tooth. *Splenials:* smaller than angular; tapered process contacting dorsal portion of angular-splenial joint absent. *Compound bone:* prearticular crest much higher than surangular.

Distribution (Fig. 15). *Xenopholis undulatus* is restricted to highland portions of the Caatinga, ecotonal zones between Caatinga and Atlantic Forest, and to riparian forests across the Cerrado. This species has a widespread distribution along the Brazilian Shield from the Maranhão to the Paraná States, reaching the Paraguayan Chaco on the other side of Paraná River.

Xenopholis werdingorum Jansen, Álvarez & Kohler, 2009

Xenopholis sp. - Marques, Eterovic, Strüssmann & Sazima, 2005:73 (Cabaceiras Farm, municipality of Poconé, 16º15'24"S 56º37'22"W, state of Mato Grosso, Brazil).

Comparative diagnosis. *Xenopholis werdingorum* can be distinguished from all congeners by the following characters: (1) dorsum of the head is black in life and after preservation (vs. reddish-brown in *X. scalaris*); (2) dorsum of the body black with three paraventral scale rows, orange in life and pale brown after preservation (vs. dorsal ground color of body red, reddish brown to orange in life and light or pale brown after preservation, with black alternate paravertebral blotches, sometimes connected forming a conspicuous cross-bands in *X. scalaris,* and dorsal covered with a conspicuous black, broad and irregular vertebral strip in *X. undulatus*); (3) dorsal scales rows 19/19/17 (vs. 17/17/17 in *X. scalaris*); (4) ventral scales in males 181–195, 180–196 in females (vs. 126–169 in males of *X. scalaris* and 160–190 of *X. undulatus*, 128–175 in females of *X. scalaris* and 168–196 *X. undulatus*); (5) subcaudal scales 46–54 in males, 38–48 females (vs. 28–45 in males of *X. scalaris* and 36–55 of *X. undulatus* and 27–42 in females of *X. scalaris* and 33- 60 *X. undulatus*); (6) two postoculars (vs. postocular single in *X. scalaris*); (7) hemipenis bilobed (vs. hemipenis unilobed with bifurcated sulcus spermaticus in *X. scalaris* and unilobed usually with undivided sulcus spermaticus in *X. undulatus*); (8) hemipenis no capitulated (vs. hemipenis strongly capitulated on the sulcate side *X. scalaris* and little capitulated in *X undulatus*); (9) Capitulum entirely papillate (vs capitulum ornamented with spinulate calyces in *X. scalaris* and papillate on distal portion of capitulum in *X. undulatus*); (10) hemipenial body ornamented with lateral spines and dispersed papillae (vs. hemipenial body ornamented with hooked spines and longitudinal plicae in *X. scalaris,* and hemipenial body ornamented with hooked spines in *X. undulatus*); (11) pupil brown (vs. pupil red in *X. scalaris*); (12) neural spine of vertebrae with a narrow septum perpendicular to the longitudinal axis of body (vs. absent in *X. scalaris*).

Color pattern in life (Fig. 18). Dorsum and background of head uniformly black to the dorsal margins of the supralabials; supralabials creamish white; ventral surface of the body creamish white to creamish yellow; dorsal ground color of the body mostly black, except for the first five or six paraventral region red, orange or yellow colored; first scale row usually creamish white, followed for two or three yellow scale rows or five red to orange-red scale rows; more rarely, the black area may be restricted to seven to eight vertebral or paravertebral scale rows, resembling the winding vertebral stripe of the *X. undulatus* — Pupil brown pupil.

Color pattern in alcohol 70% (Fig. S3). The color pattern after preservation is very similar to coloration in life, only changing to fading red, reddish-orange, and yellow pigments. The reddish-orange and red pigments become pale brown and brown, respectively; while yellow and creamish yellow pigments become cream.

Quantitative variability for secondarily dimorphic characters. Caudal length 58–82 (mean = 67.80, SD = 8.72, N = 5) in males, 25–62 (mean = 48.57, SD = 13.86, N = 7). Refers to table 5 for variables those did not present sexual dimorphism.

Hemipenial morphology (Fig. 19). Fully everted and maximally expanded hemipenes rendered a moderately bilobed, bicalyculate and non-capitate organ; capitulum with similar width than distal portion of hemipenial body; capitular crotch indistinct on both faces of organ; labels attenuate and shorter than the remaining capitular region; capitulum with approximately half-size of the hemipenial body; capitulum uniformly covered by papillate calyces; calyces transversally arranged on the sulcate and lateral faces of hemipenis, and almost irregular on the asulcate side of organ; basal region of capitulum without hooked spines delimiting capitulation region on both sides of hemipenis; hemipenial body elliptical with the narrowing toward proximal region of hemipenis; narrowing region delimited by large hooked spines; large hooked spines concentrated on lateral region of hemipenial body; hemipenial body on the asulcate side of hemipenis entirely covered with high concentration of papillae; hemipenial body on the sulcate face of organ ornamented with papillae and two longitudinal rows of hooked spines; each longitudinal rows placed on one side of sulcus spermaticus, displaying about three or four mid-sized hooked spines; sulcus bifurcates on distal third of organ within capitulum, with each branch centrolinearlly oriented running to apices of lobes; sulcus spermaticus margins narrow along all its extension, and bordered by papillae; basal naked pocket absent or indistinct; most basal region of hemipenis entirely covered by high concentration of papillae.

Skull morphology. *Premaxilla:* ascendant process broad with similar width in all its extension and blunt edge; vomerian processes with lateral lamina convergent and medial lamina parallel to the anteroposterior axis, overlapping vomer. *Septomaxillae:* conchal process not contacting nasals. *Vomers:* anterior process overlapped by vomerian process of premaxilla; mesolateral projection slightly overlapped by palatines; posterior processes with large foramen, occupying half of its high, situated in its ventral portion. *Nasals:* small in dorsal view, slightly smaller than frontals. *Frontals:* larger than nasals; contacts postorbital in its posterolateral portion; optical foramen inserted equally in the frontals and parietal, with posteroventral process of frontal delimiting the anterior lamina of the optical foramen. *Parietal:* posterior edge tapered; dorsolateral crests are contacting each other on its posterior region. *Exoccipitals:* large foramen oval located on its anteromesial portion, in the suture with prootic laterally; suture with basioccipital straight, with a small dentigerous process. *Basioccipital:* sutures with exoccipitals bear mesolateral processes in its anterior portion; three short dentigerous processes, forming a slightly developed crest on the mesial portion of the bone that continues until the mesolateral processes. *Prootics:* foramen oval situated in the suture with exoccipital; on one side of the skull, the foramen oval connected to foramen for the mandibular branch of trigeminal. *Prefrontals:* in lateral view, tapered portion right above its ventral edge. *Postorbitals:* contacting parietal and most lateroposterior point of frontal in dorsal view; about "C" shaped, with anterior border concave and posterior convex, and a small posterior process in the most ventral point. *Maxillae:* palatine process overlaps the maxillary process of palatine; bears 13 prediastemal teeth; grooved tooth larger than the prediastemal ones; diastema with a size equivalent to two-socket teeth; palatine process located on the level of teeth 9-11. *Palatines:* contacts maxilla through the maxillary process, which is overlapped by the palatine process of maxilla; bears ten teeth; maxillary process extended at the level of 5-9 teeth; choanal process extended from nine teeth to posterior portion of the bone. *Ectopterygoids:* expanded portion corresponds to about 1/4 of its extension; first forth of the bone (from its posterior edge) contacts pterygoid, with about half of the extension of pterygoid. *Pterygoids:* bears 23 teeth; gets wider abruptly on the level of the 12th tooth; in dorsal view bears a well-developed crest from the articulation with ectopterygoid to its edge, which forms two depressions laterally. *Supratemporals:* The posterior boundary does not surpass the posterior limit of the braincase. *Dentaries:* dorsal process extending from the level of tooth 16 to 24th; ventral process on the level of tooth 16 to 21; mental foramen on the level of 13th tooth. *Splenials:* tapered process in contact with the dorsal portion of angular-splenial joint absent. *Compound bone:* prearticular crest much higher than surangular.

Distribution (Fig. 16). *X. werdingorum* is associated with dry formations of the Chiquitanos dry-forests and the western Cerrado, reaching the edges of the Pantanal depression (Marques *et al.,* 2005). Marques *et al.,* (2015) mentioned that the specimen of *X. werdingorum* illustrated in page 89 (Marques *et al.,* 2015:241) came from Luiz Eduardo Magalhães Power Plant (ca. 09º45'22"S 48º22'23"W), municipality of Palmas, state of Tocantins, Brazil and, as consequence, inadvertently expanded its distribution 1,100 km airline northeastern from the municipality of Poconé (16º15'24"S 56º37'22"W), state of Mato Grosso, Brazil. Powell *et al.,* (2016) expanded the distribution of *X. werdingorum* to the region of Beni, Bolivia, and considered the record mentioned above to Palmas into the species corology (Powell *et al.,* 2016). However, such mention was likely due to an error since there is no cataloged voucher of this species for this region in ZUEC or UFMT collections (the only collections with available material for this species in Brazil). In fact, this was confirmed to us (O.A.V. Marques pers. comm. with P. Passos in July 2019). Recently, Thus, we exclude the Palmas record from the distribution of *X. werdingorum.*

DISCUSSION

Phenotypic characters and species boundaries

The results obtained with the study of distinct and putatively non-correlated qualitative (color pattern, pholidosis, osteology, and male genital features) and quantitative (meristic and morphometric traits) morphological characters entirely corroborates the current taxonomy of the genus *Xenopholis*. The disjunct pattern of distribution of *X. scalaris* in the Amazon basin and in the Atlantic Forest, and the extensive distribution of the genus in South America would suggest additional cryptic species in this the genus (Jansen *et al*., 2009). Surprisingly, we found that the phenotypic characters analyzed here together with niche overlap analyses are congruent with the current taxonomy.

Each *Xenopholis*' species present a unique combination of qualitative features in male genital morphology, skull and vertebral osteology, coloration, and pholidosis. However, we found that coloration and the number of postoculars are both polymorphic, at least for *X. undulatus.* Additionally, the vertebral morphology and the number of segmental scales (ventral and subcaudals) of *X. undulatus* and *X. werdingorum* are indistinguishable, which is contrasting to what is available in the literature (Hoge and Federsoni Jr., 1974 and Jansen *et al*., 2009 vs. this study). Zaher (1999:83, fig. 95) briefly described and illustrated a few differences between the hemipenial morphology of *X. scalaris* and *X. undulatus*. In the case of *X. werdingorum*, Jansen *et al*. (2009) had access only to females specimens and could not prepare the hemipenis of this species, which is very distinct from the other two species of the genera. Thus, the additional data for *X. werdingorum* from our study provides key diagnostic characters not available in the literature to separate all three species of this genus.

The variation of male genitalia (cylindrical vs. elongated organs; Fig. 6) observed in the hemipenial body in the specimens in the Amazon, and the Atlantic forests highlighted the higher polymorphism in the coastal populations of *X. scalaris*. A similar pattern was recovered to *Epicrates cenchria*, which also is distributed to both ecoregions (see Passos and Fernanades, 2009). However, to access if this polymorphism is geographically structured, more samples representative of a wider geographical distribution in the Atlantic Forest are needed.

Several studies have previously corroborated the monophyly of *Xenopholis* (Dowling and Pinou, 2003; Vidal *et al*., 2010; Grazziotin *et al*, 2012; Zaher *et al*., 2019). However, the position of the genus inside the family Dipsadidae has been very unstable, being recovered in several different clades or even distinct tribes and subfamilies. Based on osteological features and the hemipenial traits gathered herein, we find no particular similarities among *Xenopholis* spp., *Caaeteboia amarali Hydrodynastes* spp. (corresponding to a clade recovered in Grazziotin et al., 2012). Nonetheless, we are aware that more morphological (and molecular) data from other species are necessary to test the phylogenetic position of *Xenopholis* within the family Dipsadidae. Therefore, the best solution to date is to consider the genus as Dipsadidae *insert sedis*, awaiting for a robust phylogenetic hypothesis.

Niche overlapping and species delimitations

The distinct environmental niche space occupied by the three *Xenopholis'* species potentially indicate niche divergence as a mechanism for diversification of this genus, with each species adapted to a significantly distinct set of environmental conditions (Pyron and Burbrink, 2009). The uplift of the central Brazilian Plateau and the expansion of open vegetation ecosystems since the Miocene have promoted both the diversification of organisms in these dry environments, as well as the geographical isolation of organisms in the disjunct forests (Oliveira-Filho and Ratter 1995; Prado *et al.,* 2012). This pattern is supported by several other groups of snakes and lizards that also present this allopatric pattern along Amazonia, the dry diagonal (Caatinga, Cerrado, Chaco) and the Atlantic Forest (Dixon *et al*., 1993; Rodrigues *et al*., 2014). Additionally, niche predictions for *X. werdingorum* also indicate that environmental conditions in western Cerrado, Pantanal basin, and Bolivian dry-forests are distinct from the Cerrado core area, again supporting niche divergence as a possible mechanism of speciation. This Pantanal/Cerrado pattern is also very similar to the distribution of many other reptiles and amphibians in this region (Azevedo *et al.,* 2016). With the increase in molecular data availability, alternative mechanisms to explain diversification in these groups could be adequately tested, for example, niche conservatism versus niche divergence across geographical barriers (Raxworthy *et al.,* 2007).

The set of broad-scale bioclimatic variables used here may not necessarily capture the set of determinant environmental conditions for some species (Ficetola *et al.,* 2018). For example, the microclimatic niche space experienced by *X. undulatus* in gallery forests may not be very different from the one experienced by *X. scalaris* in the Atlantic Forest or Amazonia. Besides, the elevation is the most important variable to explain the distribution of *X. undulatus* (Table S2)*,* indicating that essential characteristics of the environmental niche were not completely captured by the bioclimatic and soil layers used here (Guisan *et al.,* 2017). On the other hand, if the edge effect is more intense in forest patches and in gallery forests of highly seasonal areas such as the Cerrado and Caatinga if compared to core forest biomes, the microclimate experienced by species in these two environments will be different. Moreover, gallery forests in high elevation areas in Cerrado tend to be narrower than in the lowlands (Silva, 1996), and the size of forest fragments is also known to influence their microclimate and vegetation structure (Magnago *et al.,* 2015). Therefore, the bioclimatic variables used here may still correctly characterize the broad-scale geographical distribution of these species (Lembrechts *et al.,* 2018). Yet, studies on the specific microhabitat requirements for these snakes and the characterization of microenvironments in gallery forests versus forest biomes are necessary to test these alternative ideas.

The relictual or disjunct distributions of several taxa in South America suggests the role of past climate conditions in their current distribution (Gainsbury and Colli 2003). Our results indicate that during the LGM, highly suitable areas for *X. undulatus* were more widespread, connecting portions of the range that are currently isolated in the Caatinga domain. On the other hand, for *X. scalaris*, highly suitable areas are more widely distributed under the current warmer temperatures than in the LGM (Fig. 7). Past connections between Amazonia and Atlantic Forest have been proposed to explain the distribution of several taxa (Le do and Colli, 2017; Moritz *et al.*, 2000; Colinvaux *et al.*, 2001; Bush and Oliveira, 2006; Pellegrino *et al.*, 2011; Prates *et al*, 2015; Rodrigues *et al.*, 2014; Bush, 2017; Marques-Souza *et al.*, 2018). In fact, the putative past forest bridges connecting Amazonia with coastal Atlantic Forest has returned to biogeographical debate

(Leite *et al*., 2016; Prates *et al*., 2016; Dal Vechio *et al*., 2018). However, in the case of *X. scalaris*, neither the current or the LGM predictions indicates highly suitable areas connecting these two domains. Interestingly, the overlap in the niche space occupied by these disjunct *X. scalaris* populations is not different from random, which indicates that beyond the current geographical isolation, these populations are evolving in distinct climatic spaces.

Recent advance in snakes' taxonomy and the past connections between Amazonia and Atlantic forest

Disparate groups of snakes present species with disjunct ranges between Amazonia and Atlantic Forest, even after detailed taxonomic reviews, for example: *Dipsas catesbyi* (Peters, 1960; Harvey and Embert, 2009; Lima and Prudente, 2009); *Chironius carinatus* and *Chironius fuscus* (Dixon *et al*., 1993); *Liophis taenigaster* (Fernandes *et al*., 2002); *Lachesis muta* (Fernandes *et al*., 2004); *Epicrates cenchria* (Passos and Fernandes, 2009); *Drymoluber dichrous* (Costa *et al*., 2013); *Cercophis auratus* (Hoogmoed *et al*., 2019); and *Xenopholis scalaris* (present study). There are possibly many more snake species sharing a similar disjunct ranges, if we consider taxa still not assessed in detail as *Imantodes cenchoa, Sibon nebulata* and *Siphlophis compressus* (Peters and Orejas-Miranda, 1970; Wallach *et al*., 2014); *Thamnodynastes pallidus* (Franco and Ferreira, 2002); *Spilotes sulphureus* (Jadin *et al*., 2014); *Xenodon rhabdocephalus* (Myers and McDowell, 2014); and *Bothrops bilineatus* (Dal Vechio *et al*., 2018). On the other hand, many of these taxa can be potentially separated in more than one species restrict to a single ecoregion, as occurred with *Chironius multiventris/Chironius foveatus, Dendrophidion dendrophis/D. atlantica* (Hollis, 2006; Freire *et al*., 2010). These emerging patterns of snake distribution highlight the importance of detailed taxonomic work to the understanding the processes underlying biodiversity in South America.

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TABLES

Table 1. Analysis of 95% confidence intervals: Group 1 (*Xenopholis scalaris* - Atlantic Forest); Group 2 (*Xenopholis scalaris* – AM, South of the Amazon River and West of Rio Negro); Group 3 (*Xenopholis scalaris* - AM, Northern Amazon River and East Black River), Group 4 (*Xenopholis undulatus*) and Group 4 (*Xenopholis werdingorum*). Abbreviations: F = Female; M = male; max = maximum; = average; min = minimum; N = sample number; sd = standard deviation; -95% = lower limit of the 95% confidence interval, + 95% upper limit of the 95% confidence interval.

		Dorsal spots	Ventral (M)	Ventral (F)	Subcaudals (M)	Subcaudals (F)	Dorsal I	Dorsal II	Prefrontal
Group 1	Min	28.00	126	128	29	27	17	17	1
	Max	41.00	144	151	41	36	17	17	1
	Mean	34.19	132.98	135.37	34.98	31.65	17	17	1
	Sd	2.61	4.66	4.36	2.44	2.26			0
	-95%	33.69	132	134	34	31			1
	+95%	34.69	134	136	36	32			1
	N	106	44	62	44	62	106	106	105
Group 2	Min	24.00	127	130	28	29	17	17	1
	Max	40.00	169	175	45	42	17	17	1
	Mean	32.27	136.66	142.90	36.53	32.48	17	17	1
	Sd	2.97	6.55	7.65	4.26	2.58			0
	-95%	31.77	135	141	36	32			1
	+95%	32.76	138	145	38	33			1
	N	139	70	69	70	69	139	139	126
Group 3	Min	35.00	160	168	36	33	19	19	2
	Max	79.00	190	196	55	60	19	19	2
	Mean	69.32	178.86	181.02	44.59	41.47	19	19	2
	Sd	5.90	7.04	6.36	5.53	5.22			0
	-95%	67.93	176	179	42	40			2
	+95%	70.72	182	183	47	43			2
	N	71	22	49	22	49	71	71	70
Group 4	Min		181	180	46	38	19	19	2
	Max		195	196	54	48	19	19	2
	Mean		190	187.14	49.50	42.57	19	19	2
	Sd		6.63	5.90	3.42	3.64	0	0	0
	N		4	7	4	7	11	11	11
Group 5	Min	28	131	133	31	29	17	17	1
	Max	32	137	142	32	32	17	17	1
	Mean	29.83	135	137.33	31.67	30.33	17	17	1
	Sd	1.47	3.46	4.51	0.58	1.53			
	N	6	3	3	3	3	6	6	6

Table 2. Niche overlap (Schoener's D metric) results for each pair of species and for the for the disjunct *Xenopholis scalaris* populations from Amazonia and Atlantic Forest. P-Values are indicated for the two distinct randomizations= Niche Equivalency and Niche Similarity. Most results indicate no significant niche overlap, not even for the disjunct populations of *X. scalaris* in Amazonia and the Atlantic Forest.

Species pair	D-metric	Equivalency	Similarity
X. scalaris – X. undulatus	0.21	1.00	0.11
X. scalaris – X. werdingorum	0.08	0.91	0.17
X. undulatus – X. werdingorum	0.14	0.91	0.02
X. scalaris Amazonia – Atl. Forest	0.24	0.91	0.08

Table 3. Selected variables synthesizing the meristic and morphometric variation of *Xenopholis scalaris*. Abbreviations are as follow: CL = caudal length; SVL = snout-vent length; TL = total length; IL cont. 1º/2º ment. = Infralabial contact with the first/second mentonian; Max = Maximum; Min = Minimum; *N* = sample size; SD = standard deviation; -95% = lower limit of the confidence interval; + 95% = upper limit of the confidence interval.

	Min	Max		SD	-95%	95%	N
SVL (mm)	110	395	243.30	42.27	238.35	248.51	247
CL (mm)	15	75	48.36	9.80	47.13	49.59	247
TL (mm)	125	433	292.07	50.43	285.75	298.39	247
Distance nostril (mm)	1.10	2.72	1.92	0.31	1.88	1.96	229
Eye circumference (mm)	0.85	2.27	1.22	0.16	1.20	1.24	230
Dist. nostril-eye (mm)	1.14	3.73	2.37	0.41	2.32	2.43	229
Dist. rostral-eye (mm)	2.00	4.35	3.35	0.47	3.29	3.42	229
Dist. eye (mm)	2.15	4.47	3.20	0.36	3.15	3.25	230
Head length (mm)	6.75	14.70	10.87	1.35	10.69	11.05	230
Head width (mm)	3.30	7.49	5.38	0.74	5.29	5.48	230
Head height (mm)	2.27	4.67	3.45	0.45	3.39	3.57	229
Dorsal I	17	17	17	0	-	-	247
Dorsal II	17	17	17	0	-	-	247
Dorsal III	17	17	17	0	-	-	247
First temporal	1	1	1	0	-	-	246
Second temporal	1	3	2	0.14	1.96	2.00	246
third temporal	2	4	3	0.30	2.86	2.94	246
Supralabial	7	8	7.99	0.08	8	8	246
Larger supralabial	6	7	6.99	0.10	6.97	7.00	246
Geniais	4	4	4	0	-	-	246
Infralabial	8	9	8.99	0.09	8.98	9.00	244
1º supralabial-eye	3	4	3.99	0.09	3.97	4.00	232
2º supralabial-eye	4	5	4.99	0.09	4.97	5.00	232
Postocular	1	3	1.94	0.24	1.91	1.97	231
IL cont. 1º ment.	1	4	1	0	-	-	231
IL cont. 2º ment.	4	5	4.01	0.11	3.99	4.02	231
Prefrontal	1	3	1.01	0.18	0.99	1.04	232
Maxillary teeth	10	12	11.85	0.36	11.81	11.90	225
Number of spots	24	41	33.08	2.97	32.70	33.45	246

Table 4. Selected variables synthesizing the meristic and morphometric variation of *Xenopholis undulatus*. Abbreviations are as follow: CL = Caudal Length; SVL = snout-vent length; TL = total length; IL cont. 1º/2º ment. = infralabial contact with the first/second mentonian; Max. = Maximum; Min. = Minimum; = ; *N* = sample size; SD = standard deviation; -95% = lower limit of the confidence interval; + 95% = upper limit of the confidence interval.

	Min	Max		SD	-95%	95%	N
SVL (mm)	134	390	267.94	63.69	252.86	283.01	71
CL (mm)	21	69	47.76	12.47	44.80	50.71	71
TL (mm)	156	452	315	75.19	297.97	333.57	71
Distance nostril (mm)	1.16	2.95	2.19	0.42	2.09	2.29	67
Eye circumference (mm)	1.00	1.80	1.37	0.16	1.33	1.41	68
Dist. nostril-eye (mm)	1.47	3.60	2.34	0.43	2.23	2.44	67
Dist. rostral-eye (mm)	2.23	4.61	3.52	0.54	3.38	3.65	67
Dist. eye (mm)	2.54	4.90	3.50	0.50	3.38	3.63	68
Head length (mm)	8.18	14.74	11.64	1.75	11.22	12.07	67
Head width (mm)	4.10	7.80	5.78	0.74	5.54	6.01	68
Head height (mm)	2.63	5.33	3.87	0.65	3,71	4.03	67
Dorsal I	19	19	19	0	-	-	71
Dorsal II	19	19	19	0	-	-	71
Dorsal III	17	17	17	0	-	-	71
First temporal	1	1	1	0	-	-	70
Second temporal	2	2	2	-	-	-	70
Third temporal	2	3	2.84	0.36	2.75	2.93	70
Supralabial	7	8	7.98	0.11	7.95	8.01	70
Larger supralabial	6	7	6.67	0.47	6.55	6.78	70
Geniais	4	4	4	0	-	-	71
Infralabial	8	9	8.95	0.20	8.90	9.00	70
1º supralabial-eye	3	4	3.97	0.16	3.93	4.01	70
2º supralabial-eye	4	5	4.95	0.20	4.90	5.00	70
Pos-ocular	1	2	1.02	0.16	0.98	1.06	70
IL cont. 1º ment.	1	4	-	-	-	-	70
IL cont. 2º ment.	4	5	-	-	-	-	70
Prefrontal	1	2	1.98	0.11	1.95	2.01	70
Maxillary teeth	11	12	11.84	0.36	11.75	11.93	64
Number of spots	35	79	69.32	5.90	67.92	70.72	71

Table 5. Selected variables synthesizing the meristic and morphometric variation of *Xenopholis werdingorum*. Abbreviations are follow: CL = caudal length; SVL = snout-vent length; TL = total length; IL cont. 1º/2º ment. = Infralabial contact with the first/second mentonian Max. = maximum; Min. = minimum; *N* = sample size; SD = standard deviation; -95% = lower limit of the confidence interval; + 95% = upper limit of the confidence interval.

	Min	Max		SD	-95%	95%	N
SVL (mm)	160	370	298.50	62.80	258.58	338.42	12
CL (mm)	25	82	56.58	15.18	46.93	66.23	12
TL (mm)	185	452	355.08	76.58	306.42	403.74	12
Distance nostril (mm)	180	196	187.58	6.09	183.70	191.45	12
Eye circumference (mm)	38	54	44.58	4.96	41.43	47.73	12
Dist. nostril-eye (mm)	1.34	3.23	2.41	0.51	2.09	2.74	12
Dist. rostral-eye (mm)	1.12	1.76	1.48	0.18	1.36	1.59	12
Dist. eye (mm)	1.93	3.91	2.58	0.50	2.26	2.90	12
Head length (mm)	2.72	4.64	3.73	0.49	3.41	4.04	12
Head width (mm)	2.86	4.73	3.70	0.48	3.40	4.01	12
Head height (mm)	8.46	15.00	12.44	1.64	11.39	13.48	12
Dorsal I	4.42	7.72	6.36	0.81	5.84	6.87	12
Dorsal II	2.86	4.83	3.95	0.58	3.58	4.32	12
Dorsal III	19	19	19	0	-	-	12
First temporal	19	19	19	0	-	-	12
Second temporal	17	17	17	0	-	-	12
Third temporal	1	1	1	0	-	-	11
Supralabial	2	2	2	-	-	-	11
Larger supralabial	3	3	3	-	-	-	11
Geniais	8	8	8	-	-	-	11
Infralabial	6	7	6.81	0.40	6.54	7.08	11
1º supralabial-eye	4	4	4	-	-	-	11
2º supralabial-eye	8	9	8.90	0.30	8.70	9.11	11
Pos-ocular	4	4	4	-	-	-	11
IL cont. 1º ment.	5	5	5	-	-	-	11
IL cont. 2º ment.	1	1	1	-	-	-	11
Prefrontal	2	2	2	-	-	-	10
Maxillary teeth	2	2	2	-	-	-	12
Number of spots	11	12	11.83	0.38	11.58	12.08	12

Table 6. Diagnostic variability table for species of the genus *Xenopholis.* Mean values in parentheses. Abbreviations are follow: M = Male; F = Female; Un. = Unilobed; Bi = Bilobed.

	Dorsal spots	Ventral (M)	Ventral (F)	Subcaudals (M)	Subcaudals (F)	Dorsal I	Dorsal II	Prefrontal	Hemipenis
Xenopholis scalaris	24–41 (34.5)	126–169 (147.5)	128–175 (151.5)	29–45 (37)	27–42 (34.5)	17–17 (17)	17–17 (17)	1–1 (1)	Un.
Xenopholis undulatus	35–79 (57)	160–190 175)	168–196 (182)	36–55 (45.5)	33–60 (46.5)	19–19 (19)	19–19 (19)	2–2 (2)	Un.
Xenopholis werdingorum	0	181–195 (188)	180–196 (188)	46–54 (50)	38–48 (43)	19–19 (19)	19–19 (19)	2–2 (2)	Bi.

FIGURES

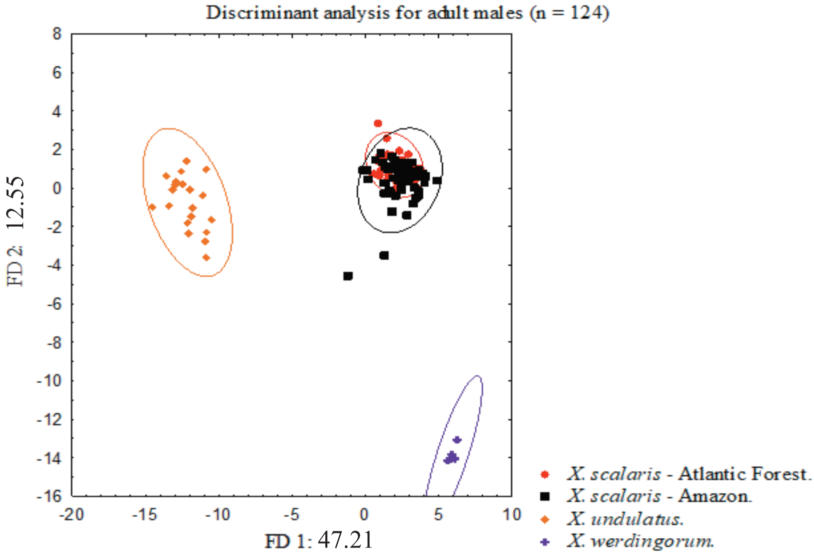

Figure 1. Bivariate plot derived from first two axes from scores of linear discriminate analyses performed for adult males (n = 124) of *Xenopholis scalaris* - Atlantic Forest; *Xenopholis scalaris* - Amazonia; *Xenopholis undulatus,* and *Xenopholis werdingorum.*

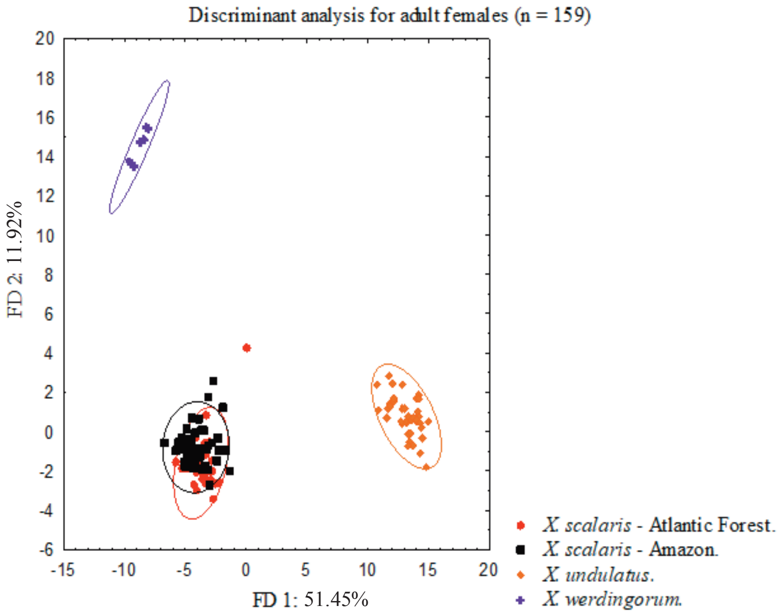

Figure 2. Bivariate plot derived from first two axes from scores of linear discriminate analyses performed for adult females (n = 159) of *Xenopholis scalaris* - Atlantic Forest; *Xenopholis scalaris* - Amazonia; *Xenopholis undulatus* and *Xenopholis werdingorum.*

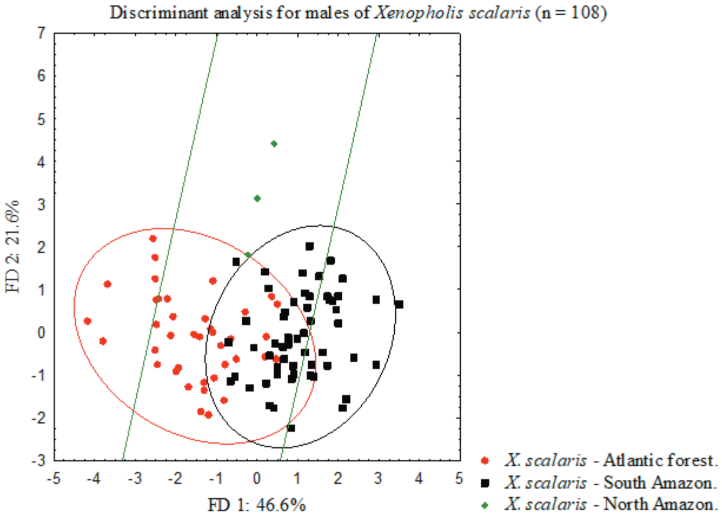

Figure 3. Bivariate plot derived from first two axes from scores of linear discriminate analyses performed for adult males (n = 108) for subpopulations of *Xenopholis scalaris* - Atlantic Forest, Northern Amazonia and Southern Amazonia.

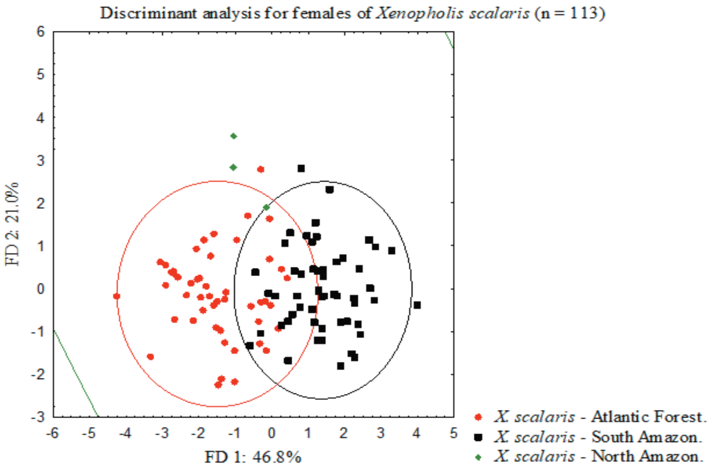

Figure 4. Bivariate plot derived from first two axes from scores of linear discriminate analyses performed for adult females (n = 113) for subpopulations of *Xenopholis scalaris* - Atlantic Forest, Northern Amazonia and Southern Amazonia.

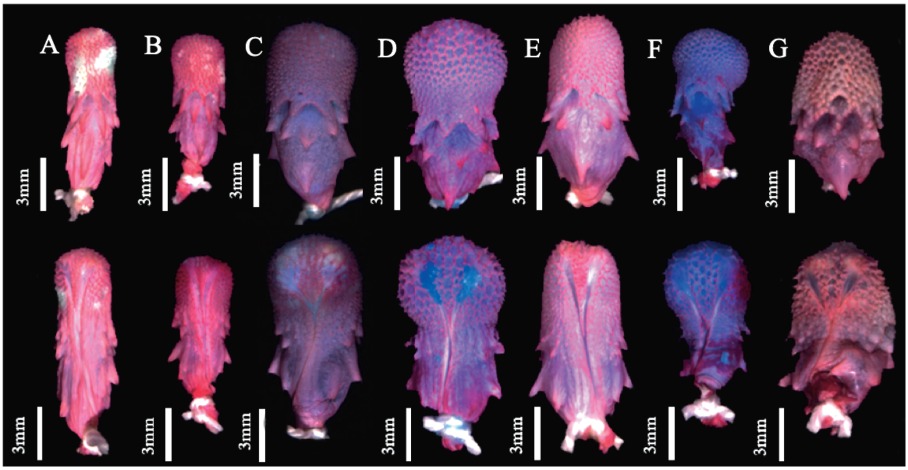

Figure 5. Hemipenial morphology variability in asulcate (upper) and sulcate (lower) sides of organs of *Xenopholis* spp. *X. scalaris* from municipality of Magé, state of Rio de Janeiro, Brazil (A- IVB 3552); from municipality of Jaqueira, state of Pernambuco, Brazil (B- URCA 6210); from municipality of Canavieiras, state of Bahia, Brazil (C- CZGB 1089); from region of Loreto, province of Loreto, district of Trompeteros, locality of Campamento San Jacinto, Peru (D- CORBIDI 1512); from municipality of Paranaita, state of Mato Grosso, Brazil (E- ZUEC 3443,); from region of Loreto, province of Putumayo, district of Putumayo, locality of Campamento Bajo-agodon Peru (F- CORBIDI 17429); and from region Loreto, province Requena, district of Yaquerana, locality Sierra del Divisor Peru (G- CORBIDI 2447).

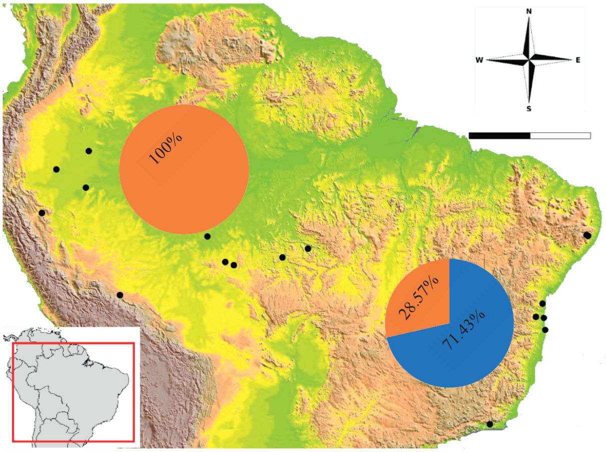

Figure 6. Population frequency of the hemipenes features through distribution of *Xenopholis scalaris*, considering its disjunct set of populations on the Amazonia and Atlantic Forest. Graphs referring to the hemipenian variability of *X. scalaris*. For the population of the Atlantic Forest 71.43% presented elongated hemipenis (blue) and 28.57% cylindrical (orange) (100% of Amazonian hemipenis are cylindrical).

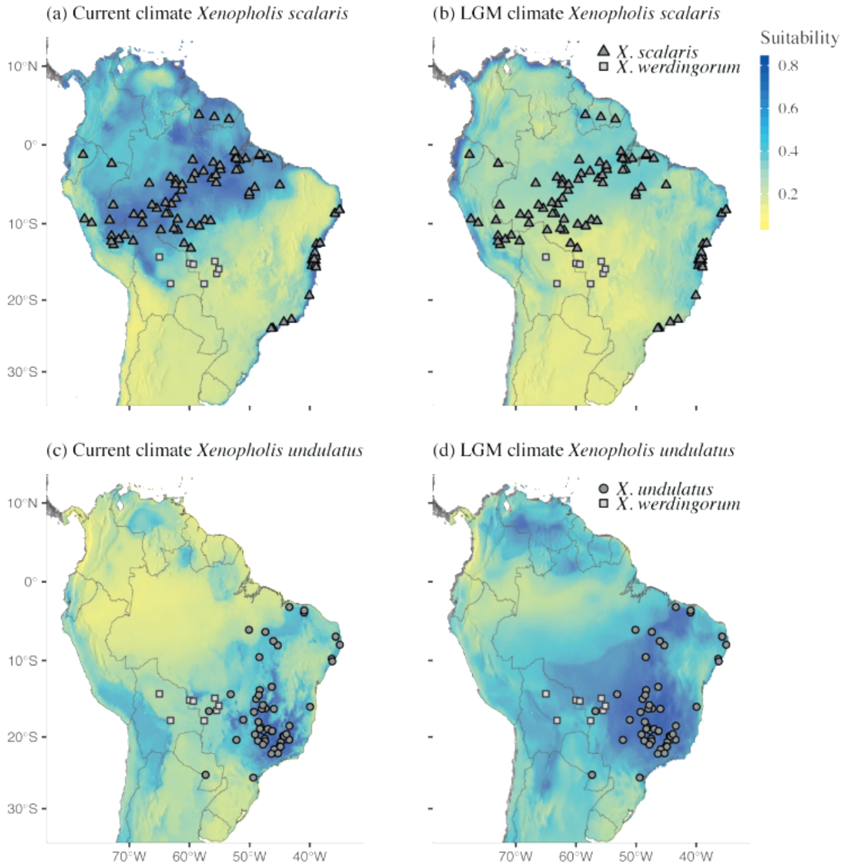

Figure 7. Predictions of species distribution models for: (a) Current climate and (b) Last Glacial Maximum for *Xenopholis scalaris*. (c) Current climate and (d) Last Glacial Maximum for *X. undulatus*. Model predictions indicate an expansion of suitable habitats since the Last Glacial Maximum for *X. scalaris* and the opposite pattern for *X. undulatus*. In general, highly suitable areas for each species do not overlap with each other or with the presence records of *X. werdingorum*.

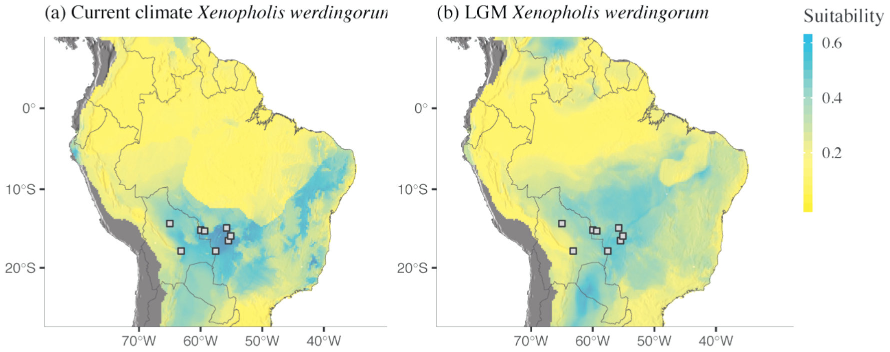

Figure 8. Predictions of species distribution for *Xenopholis werdingorum* using ensembling of small models. (a) Current climate. Areas with high suitability are distributed in the Pantanal basin and also beyond the known range of this species in the lowlands of Caatinga, Cerrado and Chaco. (b) Last Glacial Maximum (LGM). The model for the LGM does not differ considerably, but suitability values are generally smaller for the past conditions.

Figure 9. Color variability of the *Xenopholis scalaris* in life. A– tributary between Madeira and Puru Rivers, state of Amazonas, Brazil; B–D municipality of Juara, state of Mato Grosso, Brazil.; E– municipality of Uná, state of Bahia, Brazil; F– Itapuã do Oeste, state of Rondônia; G– Murici, state of Alagoas; H– Assis Brasil, state of Acre. Photos by V. Carvalho (A); T. Rodrigues (B–D); M. A. Freitas (E); D. Meneghelli (F); M. A. Freitas (G–H).

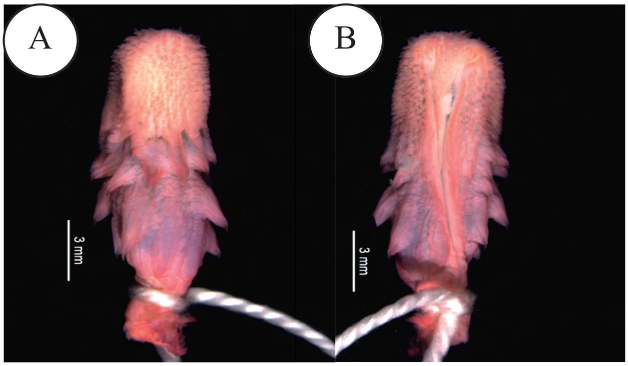

Figure 10. Asulcate (left) and sulcate side of the hemipenis of *Xenopholis scalaris* from municipality of Almadina, state of Bahia, Brazil (CZGB 13474).

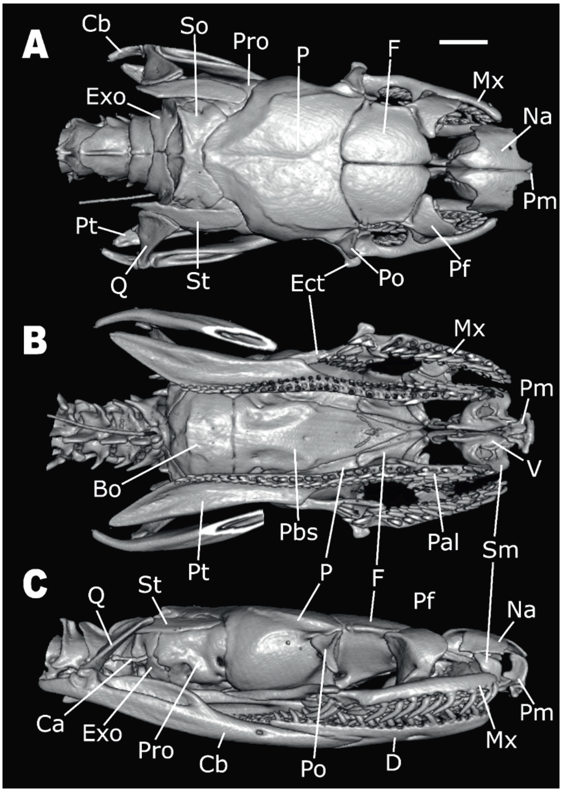

Figure 11. Dorsal (A), ventral (B), and lateral (C) views of the skull of *Xenopholis scalaris* (MNRJ 17070) from Cabo de Santo Agostinho, state of Pernambuco. Abbreviations are as follow: Cb=Compound bone; So=supraoccipital; Pro=prootic; P=parietal; F=frontal; Mx=maxilla; Na=nasal; Pm=premaxilla; Pf=prefrontal; Po=postorbital; Ec=ectopterygoid; St=supratemporal; Q=quadrate; Pt=pterygoid; Exo=exoccipital; V=vomer; Sm=septomaxilla; Pal=palatine; Pbs=parabasisphenoid; Bo=Basioccipital; Ca=columella auris; and D=dentary.

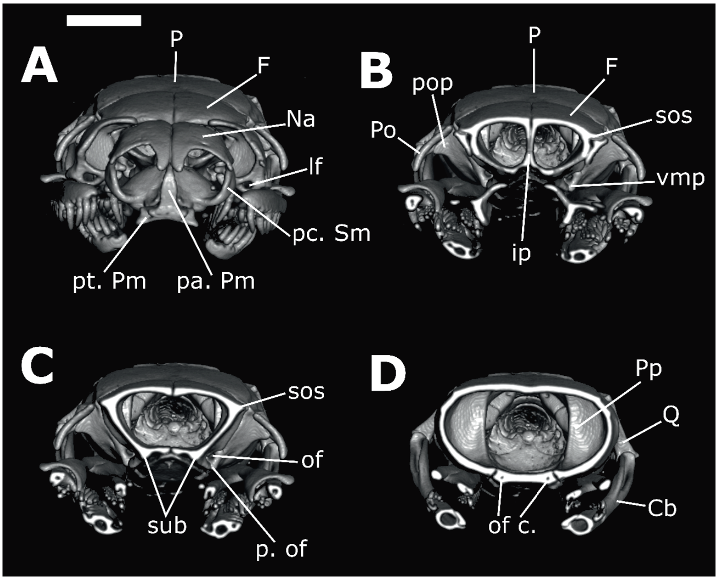

Figure 12. Three-dimensional cutaway views along the transverse axis of *Xenopholis scalaris* (MNRJ 17070), from the anterior edge of the skull. Abbreviations are as follow: pt. Pm=transverse process of premaxilla; pa. Pm=ascendant process of premaxilla; pc. Sm=conchal process of septomaxilla; lf=lacrimal foramen of prefrontal; Po=postorbital; P=parietal; sos=frontal supraorbital shelf; ip=interolfactory pillar of frontal; of=optic foramen; p. of=parietal process of optic foramen; sub=subolfactory process of frontal; of c.=optic foramen canal of parabasisphenoid; Cb=compound bone; Q=quadrate; Pp=parietal pillar.

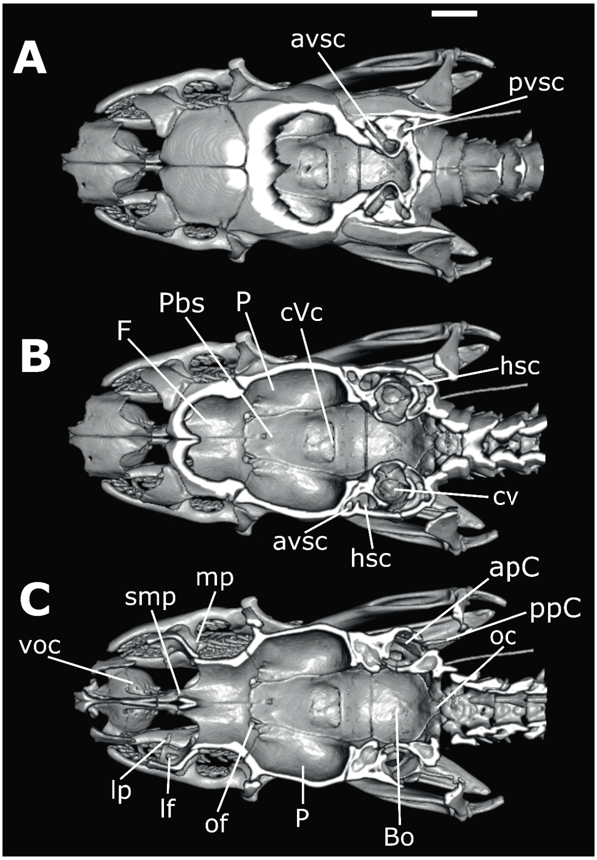

Figure 13. Three-dimensional cutaway views along the longitudinal axis of *Xenopholis scalaris* (MNRJ 17070), from the dorsal edge of the skull. Abbreviations are as follow: avsc=anterior vertical semicircular canal; pvsc=posterior vertical semicircular canal; cVc=crest of the Vidian foramina; hsc=horizontal semicircular process; cv=cavum vestibuli; mp=mesomedial process of prefrontal; smp=septomaxilar process of frontal; voc=vomeronasal organ capsule; lp=lacrimal process of prefrontal; lf=lacrimal foramen of prefrontal; of=optic foramen; P=parietal; Bo=basioccipital; oc=occipital condyle.

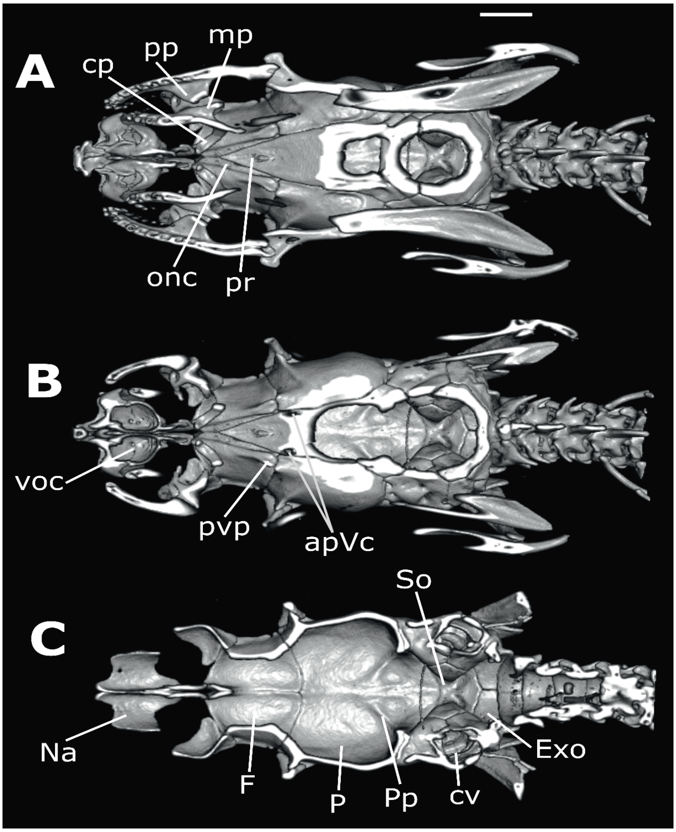

Figure 14. Three-dimensional cutaway views along the longitudinal axis of *Xenopholis scalaris* (MNRJ 17070), from the ventral edge of the skull. Abbreviations are as follow: cp=choanal process of palatine; pp=palatine process of maxilla; mp=maxillary process of palatine; onc=optic nerve canal; pr=parasphenoid rostrum; voc=vomeronasal organ capsule; pvp=posteroventral process of frontal; apVc=anterior opening of Vidian canal; So=supraoccipital; Exo=exoccipital; cv=cavum vestibuli; Pp=parietal pillar; P=parietal; F=frontal; Na=nasal.

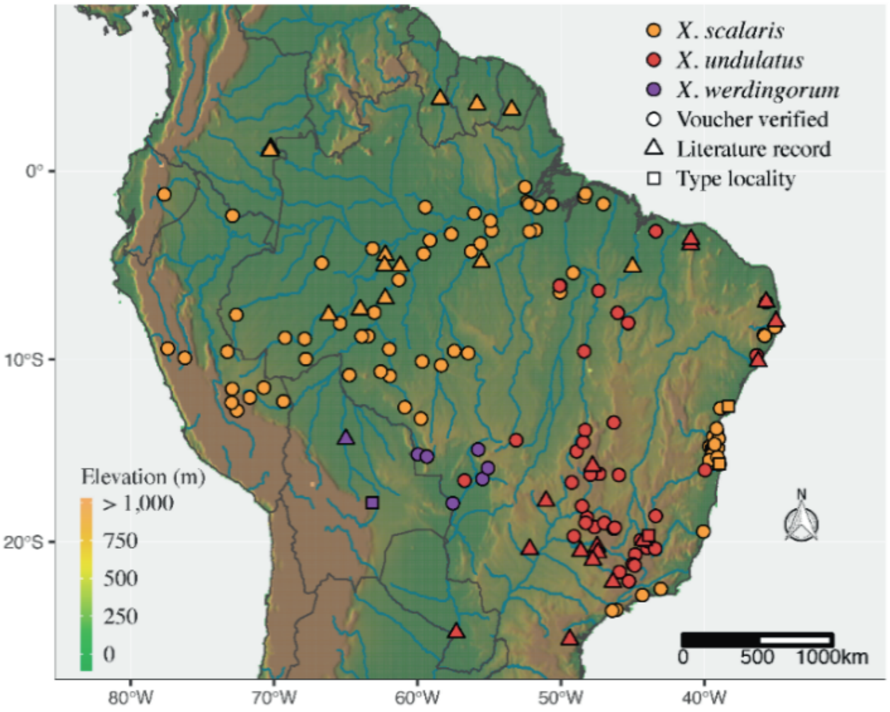

Figure 15. Known distribution of the genus *Xenopholis*. *Xenopholis undulatus* is mostly distributed in riparian forests of the Cerrado and Caatinga highlands, whereas *X. scalaris* is mostly distributed in lowland tropical forests. *Xenopholis werdingorum* is distributed mostly on the borders of the Pantanal and in the Chiquitanos forests.

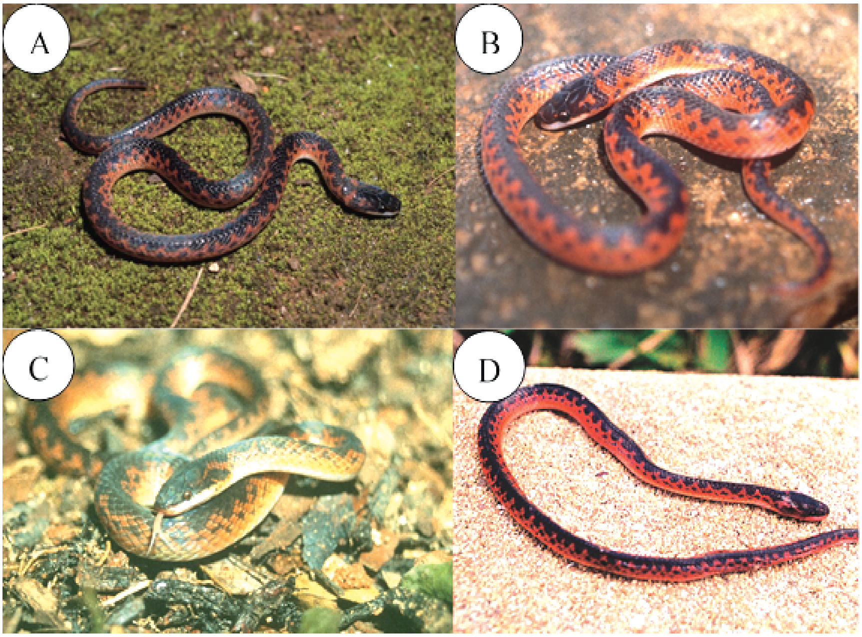

Figure 16. Color variability of *Xenopholis undulatus* in life. A– Palmas, state of Tocantins; B– Lajeado, state of Tocantins; C– Lindóia, state of São Paulo; D– Salto da Divisa, state of Minas Gerais. Photos by P. Passos (A); M. R. Duarte (B, C); M. A. Freitas (D).

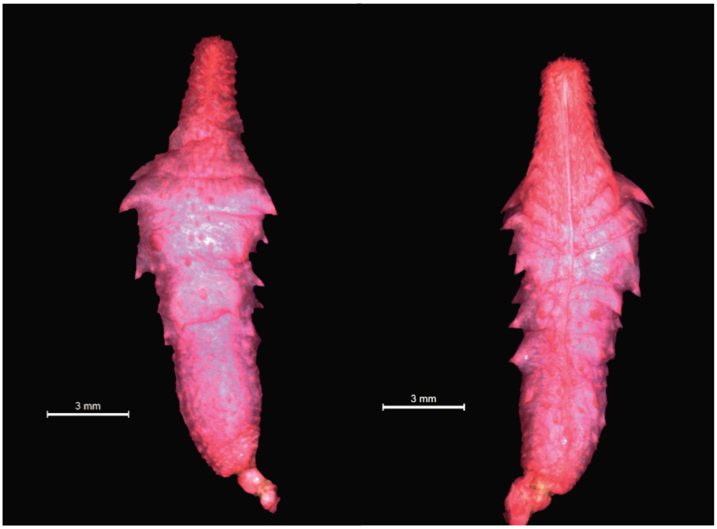

Figure 17. Asulcate (left) and sulcate side of the hemipenis of *Xenopholis undulatus* from state of Minas Gerais, Brazil (FUNED 2180).

Figure 18. Color variability of the *Xenopholis werdingorum* in life. A–B Poconé, state of Mato Grosso, Brazil; C– Pirizal, Nossa Senhora do Livramento, state of Mato Grosso, Brazil; D– Poconé, state of Mato Grosso, Brazil. Photos by Andrade-jr (A–B); C. Strussman (C) and P. Passos (D).

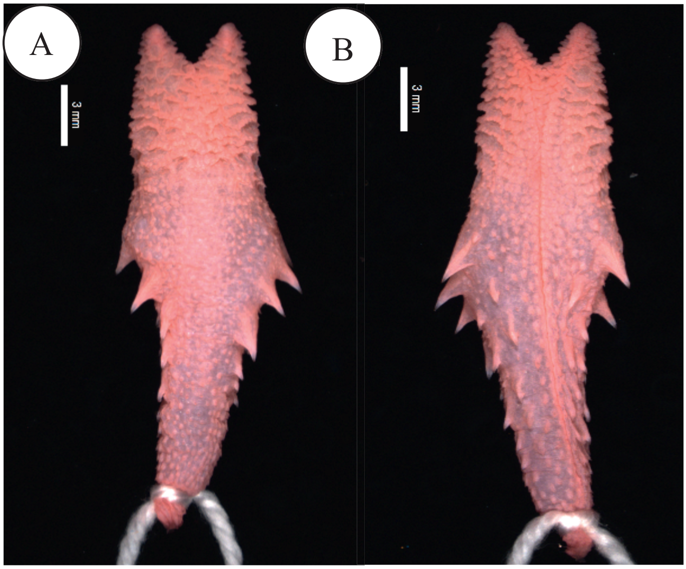

Figure 19. Asulcate (left) and sulcate side of the hemipenis of *Xenopholis werdingorum* from Corumbá, state of Mato Grosso, Brazil (UFMT 1193).

SUPPLEMENTARY MATERIAL

Table S1 – List of modelling methods used for the ensembling prediction and the respective references. Asterisks indicate models used for the small models in *Xenopholis werdingorum*.

Algorithms	References
BIOCLIM	Busby, J. R. BIOCLIM – a bioclimate analysis and prediction system. in Nature Conservation: Cost Effective Biological Surveys and Data Analysis (eds. Margules, C. R. & Austin, M. P.) 64–68 (CSIRO, 1991)
Boosted Regression Trees (BRT)	Elith, J., Leathwick, J. R. & Hastie, T. A working guide to boosted regression trees. J. Anim. Ecol. 77, 802–813 (2008).
DOMAIN*	Carpenter, G., Gillison, A. N. & Winter, J. DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. Biodivers. Conserv. 2, 667–680 (1993).
Generalized Linear Models in elastic NET model paths (GLMNET)	Friedman, J., Hastie, T. & Tibshirani, R. Regularization paths for generalized linear models via coordinate descent. J. Stat. Softw. 33, 1 (2010).
Flexible Discriminant Analysis (FDA)	Hastie, T., Tibshirani, R. & Buja, A. Flexible discriminant analysis by optimal scoring. J. Am. Stat. Assoc. 89, 1255–1270 (1994).
Generalized additive models (GAM)*	Hastie, T. & Tibshirani, R. Generalized Additive Models. Monographs on Statistics & Applied Probability. Chapman and Hall/CRC 1, (1990).

Generalized linear models (GLM)*	McCullough, P. & Nelder, J. A. Generalized linear models. Monographs on Statistics & Applied Probability. Chapman and Hall/CRC (1989).
Maxlike - Likelihood-based Maxent*	Royle, J. A., Chandler, R. B., Yackulic, C. & Nichols, J. D. Likelihood analysis of species occurrence probability from presence-only data for modelling species distributions. Methods Ecol. Evol. 3, 545–554 (2012).
Mixture Discriminant Analysis (MAD)	Royle, J. A., Chandler, R. B., Yackulic, C. & Nichols, J. D. Likelihood analysis of species occurrence probability from presence-only data for modelling species distributions. Methods Ecol. Evol. 3, 545–554 (2012).
Multivariate Adaptive Regression Spline (MARS)	Friedman, J. H. & others. Multivariate adaptive regression splines. Ann. Stat. 19, 1–67 (1991).
Random Forests (RF)	Breiman, L. Random forests. Mach. Learn. 45, 5–32 (2001).
Recursive Partitioning (RPart)	Therneau, T., Atkinson, E. & Ripley, B. rpart: Recursive partitioning for classification, regression and survival trees. R Packag. version 3, (2005).
Multi-layer Perceptron (MPL)	Rumelhart, D. E., Hinton, G. E. & Williams, R. J. Learning internal representations by error propagation. (1985).
Support Vector Machine (SVM)	Vapnik, V. N. The Nature of Statistical Learning Theory. (1995).
Radial Basis Function (RBF)	Acosta, F. M. A. Radial basis function and related models: an overview. Signal Processing 45, 37–58 (1995).

Table S2 – Variable importance calculated with AUC-based permutations from Random Forest models for each possible set of three environmental variables (median values per variable across all rounds). Asterisks* indicate selected six variables for the final model of each species.

Bioclim code	Relative variable importance			Variable descriptions
	X. undulatus	*X. scalaris*	*X. werdingorum*	
bio_1	0.06	0.12	0.21	Annual Mean Temperature
bio_2	0.11*	0.39*	0.18	Mean Diurnal Range
bio_3	0.09*	0.15	0.27*	Isothermality
bio_4	0.15*	0.20*	0.20	Temperature Seasonality
bio_5	0.06	0.16	0.22*	Max Temperature of Warmest Month
bio_6	0.06	0.21*	0.28	Min Temperature of Coldest Month
bio_7	0.07	0.35	0.26*	Temperature Annual Range
bio_8	0.11*	0.13	0.26*	Mean Temperature of Wettest Quarter
bio_9	0.06	0.12	0.20	Mean Temperature of Driest Quarter
bio_10	0.07	0.14	0.19	Mean Temperature of Warmest Quarter
bio_11	0.05	0.15	0.23	Mean Temperature of Coldest Quarter
bio_12	0.14	0.18*	0.16	Annual Precipitation
bio_13	0.11*	0.12	0.18	Precipitation of Wettest Month
bio_14	0.08	0.18*	0.16	Precipitation of Driest Month
bio_15	0.13*	0.17	0.17	Precipitation Seasonality
bio_16	0.11	0.12	0.19	Precipitation of Wettest Quarter
bio_17	0.09	0.18	0.15	Precipitation of Driest Quarter
bio_18	0.06	0.11	0.17	Precipitation of Warmest Quarter
bio_19	0.07	0.13	0.24*	Precipitation of Coldest Quarter
clay	0.08	0.19*	0.17	Percentage of clay in the soil
sand	0.09	0.13	0.21*	Percentage of sand in the soil
elevat	0.18	0.18	0.15	Elevation in meters

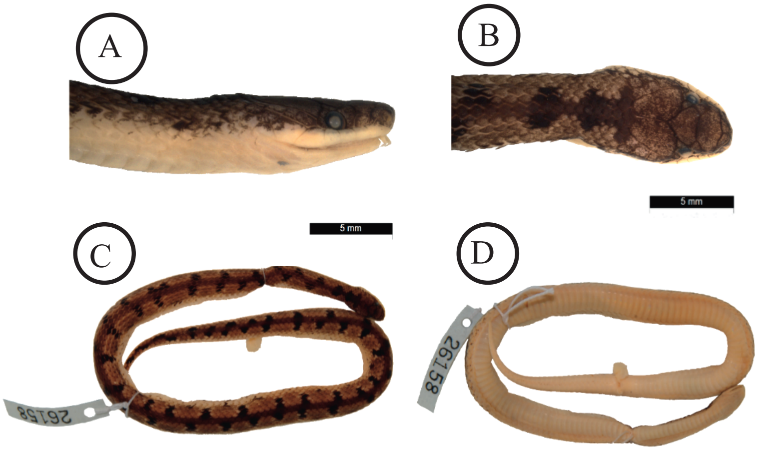

Figure S1. Preservation in alcohol, Xenopholis scalaris, MNRJ 26158, Locality: Reserva Extrativista Ararixi, comunidade Manitã, Boca do Acre, Amazonas. Legend: A - side view of the head, B - dorsal view of the head, C - dorsal view and D - ventral view.

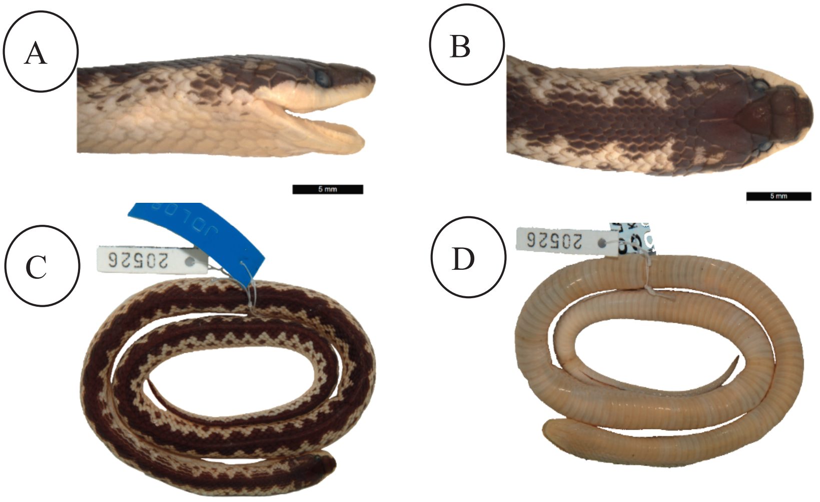

Figure S2. Preservation in alcohol, Xenopholis undulatus. Acronym: 20526. Locality: Urbano Santos, fazenda Santo Amaro, Maranhão. Legend: A - side view of the head, B - dorsal view of the head, C - dorsal view and D - ventral view.

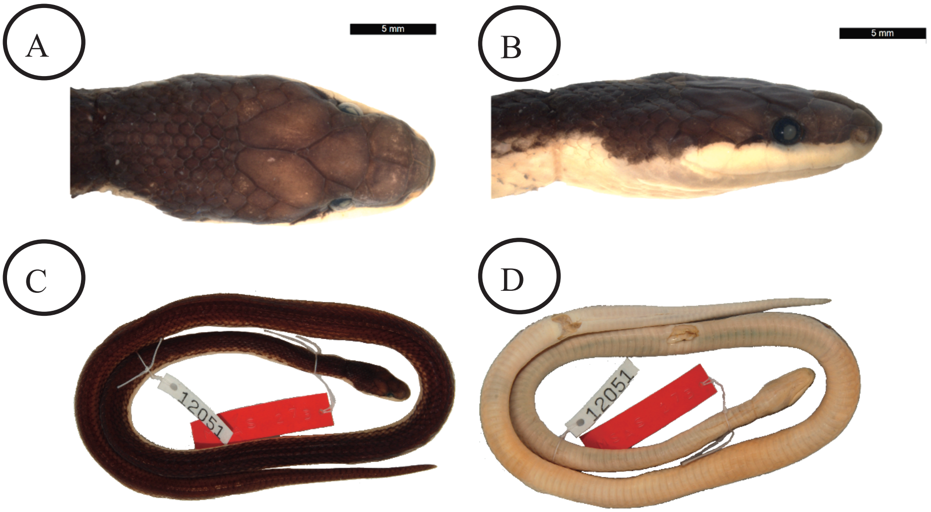

Figure S3. Preservation in alcohol, *Xenopholis werdingorum*. Acronym: 12051. Locality: Santo Antônio do Leverger, Mato Grosso. Legend: A - side view of the head, B - dorsal view of the head, C - dorsal view and D - ventral view.

IV

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Museums and cradles of diversity are geographically coincident for narrowly distributed Neotropical snakes

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ABSTRACT

Factors driving the spatial configuration of centres of endemism have long been a topic of broad interest and debate. Due to different eco-evolutionary processes, these highly biodiverse areas may harbour different amounts of ancient and recently diverged organisms (paleo- and neo-endemism, respectively). Patterns of endemism still need to be measured at distinct phylogenetic levels for most clades and, consequently, little is known about the distribution, the age and the causes of such patterns. Here we tested for the presence of centres with high Phylogenetic Endemism (PE) in the highly diverse Neotropical snakes, testing the age of these patterns (paleo- or neo-endemism), and the presence of PE centres with distinct phylogenetic composition. We then tested whether PE is predicted by topography, by climate (seasonality, stability, buffering and relictualness), or biome size. We found that most areas of high PE for Neotropical snakes present a combination of both ancient and recently diverged diversity, which is distributed mostly in the Caribbean region, Central America, the Andes, the Atlantic Forest and on scattered highlands in central Brazil. Turnover of lineages is higher across Central America, resulting in more phylogenetically distinct PE centres compared to South America, which presents a more phylogenetically uniform snake fauna. Finally, we found that elevational range (topographic roughness) is the main predictor of PE, especially for paleo-endemism, whereas low paleo-endemism levels coincide with areas of high climatic seasonality. Our study highlights the importance of mountain systems to both ancient and recent narrowly distributed diversity. Mountains are both museums and cradles of snake diversity in the Neotropics, which has important implications for conservation in this region.

Keywords: CANAPE, neo-endemism, paleo-endemism, phylogenetic endemism, phylogenetic turnover, squamates

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INTRODUCTION

Areas with narrowly distributed organisms have always caught the attention of naturalists and are frequently used to interpret biogeographical history and to guide conservation prioritization (Rosen 1978, Kruckeberg and Rabinowitz 1985, Morrone 2018). Beyond geographical rarity, endemism can also be characterized by whether taxa are ancient or recently diverged (paleo- and neo-endemism, respectively – Stebbins 1974). Areas of paleo-endemism support the persistence of lineages extinct elsewhere (e.g. tuataras in New Zealand, Jones et al. 2009), and therefore potentially represent biodiversity museums (Nekola 1999). Conversely, areas of neo-endemism are inhabited by recently diverged lineages, such as the East African great lakes and the high-altitude Andean Páramos, both comprising species-rich radiations of cichlids and plants respectively, and therefore represent biodiversity cradles (Johnson et al. 1996, Madriñán et al. 2013). Besides these well-known examples, Earth's museums and cradles of narrowly distributed biodiversity still await to be precisely delimited (Rosauer and Jetz 2015).

Species richness, phylogenetic diversity, and the different species endemism metrics are traditionally used in the delimitation of biodiversity patterns (Faith 1992, Crisp et al. 2001). However, such metrics do not integrate information on geographical rarity and phylogenetic divergence among all taxa in a given region. This integration is better achieved by the Phylogenetic Endemism (PE – Rosauer et al. 2009), a metric that weights the branch lengths of each lineage by their respective geographic ranges. Additionally, different kinds of endemism can be distinguished with a method built upon PE, the Categorical Analysis of Neo- And Paleo-Endemism (CANAPE, Mishler et al. 2014). Furthermore, the biodiversity patterns highlighted by PE and CANAPE allow the characterization of environmental conditions that enable evolutionary persistence and biotic diversification (Rosauer and Jetz 2015, Thornhill et al. 2017).

Several distinct topographical and climatic variables have been associated with high endemism levels (Steinbauer et al. 2016, Badgley et al. 2017). For example, strong environmental gradients provide opportunities for species to move in case of climate fluctuations (climatic buffering), potentially reducing local extinctions (Ohlemüller et al. 2008). The presence and survival of paleo-endemics may be associated with long-term geographical isolation and climatic stability (Sandel et al. 2011, Irl et al. 2015, Antonelli 2017). In contrast, the presence of neo-endemics might be related to recent dispersal to new areas or the formation of new habitats (Antonelli 2015, Merckx et al. 2015). Although the evolutionary processes that promote the accumulation of paleo- and neo-endemics are distinct (e.g. local low extinction rates vs. peripatric speciation), they are not necessarily mutually exclusive in geographical space (Mittelbach et al. 2007). For instance, current climatic conditions determine the distributions of terrestrial biomes, thus shaping the range sizes of their endemic lineages (Gallagher 2016) regardless of whether lineages have diverged as early as the Cretaceous or as recently as the Pleistocene. The role of evolutionary history in patterns of endemism remains to be tested, especially for most tropical areas and organisms, for which endemism patterns are not fully mapped or understood.

In the Neotropics, the diversity of terrestrial vertebrates reaches the highest levels throughout tropical forests of Central America, Amazonia, the Atlantic Forest, and the Cerrado savannas (Roll et al. 2017). In contrast, species endemism and PE often do not coincide with species richness, with high PE in mammals occurring mostly on islands (e.g. the Caribbean Islands) and mountainous areas (e.g. the Andes and Pantepui) (Rosauer and Jetz 2015). However, it is difficult to assess if this pattern is a trend for other vertebrates that might present different responses to environmental correlates of range size and geographical turnover (Saladin et al. 2019). In Neotropical snakes, patterns of species richness were only recently described and follow the same trends as other terrestrial vertebrates (Roll et al. 2017, Guedes et al. 2018). The extreme diversity of Neotropical snakes is the result of a long and complex history of origin, colonization and diversification of distinct snake clades in the region (Head et al. 2009, Zaher et al. 2018, 2019), which have resulted in uneven patterns of diversity (Cadle and Greene 1993, Fenker et al. 2014); however, very little is known about the resulting patterns of endemism.

Here we map PE in Neotropical snakes, classifying the different kinds of endemism using the CANAPE protocol for spatial phylogenetic analyses described in Mishler et al. (2014). Specifically, we address four main questions:

(1) *Where are the centres of PE for Neotropical snakes?* We expect to find high endemism levels in islands and highlands, following the same trends of PE as in terrestrial mammals (Rosauer and Jetz 2015);

(2) *Where are the centres of paleo- and neo-endemism?* Based on the presence of several relict taxa in Central America (e.g. Loxocemidae, *Loxocemus*) (Smith 2013) or in the Atlantic Forest (e.g. Tropidophiidae, *Tropidophis*) (Curcio et al. 2012), it is possible that these areas represent museums of snake diversity, thus potentially harbouring paleo-endemics. Areas with high species richness in the Andes and Amazonia might represent cradles of diversity (Rangel et al. 2018), thus potentially harbouring neo-endemics;

(3) *Do the PE centres have distinct phylogenetic community structure in different portions of the Neotropics?* The uneven distribution of the major snake clades in local communities suggests phylogenetic differentiation of the Neotropical snake fauna (Cadle and Greene 1993, Moura et al. 2017). Alternatively, if biotic interchange was sufficiently high (Antonelli et al. 2018a) – e.g. especially after the faunal interchanges related to the Panama isthmus formation – very distant areas might contain phylogenetically similar faunas;

(4) *Which environmental characteristics are associated with high PE?* We tested several known predictors of endemism, including topographic roughness, climate stability, climatic buffering and current climate (seasonality and biome sizes). We expect topographical-related variables to be the most important predictor of endemism, as suggested by regional species-level patterns of endemism (Nogueira et al. 2011, Guedes et al. 2014).

MATERIAL AND METHODS

Spatial data

To address all four questions, we mapped the geographical distribution of Neotropical snake species using the most complete database of occurrence records available for the region (Guedes et al. 2018). This database includes a curated dataset constructed through taxonomic work and literature review as part of the ongoing project of the Atlas of Brazilian snakes (details in Guedes et al. 2018) supplemented by a taxonomically and geographically revised dataset downloaded from the Global Biodiversity Information Facility (GBIF) (updated to https://doi.org/10.15468/dl.qsynre). The final dataset comprises the records of 1,087 species, which represents about 81% of the total number of species currently recognized for the Neotropics (Supplementary material, Appendix 1). We followed the delimitation of the Neotropical region from Olson et al. (2001) excluding southern Florida, which harbours an exclusively Nearctic snake fauna, with the exception of exotic taxa recently introduced (Meshaka et al. 2004).

We used alpha hulls to delimit the geographical ranges of species with more than two records. Alpha hulls generate a geometrical representation of a range that closely matches the outermost presence records of each species, decreasing systematic biases associated with convex-hulls (Meyer et al. 2017). Alpha values were set as the minimal value resulting in one continuous range per species (Cox et al. 2016), except for species with naturally disjunct ranges, for which we allowed the formation of more than one alpha hull (details in Supplementary Material, Appendix 1). The remaining species with only one or two records were represented as points and lines, respectively. The distribution of each species was then mapped on a gridded cylindrical equal-area Behrmann projection, with a resolution equivalent to $1^o \times 1^o$ at the 30th degree of latitude (hereafter one-degree resolution). This resolution is the same used in previous studies of snake diversity and PE in the region (Rosauer and Jetz 2015, Guedes et al. 2018), thus allowing direct comparisons. Additionally, to measure the sensitivity of our results to the chosen resolution, different grid sizes were tested (0.5° and 2° resolution). In order to verify whether the patterns observed for the Categorical Analysis of Neo- And Paleo-Endemism (CANAPE) were robust enough to be recovered regardless of the mapping method, the same procedure was repeated considering point records only.

Analyses

For the phylogenetic measures, we used the time-calibrated phylogeny of Squamata reptiles from Tonini et al. (2016), which we trimmed for the Neotropical snake species. This phylogeny also includes species with no molecular information available, which were randomly assigned at the genus level (54% of the total). To account for the variation related to the taxonomic assignment, we ran all analyses from a distribution of 100 separate phylogenies and used the median values in subsequent analyses. We tested the sensitivity of our results to this random taxonomic assignment by removing species within both: (1) the 20% highest variation in the tip ages across the sampled phylogenies (standard deviation values); and (2) species with the 20% smallest range sizes. We also repeated the analysis using only the backbone molecular phylogeny (Tonini et al. 2016). As genus ages and therefore the respective variation of species ages within each genus represent a very small proportion of the total amount of branch lengths, we predict that this variation will be less important to our results.

To address *Question 1 – Where are the centres of PE for Neotropical snakes?* – we followed the CANAPE protocol for spatial phylogenetic analyses in Biodiverse 2.0 (Laffan et al. 2010, Mishler et al. 2014). First, two main measures of endemism were generated: Phylogenetic Endemism (PE – Rosauer et al. 2009) and Relative Phylogenetic Endemism (RPE – Mishler et al. 2014). Phylogenetic endemism is the total branch length (from the time-calibrated phylogeny) of the lineages present at a grid cell divided by the range sizes of the respective lineages. Relative Phylogenetic Endemism is the ratio between PE measured from the original phylogeny in relation to the PE estimated from a phylogeny with equally distributed branch lengths (see more details in Mishler et al. 2014). When compared to a null distribution (see below), high RPE values represent grid cells with a predominance of longer branches than in the null phylogeny, indicating paleo-endemism. Likewise, low values of RPE indicate grid cell with neo-endemism.

To assess the statistical significance of PE and RPE, we followed the approach suggested by Mishler et al. (2014) in comparing the actual PE and RPE values of each grid cell to the 999 values of a null distribution. The null distribution was calculated by shuffling the terminals of the phylogeny while keeping constant the species richness of each grid cell and the range sizes of each lineage. P-values were estimated from a two-tailed distribution of values to identify areas with higher (> 0.975) or lower (< 0.025) PE or RPE than the null distribution.

To address *Question 2 – Where are the centres of paleo- and neo-endemism?* – we used PE and RPE for classifying grid cells relative to the amount of paleo- and neo-endemics with CANAPE (Mishler et al. 2014). Grid cells with a higher or lower PE/RPE ratio than the null distribution indicate paleo- or neo-endemism, respectively. Grid cells with PE significantly higher than the null distribution but intermediate values of RPE indicate mixed-endemism (i.e. grid cells with high levels of both paleo- and neo-endemism). For simplicity, we also call grid cells below the 0.01 level of significance mixed-endemic, instead of using the term "super-endemism" suggested in Mishler et al. (2014).

We tested the sensitivity of the CANAPE results to the total extent of our study area by the stepwise exclusion of: (1) the Caribbean Islands – since islands are naturally more isolated and might contain an unequal representation of lineages; (2) the Mesoamerican transition zones and Central American dominion (*sensu* Morrone 2014), ranging approximately from Mexico to the Costa Rican border with Nicaragua – because the northern parts of the Neotropics contain relatively more taxa with centres of diversity mainly located in the Nearctic or Palearctic regions, thus, with only a few species marginally entering the Neotropics; and (3) the South American ecoregions west of the Andes – trans-Andean South America, as the Andes are a geographical barrier for many Central American taxa to disperse further into South America, and vice versa (Cadle and Greene 1993). We then evaluated how these exclusions affected the patterns of endemism in South American ecoregions east of the Andes (cis-Andean South America).

To help in the interpretation of *Questions 1* and *2*, we tested whether PE and RPE patterns are similar to equivalent non-range weighted patterns, i.e. Phylogenetic Diversity (PD – as in Faith 1992) and Relative Phylogenetic Diversity (RPD – Mishler et al. 2014). We measured these metrics against null models as described above. For comparison, we additionally measured species richness and species richness weighted by the inverse of the range size of each species in a grid cell – weighted endemism (Crisp et al. 2001, Guerin and Lowe 2015). All analyses were performed using Perl wrapper functions to run Biodiverse in the R environment modified from <https://github.com/NunzioKnerr/biodiverse_pipeline>.

To address *Question 3 – Do the PE centres have distinct phylogenetic community* *structure in different portions of the Neotropics?* – we measured the turnover component of phylogenetic beta diversity among grid cells with the Simpson derived pairwise phylogenetic dissimilarity index (Leprieur et al. 2012). Grid cells were then clustered using unweighted arithmetic mean (UPGMA), which performs well in clustering dissimilarity matrices (Kreft and Jetz 2010).

Correlations of endemism

To address *Question 4 – Which environmental characteristics are associated with high PE?* – we initially calculated all environmental variables in a resolution equivalent to 0.1 degrees. We then aggregated the resulting values by median to the main resolution adopted in our study (one-degree). Bioclimatic variables were downloaded from CHELSA (Karger et al. 2016). A more detailed explanation on how each potential predictor of endemism was calculated is available in the Supplementary material, Appendix I. We calculated: (1) *Topographic roughness* (or elevational range) of a grid cell – a surrogate for both habitat heterogeneity and geographical isolation (Ruggiero and Hawkins 2008); (2) *Climatic buffering* – the availability of distinct climatic conditions within a short geographical distance (200 km as in Ohlemüller et al. 2008); (3) *Relictual climate* – a measure of whether climatic conditions are very different from the surrounding areas (1,000 km as in Ohlemüller et al. 2008); (4) *Distance to the border of a biome* (*sensu* Olson et al. 2001) – to determine whether a grid cell contains, or is part of, small, isolated biome patches or if it is located in the middle of a large extension of biome; (5) *Precipitation and temperature seasonality* – a measure of whether high seasonality selects lineages with larger ranges (due larger climatic niches, a potential result of higher climatic tolerances, Addo-Bediako et al. 2000); and (6) *Climate change velocity* since the Last Glacial Maximum (LGM; 21,000 years ago) based on the mean annual temperature (Sandel et al. 2011) – as an estimation of climatic stability.

We then calculated the standardized effect size scores of PE and RPE (Molina-Venegas et al. 2017). This score is the actual PE or RPE in a grid cell minus the mean value of the null PE distribution (from the 999 randomizations for each phylogeny) divided by the standard deviation of the null PE distribution. This procedure allowed us to incorporate information on how much these values deviate from the null distribution as well as to minimize the effects of the number of terminals of the phylogeny occurring in a grid cell, which generally affects PE scores (Rosauer and Jetz 2015).

To test relationships among PE and RPE with the mentioned predictors of endemism, we regressed their respective standardized effect size scores in each grid cell using generalized linear models (GLM) and spatial autoregressive (SAR) models with spatially autocorrelated errors (SAR_{err}) to account for spatial autocorrelation (Kissling and Carl 2008). We inferred the degree of spatial autocorrelation through correlograms of the residuals of both SAR_{err} and GLM models using the R package *spdep* (Bivand et al. 2011). First, the number of neighbours for the SAR_{err} models was selected in order to minimize the corrected Akaike Information Criterion (AICc). Then, we tested models for all combinations of variables, excepting those that included both climate change velocity and topographic roughness, which are highly correlated ($r = -0.8$), and selected the best model (lower AICc). We also considered any additional model with ΔAICc smaller than two as an equally supported model. The values of all environmental predictors were standardized so that the resulting model coefficients indicated the relative contributions of each variable to explain PE or RPE.

RESULTS

Phylogenetic endemism in Neotropical snakes is significantly high in the entire Central America and in the Andes, in scattered grid cells in the Atlantic Forest, Cerrado and Caatinga, and significantly low in lowland Amazonia (*Question 1*; Fig. 1a). For RPE, only a few scattered grid cells across the Neotropics were significantly high, indicating that paleo-endemism is not concentrated in any major region (Fig 1b). In contrast, there is a concentration of grid cells with low RPE in the central and in the southern Andes (especially at the western slopes).

There is a spatial mismatch between the non-range weighted patterns (PD and RPD – Figs. 1c–d) and the narrowly distributed patterns (PE and RPE – Figs. 1a–b). The only exceptions apply to a small number of grid cells in the Caribbean Islands and Central America with significantly high PE, RPE, PD, and RPD. An additional area in the Guiana Shield presents significantly high PD and RPD, indicating the distribution of ancient widely distributed diversity. Such spatial mismatch between widely and narrowly distributed diversity is also indicated by species richness and weighted endemism (Supplementary material, Appendix 1, Fig. A1a–d).

CANAPE classified most grid cells with significantly high PE (Fig. 1a) and non-significant RPE (Fig 1b) as mixed-endemism (Fig. 2a), i.e. significantly high neo- and paleo-endemism (*Question 2*). Most of the neo- (92 grid cells), paleo- (10 grid cells), and mixed-endemism grid cells (483 in a total of 2150) are located in (or close to) mountainous areas in Central America and in the Andes (Fig. 2d). The CANAPE analysis restricted to the cis-Andean South America results in an increased extension of mixed-endemism grid cells, especially in the southern portion of the Atlantic Forest, Patagonia, and the eastern Andean slopes (Fig. 2b and sensitivity tests below). The CANAPE grid cells are clustered into three main regions according to their phylogenetic similarity (*Question 3*): 1 – The Caribbean, 2 – the northern portions of Central America, and 3 – all remaining grid cells in Central and South America (Fig. 2c). Additionally, the turnover of lineages is considerably smaller within South America than in Central America (Fig. 2c).

The CANAPE results are robust to the removal of species with both small ranges and high age variation across the distribution of phylogenies (Appendix 1, Fig. A2). Even after removing all species without molecular data, the CANAPE results keep the general pattern of mixed-endemism for most grid cells of high PE (Appendix 1, Fig. A2 d). Overall, our results are also robust to the grid sizes, always capturing the overall pattern of high mixed-endemism in Central America and in the northern Andes (Appendix 1, Figs. A3 and A4). The sequential removal of portions of the study area has only a small effect on the patterns of endemism in the cis-Andean South America, except when this area is analysed alone (Appendix 1, Fig. A5).

The Spatial Autoregressive Error Models indicate that topographic roughness is the most important correlate of both PE ($R^2 = 0.44$) and RPE ($R^2 = 0.64$) (*Question 4* – Table 1). Additional models are within the threshold of ΔAICc = 2, all of them indicating topographic roughness as the main predictor of PE and RPE (Appendix 1, Table A1). All the additional models also include climatic buffering and low precipitation seasonality as additional predictors of PE, and low precipitation and temperature seasonality for high RPE. Topographic roughness remained the strongest coefficient to explain PE and RPE across the half and two-degrees resolution (Appendix 1, Table A2). Besides the high correlation between topographic roughness and climate change velocity, none of the models within the ΔAICc threshold included the last variable (Appendix 1, Table A2). Correlograms indicate that the SAR_{err} models removed the spatial autocorrelation in the variables in comparison with linear models (Appendix 1, Table A3, Fig. A6). Therefore, we discuss our results based on the SAR_{err} models only.

DISCUSSION

Diversity patterns and types of endemism

The geographical distribution of Phylogenetic Endemism (PE) in Neotropical snakes matches an emerging general trend found in mammals (Rosauer and Jetz 2015, López-Aguirre et al. 2018) – that of high PE values prevailing across Central America, the Caribbean Islands, the Andes, and the Atlantic Forest (*Question 1* – Fig. 2a). Coincident patterns of endemism among such distinct groups of organisms in terms of thermophysiology (ectotherms vs. endotherms) or dispersal abilities (bats vs. snakes and non-flying mammals) are suggestive of a common evolutionary process producing geographically and historically unique biotas (Jetz et al. 2004, Hughes et al. 2013). Most significantly, high PE for Neotropical snakes predominantly comprises a mix of both ancient and young narrowly distributed diversity (i.e. a mixed-endemism pattern – *Question 2*). This result indicates that rare, relictual components of diversity are co-occurring with a more recently evolved narrowly distributed diversity (Harrison 2013, Thornhill et al. 2017).

Centres of neo-endemism potentially indicate places where peripheral isolation and recent diversification tend to occur (Mishler et al. 2014). The neo-endemism in grid cells along the western slopes of the southern Andes represents the main deviation from the mixed-endemism trend in Neotropical snakes (Fig. 2a). Additionally, neo-endemism is not a phenomenon seen in plants in the western Andean slopes (Scherson et al. 2017), and for bats, this area is actually a centre of paleo-endemism (López-Aguirre et al. 2018). This indicates that the neo-endemism for snakes in the southern Andes is related to the unique biogeographical history of the group in this region rather than by environmental predictors of endemism alone. For instance, neo-endemism in snakes in this area is explained by the presence of only two endemic species that belong to relatively species-rich genera (Dipsadidae: *Philodryas* and *Tachymenis*) (Cadle 1985, Núñez 1992). Interestingly, the eastern Andean slopes and the Patagonia region at the same latitudes present both mixed- and neo-endemic grid cells (Fig. 2b). These southernmost parts of South America were strongly affected by several glaciation cycles since the Late Miocene (~ 7 Ma) (Rabassa et al. 2005). These events triggered the extinction of several snake lineages in Patagonia (indicated by the fossil record in the Miocene, Albino 2011) and probably prevented the colonization of the western Andean slopes by additional snake lineages.

The very low number of paleo-endemic grid cells, which are distributed predominantly amongst extensive mixed-endemism centres seems to indicate that mixed-endemism is the main regional trend. Even the presence of relict snake taxa in Central America has not driven distinct levels of paleo-endemism in relation to neo-endemism in the region. Instead, northern

Amazonia along the Guiana Shield emerges as an additional area with an over-representation of long branches (high PD and RPD), but which are not geographically rare (although with mixed-endemism grid cells in the cis-Andean analyses – Fig. 2b). The high PD and RPD is probably the result of biological communities being formed as the result of multiple colonisations of distantly related lineages (Tucker and Cadotte 2013), from competitive exclusion among closely related taxa, or from an area functioning as a biogeographical museum of non-narrowly distributed diversity (Chown and Gaston 2000). Mechanistic models of speciation/extinction and biogeographical reconstructions of ancestral ranges could be explored in future studies to test these alternative hypotheses.

The contrasting pattern between lowland Amazonia (low PE) and the Andes (high PE) was also reported at the species level for plants, with geographic clustering of rare species in highlands versus the opposite pattern across the lowlands (Zizka et al. 2018). This contrast between highlands and lowlands was also identified for squamate reptiles in the Cerrado and the Caatinga domains in Brazil (Nogueira et al. 2011, Guedes et al. 2014, Azevedo et al. 2016). Additionally, Amazonia contains the largest extension of a biome in the Neotropics (Olson et al. 2001, Ab'Sáber 2003), implying that some of its endemic lineages have wider ranges compared to endemic species in other biomes (especially if most rivers are not important barriers to dispersal, Santorelli et al. 2018, Oliveira et al. 2019); which is also supported by the negative correlation between PE and distance to the biome border (Table 1). Although our method of computing species distributions based on alpha-hull polygons may have overestimated the ranges of some species (Graham and Hijmans 2006), thus exaggerating low PE patterns, the interpretation of our results is robust to the different mapping strategies and grid resolutions (Appendix 1, Figs. A3 and A4). Low sampling in Amazonia (Guedes et al. 2018) might account for part of the low PE, but there are still a large number of species occurring across disjunct areas; thus resulting in large ranges when estimated by alpha hulls. Cryptic diversity might play a particular role in the weak endemism of Amazonia, and additional taxonomic reviews and phylogeographic studies may reveal additional species complexes in the region (Prudente and Passos 2010, Passos et al. 2016, 2018) as well in as other parts of the Neotropics (Domingos et al. 2017). Thus, our results set the ground for additional studies on hypotheses concerning the impact of cryptic diversity on PE patterns in the Neotropics.

Phylogenetic regionalization

The regionalization of the high PE areas in Neotropical snakes (*Question 3*) offers a perspective on how processes generating endemism may differ across the region. For instance, the long-term oceanic isolation and the relatively smaller areas of islands heavily influence patterns of dispersal and extinction (Kier et al. 2009). These island characteristics might explain why the Caribbean Islands clustered separately from the rest of the Neotropics in terms of phylogenetic composition – for example, the Caribbean Islands harbour a radiation of tropidophiid snakes, while these snakes are relictual in the rest of the Neotropics (Curcio et al. 2012). In contrast, high PE in grid cells in the northernmost parts of the Neotropics (orange shades in Fig. 2c) are related to the presence of many snake taxa of which the main centres of diversity are in the Nearctic region, appearing as rare branches in the Neotropics (Pinou et al. 2004, Roll et al. 2017). High PE grid cells from southern Mexico to the entire South American continent (green shades in Fig. 2c) harbour a phylogenetically similar snake fauna, a result of the interchange of animal and plant lineages since the Miocene (Bacon et al. 2015). In a broader zoogeographical perspective, this area is also coincident with the northernmost limits of the Neotropical region found for birds, mammals, and amphibians (Kreft and Jetz 2013). Finally, grid cells of high endemism as geographically separated as the northern Andes and the Atlantic Forest in South America are more similar to one another than geographically close grid cells in Central America. This reinforces the patterns of high connectivity among different regions within South America (Antonelli et al. 2018a).

Environmental correlates of phylogenetic endemism

The predominance of mixed-endemism in our results suggests that similar environmental configurations lead to the accumulation of both paleo- and neo-endemics – for example, climatic stability enables the persistence of ancient diversity but can also promote the morphological differentiation of diverging lineages (Fjeldså and Lovett 1997). Consistent with this trend, mixed-endemism grid cells in the Brazilian Atlantic Forest (Fig. 2a) are coincident with some of the more stable portions of this domain – climate refugia (Costa et al. 2018), especially when considering the cis-Andean analysis (Fig. 2b).

High spatial heterogeneity on small scales may also account for the presence of distinct conditions needed for the accumulation of both paleo- and neo-endemics (Zuloaga et al. 2019). In this way, topographic roughness, a well-recognized correlate of low extinction rates for reptiles and a proxy for habitat heterogeneity (Araújo et al. 2008), was the most important predictor of PE in our study. As shown in the case of the Andes, topographic roughness is associated with a continuous history of geological changes (Jetz and Rahbek 2002, Antonelli et al. 2018b). Such topographical changes occur on a deeper timescale than the available climate variables and may explain the even higher correlation between topographic roughness and high RPE (paleo-endemism). Our findings also support the idea that low extinction rates are related to higher climatic buffering in mountainous regions (Table 1), also an indirect measure of environmental heterogeneity (Ohlemüller et al. 2008). The combined effects of topography and climate on PE patterns are not only a Neotropical phenomenon but were also recorded for a variety of groups of organisms in different continents (Rosauer et al. 2015, Rosauer and Jetz 2015, Barratt et al. 2017). Therefore, our results add support to the idea that the interactions of topography and climate are related to the formation of museums and cradles of biodiversity along mountainous regions (*Question 4;* Rangel et al. 2018).

The relation of high PE and RPE in Neotropical snakes to current climatic variables such as low precipitation seasonality or the distribution of terrestrial biomes (Table 1) suggests some influence of niche-related processes in generating patterns of endemism (e.g. selection of lineages with narrower niches in less seasonal environments, Slatyer et al. 2013). As niche breadth and dispersal abilities are sometimes phylogenetically conserved (Wiens and Graham 2005), it is possible that even if the environmental conditions are similar, grid cells with phylogenetically distinct communities may display distinct patterns of endemism (Zuloaga et al. 2019). In line with this, the high spatial autocorrelation in the residuals of the GLM models indicates that PE is also linked to more local factors, which could be related to the local history of diversification (Rosauer and Jetz 2015). For example, although the increase of high PE grid cells in the cis-Andean analysis (Fig. 2b) might be the result of pruning taxa that are more widely distributed outside this area, it could also suggest a dynamic of higher dispersal (resulting in larger ranges) of snake lineages in this area. Therefore, our results set the ground for potential studies focusing on particular clades of snakes (with the increase of available molecular data) or in particular regions (e.g. within Amazonia, with the increase of distribution data and taxonomic knowledge). Thus, it will be possible to test whether PE in Neotropical snakes is the result of an emerging trend or the average of disparate individual biogeographical histories.

CONCLUSIONS

In relation to the long-standing discussion on whether mountainous areas of the Neotropics act as museums or cradles of diversity (Fjeldså et al. 2012), our results indicate that for narrowly-distributed Neotropical snakes the answer is both. Topography and certain climate-related characteristics are responsible for the maintenance of both ancient and recent diversity. The areas of high PE found here add conservation value to the already recognized centres of high species richness for snakes in the Neotropics, such as Amazonia (Guedes et al. 2018), and those undergoing extreme habitat loss, such as the Cerrado savannas (de Mello et al. 2015). Some areas already identified as biodiversity hotspots, such as the Atlantic Forest (Myers et al. 2000), are also recovered as centres of mixed-endemism and, therefore, of high conservation value both for their current diversity patterns and for their respective underlying evolutionary and ecological potential (Crandall et al. 2000). Our results highlight the importance of accounting for different biodiversity metrics for a comprehensive characterization of centres of biodiversity.

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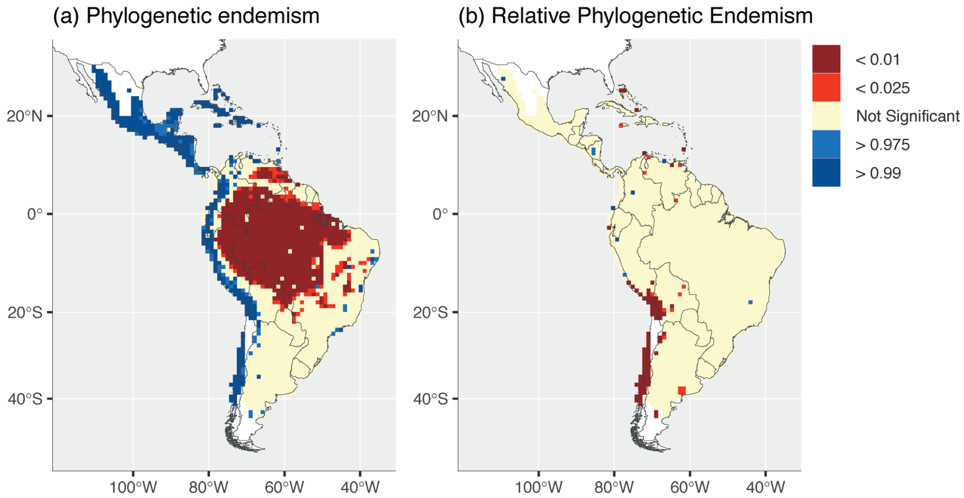
TABLES

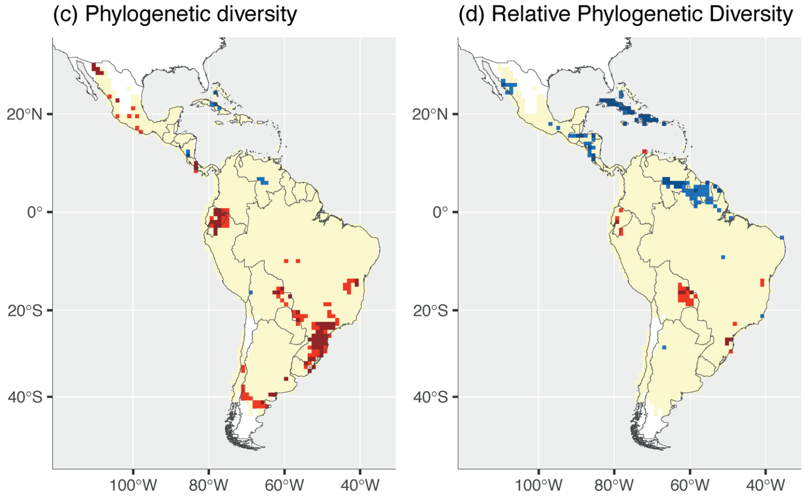
Table 1 – Best Spatial Autoregressive Error Models (SAR_{err}) for the relationships among the standardized effect size scores of Phylogenetic Endemism (PE), Relative Phylogenetic Endemism (RPE) and the respective environmental correlates (only variables selected in the respective best models). Topographic roughness was the best predictor of both high PE and RPE, whereas climatic seasonality was negatively correlated to both measures. Asterisks indicate: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Gelkerke pseudo R-squared (p-R^2). Corrected Akaike Information Criterion (AICc).

	Variables	Coefficients	p-R^2	AICc
PE ~	Topographic roughness	0.198 (0.04)***	0.44	4430
	Climatic buffering	0.153 (0.061)*		
	Precipitation seasonality	-0.143 (0.045)**		
	Distance to biome border	-0.157 (0.049)**		
RPE ~	Topographic roughness	0.262 (0.032)***	0.64	3528
	Precipitation seasonality	-0.217 (0.044)***		
	Temperature seasonality	-0.181 (0.066)**		

FIGURES


(a) Phylogenetic endemism

(b) Relative Phylogenetic Endemism


(c) Phylogenetic diversity

(d) Relative Phylogenetic Diversity

Figure 1 (a–b) – Distribution of Phylogenetic Endemism (PE) and Relative Phylogenetic Endemism (RPE) for Neotropical snakes (N = 1,087 species). (c–d) For comparison, similar plots using equivalent non range-weighted metrics: Phylogenetic Diversity (PD) and Relative Phylogenetic Diversity (RPD). Sites with both high PE and high RPE indicate paleo-endemism. High PE and low RPE indicates neo-endemism (see Fig. 2). Sites with both high PD and RPD indicate significantly high concentration of ancient PD. In general, range-weighted patterns are very distinct from non-range weighted diversity.

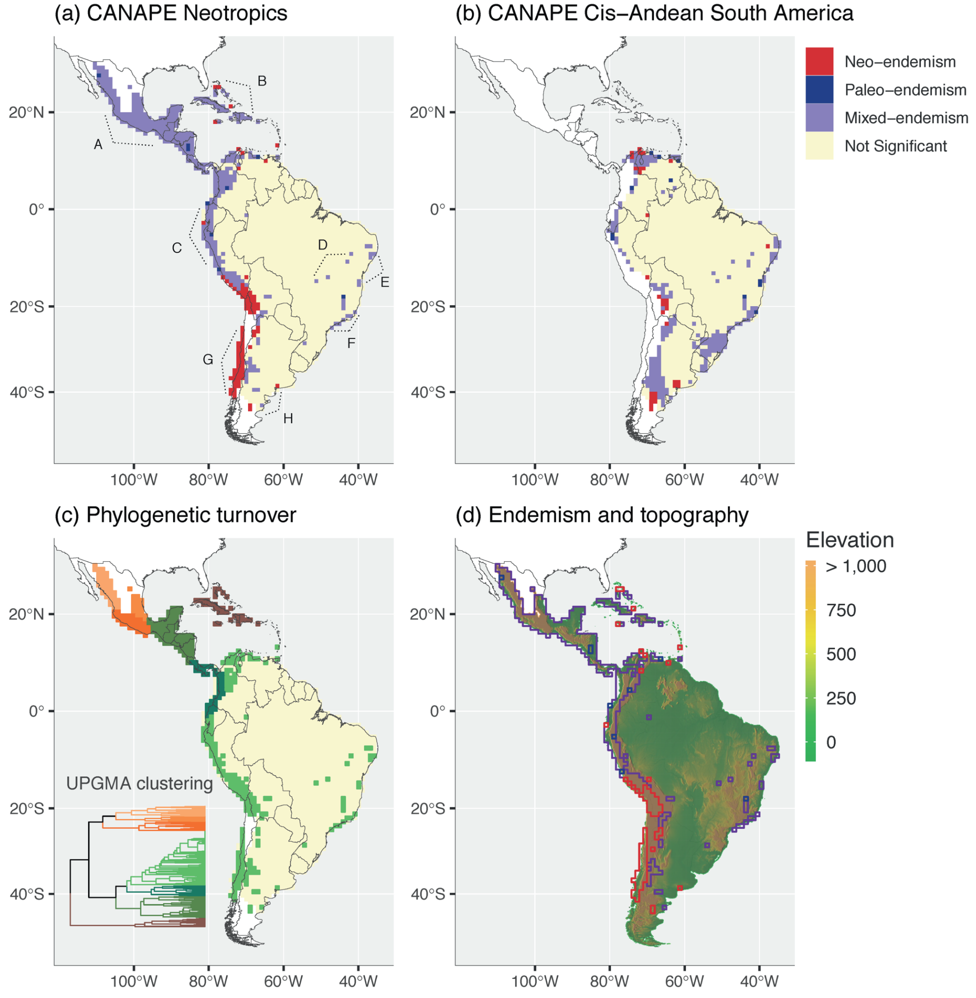


Figure 2- (a) Categorical Analysis of Neo- And Paleo-Endemism (CANAPE) for Neotropical snakes. (b) CANAPE restricted to the Cis-Andean South America. (c) Phylogenetic dissimilarity (turnover component) of high PE sites. Brown, orange and green shades indicate the first three UPGMA clusters. Each terminal in the dendrogram represents a grid cell. (d) Areas with high endemism detected in CANAPE are located mostly on mountains or plateaus. A- Central America, B- The Caribbean, C- Northern Andes, D- Cerrado and Caatinga Highlands, E- Northern Atlantic Forest, F- Southern Atlantic Forest, G- Southern Andes, H- Patagonia.

SUPPLEMENTARY MATERIAL

APPENDIX 1

Database update

We updated the dataset provided by Guedes et al. (2018) by adding new records from Global Biodiversity Information Facility (GBIF, https://doi.org/10.15468/dl.qsynre). We followed the same procedure as in Guedes et al. (2018): (1) we used speciesgeocodeR (Töpel et al. 2017) to clean biased geographical coordinates; (2) we included only voucher verified specimens, (3) we checked the taxonomic validity of each species according to the literature, (4) we checked the geographical distribution of each species according to the literature. We plotted occurrence records of each species individually and verified the distribution of each taxon using available taxonomic reviews and expert knowledge. All GBIF occurrence records located outside known species geographic ranges were removed from the database. This update resulted in additional records for 220 species.

Mapping alpha-hulls

To identify species with disjunct geographical distribution, we visually checked the presence records over a map of biomes before building the alpha-hulls (Olson et al, 2001). For the species with non-disjunct distributions, we built the alpha-hulls by sequentially increasing the alpha parameter value until we obtained only one contiguous range per species (Cox et al. 2016). For species with naturally disjunct distributions (e.g., *Bothrops bilineatus*, *Lachesis muta* and *Thamnodynastes pallidus* in Amazonia and in the Atlantic Forest), we allowed the formation of two or more polygons. We used this combination of methods to estimate the most precise ranges possible for each species according to the current state of knowledge, thus, avoiding a systematic under- or overestimation of the area occupied by each species. The spatial polygons produced in this study are available on the Dryad Digital Repository (DataDryad.org).

Correlates of endemism

Topographic roughness – We calculated the topographic roughness of a grid cell as the largest difference in elevation in a one 0.1 degree grid cell in relation to its immediate neighbours. The layers used to calculate the climatic-related environmental predictors of PE were downloaded from CHELSA (Karger et al. 2016) and represent relevant variables related to macroecological patterns for squamate reptiles (Costa et al. 2007) and with no collinearity problems (variance inflation factor smaller than 2 – Fox and Monette 1992) including: annual mean temperature, temperature seasonality, precipitation of driest quarter, and precipitation seasonality. *Climatic buffering* – We calculated climatic buffering as the sum of the maximum range of (standardized) bioclimatic variables within a 200 km radius (as suggested by Ohlemüller et al. 2008). *Relictual climate* – We measured relictual climate as the absolute difference in the values of bioclimatic variables in one cell to the average values of the surrounding cells (1,000 km - as measured in Ohlemüller et al. 2008). Ohlemüller et al. 2008 measured both climatic buffer and relictual climate with different distances without noticing important changes in the resulting patterns. *Distance to the border of a biome* – We measure this variable as the minimal distance from a 0.1 degree cell to the closest terrestrial biome border (Olson et al. 2001). *Current Climatic seasonality* – These are simply the actual values of the bioclimatic variables corresponding to temperature and precipitation seasonality. *Climate Change Velocity* – Calculated by Sandel et al. (2011), is the estimated changes in mean annual temperature since the Last Glacial maximum (LGM; 21,000 years ago) to the present. The result is the rate in km/year to track changing climate.

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SUPPLEMENTARY TABLES

Table A1 – Corrected Akaike Information Criterion (AICc) values for the top six SAR$_{err}$ models for phylogenetic endemism (PE) and relative PE (RPE) – standardized effect size scores. All combinations of variables were tested with the exception models that included both climate change velocity and topographic roughness, which are highly correlated (r = -0.8). The top four models (AICc in bold) present ΔAICc smaller than two, which indicates that they are equally suitable. Variables included: topographic roughness (top_roug), temperature seasonality (temp_seas), precipitation seasonality (precip_seas), relictual climate (rel_clim), climatic buffer (buf_clim), distance to the border a biome (dist_biome_bor).

	Environmental correlates	AICc
PE ~	top_roug + precip_seas + buf_clim + dist_biome_bor	**4429.57**
	top_roug + precip_seas + rel_clim + buf_clim + dist_biome_bor	**4429.6**
	top_roug + temp_seas + precip_seas + rel_clim + buf_clim	**4431.24**
	top_roug + temp_seas + precip_seas + buf_clim + dist_biome_bor	**4431.32**
	top_roug + precip_seas + dist_biome_bor	4433.83
	top_roug + temp_seas + precip_seas + dist_biome_bor	4435.38
RPE ~	top_roug + temp_seas + precip_seas	**3527.81**
	top_roug + temp_seas + precip_seas + dist_biome_bor	**3528.27**
	top_roug + temp_seas + precip_seas + buf_clim	**3529.06**
	top_roug + temp_seas + precip_seas + rel_clim	**3529.71**
	top_roug + temp_seas + precip_seas + buf_clim + dist_biome_bor	3529.82
	top_roug + temp_seas + precip_seas + rel_clim + dist_biome_bor	3530.11

Table A2 – Spatial Autoregressive Error Model (SARerr) for the standardized effect size scores of Phylogenetic Endemism (PE), Relative Phylogenetic Endemism (RPE) and the respective environmental correlates in half- and two-degrees resolution. Gelkerke pseudo-R-squared (p-R^2). Corrected Akaike Information Criterion (AICc). Asterisks indicate: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Topographic roughness remained the most important variable at all scales.

	Variables	Coefficients	p-R^2	AICc
PE half-degree ~	Topographic roughness	0.145 (0.019)***	0.54	15778.5
	Precipitation seasonality	-0.093 (0.036)*		
	Climatic buffer	0.093 (0.049)		
	Distance to the biome border	-0.083 (0.034)*		
PE two-degrees ~	Topographic roughness	0.246 (0.083)**	0.25	1344.2
	Precipitation seasonality	-0.222 (0.065)***		
	Climatic buffer	0.131 (0.094)		
	Distance to the biome border	-0.158 (0.067)*		
RPE half-degree ~	Topographic roughness	0.151 (0.016)***	0.70	12399.3
	Temperature seasonality	-0.029 (0.054)		
	Precipitation seasonality	-0.092 (0.032)**		
RPE two-degrees ~	Topographic roughness	0.349 (0.048)***	0.69	887.8
	Temperature seasonality	-0.178 (0.114)		
	Precipitation seasonality	-0.292 (0.064)***		

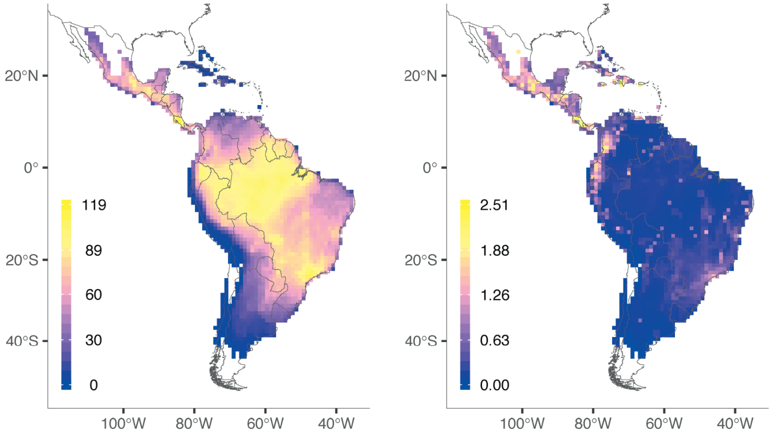
Table A3 – Generalized Linear Models (GLM) results for the standardized effect size scores of Phylogenetic Endemism (PE), Relative Phylogenetic Endemism (RPE) and the respective environmental correlates (best model in one degree resolution). Gelkerke pseudo-R-squared (p-R^2). Corrected Akaike Information Criterion (AICc). As in the case of the SAR_{err} models (Table 1), topographic roughness was the best predictor of both high PE and high RPE, whereas precipitation seasonality was negatively correlated to both measures. Asterisks indicate: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Variables	Coefficients	p-R^2	AICc
PE ~	Topographic roughness	0.207 (0.03)***	0.175	5170
	Precipitation seasonality	-0.185 (0.022)***		
	Climatic buffer	0.107 (0.03)***		
	Distance to the biome border	-0.215 (0.024)***		
RPE ~	Topographic roughness	0.285 (0.022)***	0.11	5315
	Temperature seasonality	-0.206 (0.021)***		
	Precipitation seasonality	-0.074 (0.022)**		

SUPPLEMENTARY FIGURES

(a) Species Richness

(b) Weighted Endemism



(c) Phylogenetic Diversity

(d) Phylogenetic Endemism

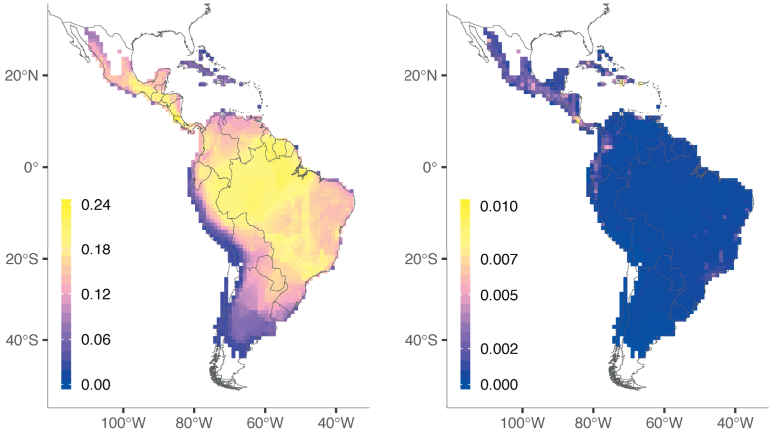
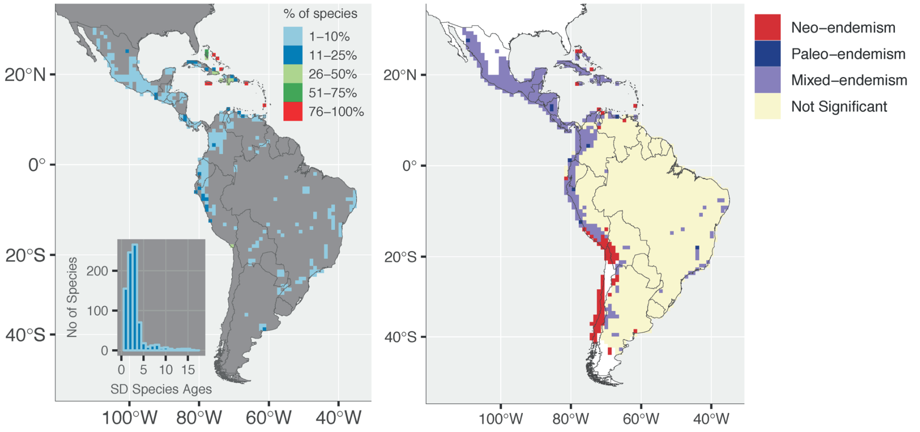
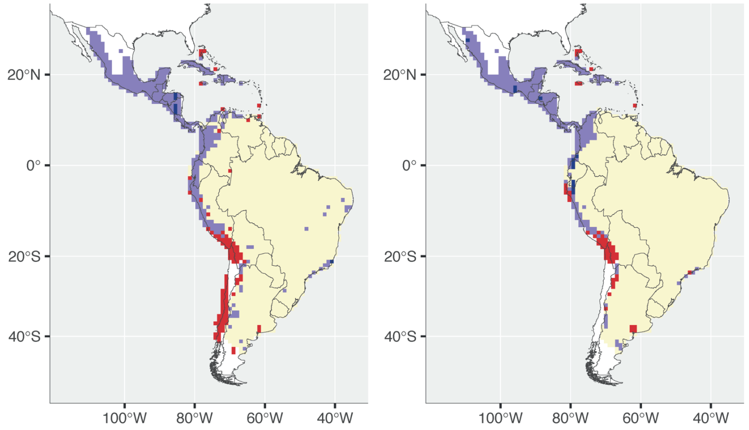


Figure A1 – Biogeographical patterns of Neotropical snakes: (a) Species richness (N = 1,087); (b) Weighted Endemism (log-transformed); (c) Phylogenetic diversity (PD); and (d) Phylogenetic endemism (PE; log-transformed). Species richness and PD are similarly distributed across space as well as PE and weighted endemism.



(a) Variation across phylogenies

(b) CANAPE Neotropics



(c) CANAPE without spp. in fig. a

(d) CANAPE molecular phylogeny only

Figure A2 – Sensitivity to the phylogenetic sampling: (a) Proportion of species per cell with both very restricted ranges (20% lowers range sizes, all occurring in less than < 5 cells) and with high variability in the estimated ages in the sample of phylogenies used in our study (Standard deviation – SD – higher than 3 million years, N = 257 spp.). Most cells do not have any of these species (grey) and most of the remaining cells contain only a small proportion of potentially problematic species (> 10%). (b) For comparison, main results of the Categorical Analysis of Neo- And Paleo-endemism (CANAPE) with all species. (c) CANAPE removing problematic species in Fig. A2(a). (d) CANAPE results removing all species with no molecular data. The overall patterns of endemism found in our work are consistent even when removing more than 50% of the species (N = 485 spp.).

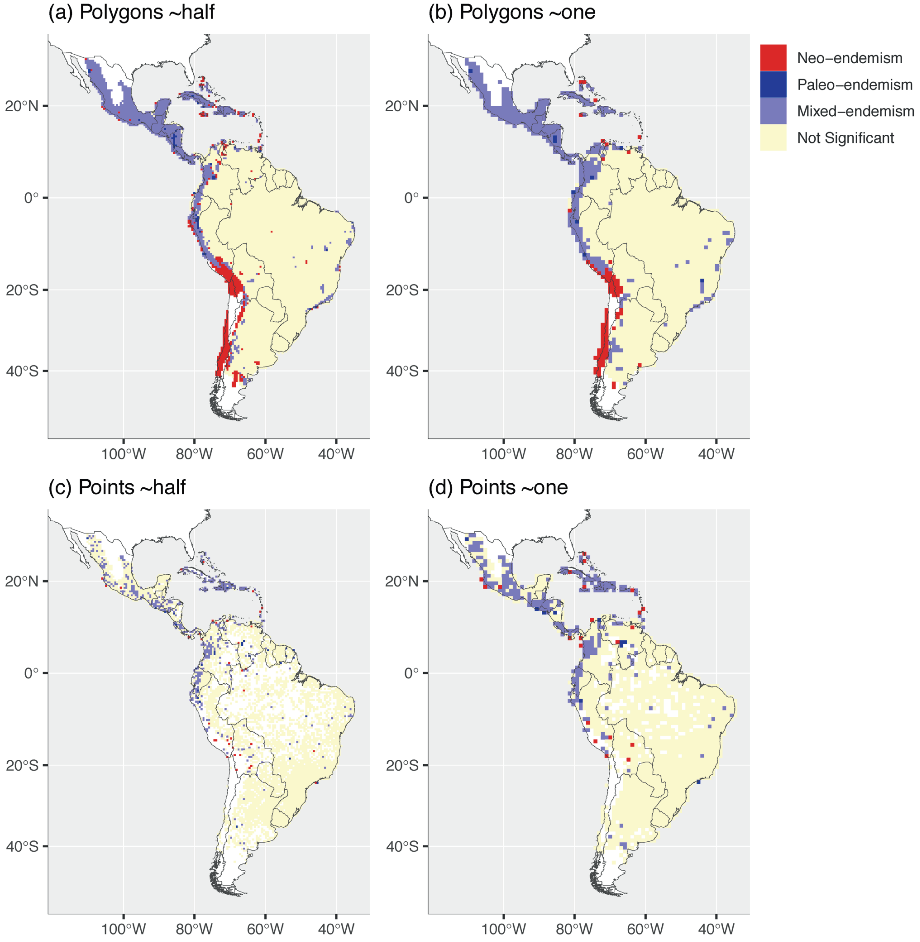


Figure A3 – Sensitivity of the Categorical Analysis of Neo- And Paleo-endemism (CANAPE) analyses to the mapping method and finer geographical scale (0.5 degree). (a–b) CANAPE results for ranges estimated with alpha hull polygons. (c–d) CANAPE results estimated from point-localities. Overall results for most regions are congruent, especially for mixed-endemism patterns, regardless the mapping technique and grid resolution.

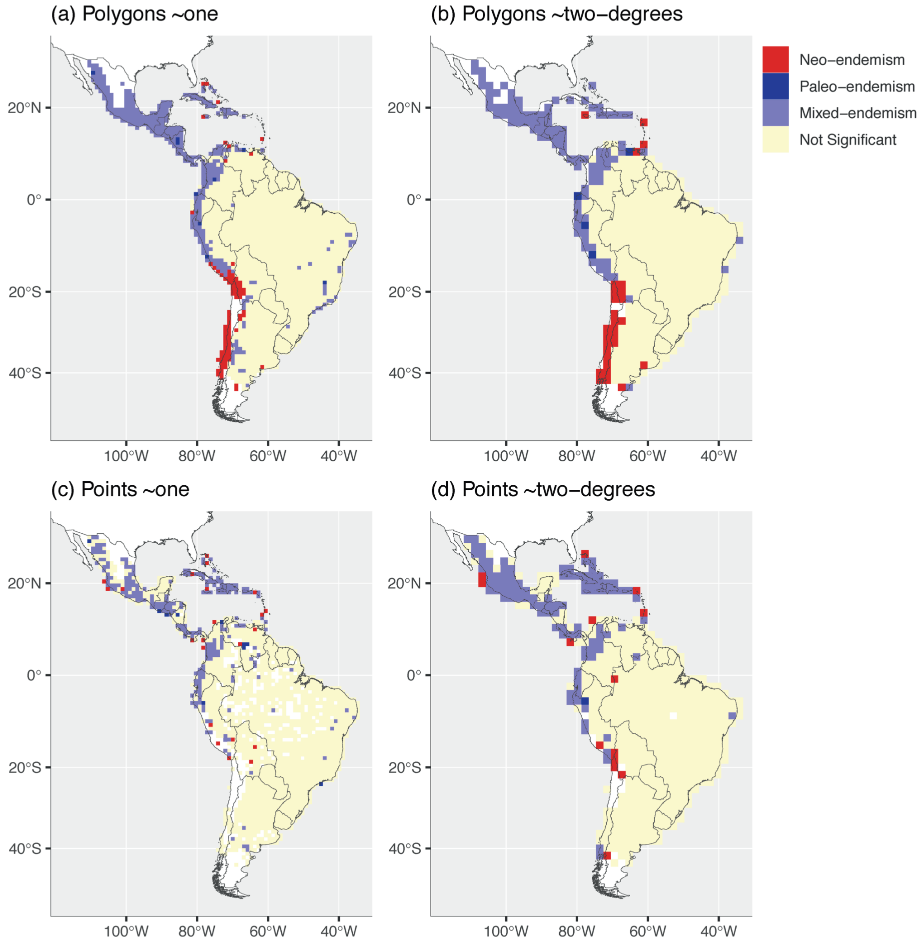


Figure A4 – Sensitivity of the Categorical Analysis of Neo- And Paleo-endemism (CANAPE) analyses to the mapping method and larger geographical scale (2 degrees). (a–b) CANAPE results for ranges estimated with alpha hull polygons in an approximately 2 degrees grid (equal area projection). (c–d) CANAPE results estimated from point-localities. Overall results for most regions are congruent, especially for mixed-endemism patterns, regardless the mapping technique and grid resolution.

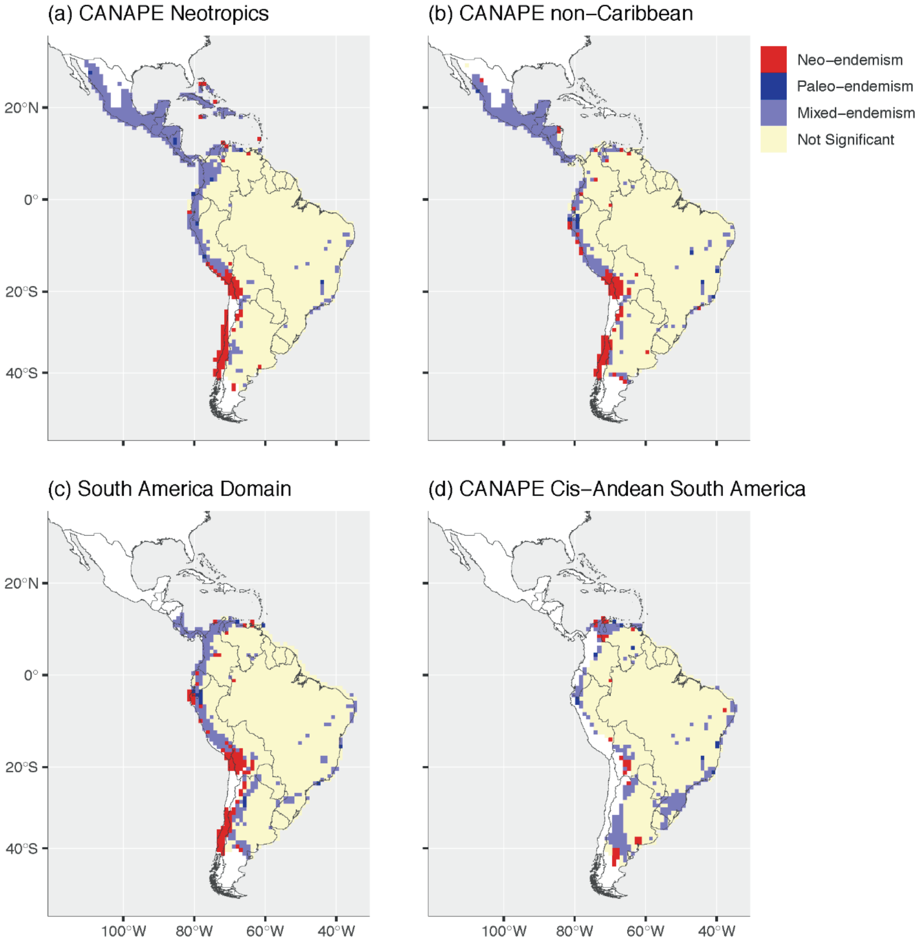


Figure A5 – Sensitivity of the Categorical Analysis of Neo- And Paleo-endemism (CANAPE) to the delimitation of the study area. (a) Complete dataset. (b) CANAPE without the Caribbean. (c) CANAPE without Mexican transition zone and Central America dominion (*sensu* Morrone 2014). (d) CANAPE including only cells in the cis-Andean South America.

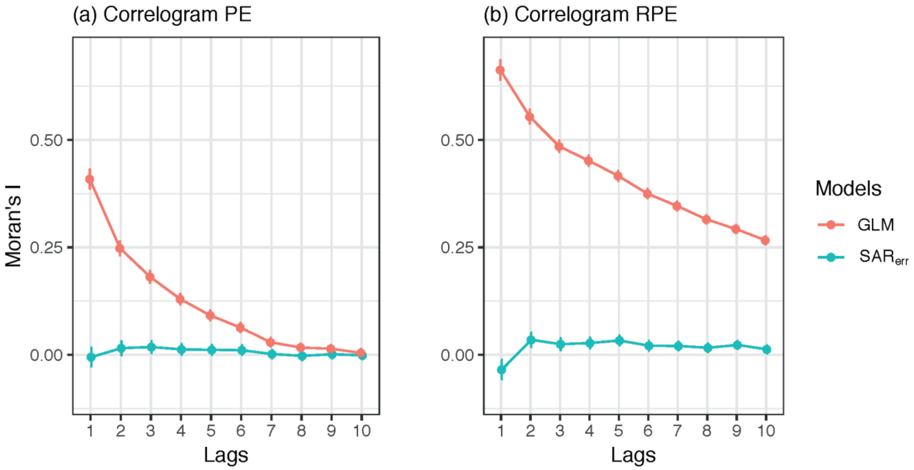


Figure A6 – (a) Correlogram of the residuals of the best Spatial AutoRegressive error model (SAR$_{err}$) compared to the respective Generalized Linear Model (GLM) for phylogenetic endemism (PE) explained by topographic roughness, precipitation seasonality, climatic buffer and distance to the biome borders. (b) Correlograms of the residuals of the best model for relative phylogenetic endemism (RPE) explained by topographic roughness, temperature seasonality and precipitation seasonality. SAR$_{err}$ models removed most of the spatial autocorrelation in the data. Lags correspond to successive neighbourhood levels.

V

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WEGE: A NEW METRIC FOR RANKING LOCATIONS FOR BIODIVERSITY CONSERVATION

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ABSTRACT

In order to implement effective conservation policies, it is crucial to know how biodiversity is distributed. However, defining optimal ways to measure biodiversity and how best to use such measures to prioritize conservation actions is still a topic of debate. One of the most widely used systems is the Key Biodiversity Areas (hereafter KBA) criteria, developed by the International Union for Conservation of Nature (IUCN). In this study we compare the KBA criteria to four primary biodiversity indices (species richness, phylogenetic diversity, weighted endemism, and phylogenetic endemism) and to EDGE score to rank the conservation importance of eight remote sites in Northern Mozambique where we sampled for amphibians and reptiles. We find that none of these metrics is able to provide a suitable ranking of the sites surveyed that would ultimately allow prioritization. We therefore develop and validate the "WEGE index" (Weighted Endemism including Global Endangerment index), which is an adaptation of the EDGE score (Evolutionarily Distinct and Globally Endangered) and allows the ranking of sites according to the KBA but in a continuous scale. For our study system, the WEGE index yields the same ranking as in the KBA assessment, but outperforms it by being able to rank sites inside the same KBA categories. Prioritization may be crucial for policy making and real-life conservation, allowing the choice between otherwise equally qualified sites according to the KBA categories. WEGE is intended to support a transparent decision-making process in conservation.

INTRODUCTION

In order to protect biodiversity and promote conservation, the decision-making process should be based more on scientific research and data, and less on empirical assumptions not supported by scientific studies (Sutherland et al., 2004). Threats to biodiversity such as conversion and degradation of natural habitats, and invasion by non-native species and overexploitation, have the potential of completely decimating biodiversity at local scales (Biofund, 2018; Mucova et al., 2018). Therefore, in recent years there has been an increased awareness of the value of protecting particular sites of high biological value, instead of focusing on large extensions of land (Butchart et al., 2012). Such decisions may ultimately determine whether biodiversity is preserved or lost. Thus, conservation planning should not only encompass the concepts of global conservation prioritization (Myers et al., 2000), but also include a more local-scale approach.

The Global Standards for the Identification of Key Biodiversity Areas (KBA) is an attempt to gather a consensus on biodiversity and conservation practices by highlighting sites that contribute significantly to the global persistence of biodiversity (IUCN 2004). The criteria and methodology for identifying KBAs was created by the IUCN World Commission on Protected Areas (IUCN, 2016). The categorization of areas is based on criteria such as presence or absence of threatened species, species' distribution ranges and ecological integrity. However, indices that directly measure biodiversity such as species richness (SR), phylogenetic diversity (PD:Faith, 1992), weighted endemism (WE:Crisp et al., 2001) and phylogenetic endemism (PE:Rosauer et al., 2009) were left out of the KBA methodology.

Although most biodiversity measures used in conservation relies on species richness, measures such as PD and PE add the evolutionary relations among species and minimize taxonomic conflicts. All these indices contribute to the understanding of how and where biodiversity is distributed in a continuous scale, and should allow the ranking of individual sites under consideration for conservation. However, their accuracy is highly dependent on the quality and availability of data, making poorly sampled areas particularly hard to evaluate (Faith, 1992; Faith et al., 2004; Rosauer et al., 2009).

Although both the KBA and the raw biodiversity indices may be useful in various ways in conservation, none of them incorporate information on the threat status of the constituent species – the IUCN's Red List Assessment parameter. One exception is the Evolutionarily Distinct and Globally Endangered (EDGE) score (Isaac et al., 2007), which combines one biodiversity index – Evolutionary Distinctiveness (ED) – with the threat category of species.

ED is a measurement of the branch lengths divided by the number of species inside each clade. The EDGE score combines ED with values for species' extinction risk in order to generate a list of species that are both evolutionarily distinct and globally endangered ('EDGE species'). The EDGE score is however tailored to rank species rather than locations. Location scores may be computed as the sum of EDGE scores for all species in a site (Safi et al., 2013). However, this is not guaranteed to maximize conservation importance of individual sites, since the presence of widespread, critically endangered species produces higher EDGE scores than a vulnerable or endangered micro-endemic restricted to very few sites, which could rapidly go extinct if those sites are damaged. One example is the Atlantic bluefin tuna, which exists in great part of the Atlantic Ocean, but nevertheless is considered an endangered species (Collette et al., 2011).

In this study we tested whether biodiversity indices can be used to rank locations inside the categories recognized by IUCN's KBA. We focus on two poorly studied vertebrate groups, amphibians and reptiles (K. A. Tolley et al., 2016; Titley et al., 2017) in eight remote and poorly sampled areas in Northern Mozambique, which we surveyed through our own extensive fieldwork. To achieve our objectives of ranking individual sites for conservation, we introduce a new spatially explicit index –WEGE (Weighted Endemism and Globally Endangered). WEGE is an adaptation of the EDGE score (Mooers et al., 2008), were we have replaced the phylogenetic component with an endemism score and is presented as a tool for attributing a continuous scale for the Global Standard of Identification of KBAs.

METHODS

Study area

This study focuses on Northern Mozambique. The country is one of the poorest countries in the world which makes conservation versus development a challenge. Exploitation of gold, ruby, coal, natural gas, and many other important minerals (Yager, 2002; FocusEconomics, 2018) is an important source of income but is also likely to have detrimental effects on the natural environment.

Being one of the most poorly known areas Africa in terms of biodiversity assessments, Northern Mozambique has recently attracted efforts leading to surveys and the discovery of many new species for science of reptiles, mammals, amphibians and plants. Many of these new species appear to be restricted to single mountains (Branch et al., 2014; Bittencourt-Silva et al., 2016), making these mountains important areas of endemism and consequently in need of effective conservation policies.

Fieldwork

To give consistence and comparable results we sampled amphibians and reptiles in eight sites in Northern Mozambique for three full days in each individual site (Figure 1). All sampling took place between October 9th and November 16th of 2017 and was carried out by 3–4 scientists each time (members of our team), with the same three members of the team being present in all 8 sites. All sites consisted of small inselbergs (400 – 1200m) with similar areas (1-2 km^2) and were sampled by the same team and methods. The inselbergs consisted of exposed bedrock and patchy vegetation (Fig. 3). The methods consisted of opportunistic and active search and trapping with funnel and glue traps. We excluded species that were recorded only once at each site in order to reduce the effects of false negatives on our analyses e.g. species might be present in locations, but we failed to record them.

Key Biodiversity Areas

Although the Global Standard for the Identification of Key Biodiversity Areas (KBA) (IUCN, 2016) has five main criteria and thresholds for the assessment, namely: A. Threatened biodiversity; B. Geographically restricted biodiversity; C. Ecological integrity; D. Biological processes; and E. Irreplaceability through quantitative analysis, only criteria A and B could be applied to our dataset consisting of a georeferenced species list. The full list of criteria and applicability in this study is provided in Appendix S1.

Biodiversity indices

To test whether we could use widely used biodiversity metrics to rank our locations, we calculated the scores of four indices: SR, PD, WE, PE and the EDGE score in a grid of 0.1 degrees (equivalent to approximately to 10 by 10 kilometers) and compared with the Global Standards for the Identification of KBAs. Details and resources used to calculate the different metrics can be found in the Appendix S1.

The WEGE index

We sought a measure that would align its results with the IUCN's KBA categorization of our locations. Since such measure has not yet been proposed to the best of our knowledge, we created an index capable of ranking locations in a continuous scale inside the categories of the KBA.

The WEGE index proposed here is an adaptation of the EDGE score (Isaac et al., 2007) using the probability of extinction risk as in Mooers et al. (2008). The idea of the EDGE score is to measure biodiversity by taking into account both the evolutionary distinctness (ED) and the Probability of Extinction (ER).

To calculate EDGE, the following formula is used in Mooers et al. (2008):

$$EDGE = \ln(ED * ER)$$

To calculate WEGE, we apply this formula:

$$WEGE = SQRT(WE*ER)$$

In order to calculate the values for the WEGE index of all the locations in this study we created a package in R, available at devtools::install_github('harithmorgadinho/wege').
We used the IUCN50 transformation for the ER as in (Davis et al., 2018), which scales the extinction risk over a 50-year period using the following extinction probabilities: LC = 0.0009, NT = 0.0071, VU = 0.0513, EN = 0.4276, CR = 0.9688.

The EDGE enables the ranking of species, rather than directly scoring areas, in regard to prioritization. The WEGE index, in contrast, allows the ranking of locations rather than individual taxa. Another advantage of the WEGE index over the EDGE score for conservation is that EDGE does not explicitly take into account distribution ranges of the constituent species, since distribution isn't alone a requirement for the attribution of a threatened status. One example is the Mount Gorongosa Pygmy Chameleon, assessed as LC but with an estimated area of occupancy of 108 km^2 (K. Tolley, Menegon, M. & Plumptre, A, 2014). Mount Gorongosa may therefore not be classified as an important conservation area based on EDGE, but the only way to protect this species is to protect this area. Using species distribution ranges can be crucial in assessing the importance of locations comprising all known populations of a species with a low threat category in IUCN's Red List, which we argue should be of higher conservation priority than a location containing a more threatened species but with larger total distribution (see fig. 3 for a schematic representation on how the WEGE index ranks locations).

Unlike amphibians, reptiles in Africa are largely unassessed. In order to avoid a biased threat status attribution, we therefore treated unassessed species as “UA” (Under Assessment) as in Tonini et al. (2016) and attributed a value of extinction risk similar “LC” (Least Concern). Our dataset didn’t contain any “DD” (Data Deficient) species, although we would recommend treating them as VU.

To showcase the main differences between EDGE and WEGE we created two hypothetical scenarios. In the first we removed the new species of *Elasmodactylus* recorded from locations 5 and 6 and in the second scenario we added a record of an endangered reptile that is expected to occur in our sampled locations, the terrapin *Cycloderma frenatum*. In order to calculate WEGE for the second scenario we downloaded the species records from GBIF (https://doi.org/10.15468/dl.u9bqnu).

RESULTS

3. Fieldwork data collection

We recorded 15 species of amphibians and 29 species of reptiles (Appendix S1) in eight locations in Northern Mozambique (Fig. 1). The most remarkable findings were a putatively new amphisbaenian, *Geocalamos* sp. nov, a putatively new species of gecko *Elasmodactylus* sp. nov and a new distribution record for Cordylus maculae (64 km east to the only previously known locality, Mount Mecula).

IUCN’s KBA fit

From the five major criteria and nine sub-criteria of IUCN’s KBA, seven sub-criteria could be applied to species inventories carried out in this study (Appendix S1). In criteria A (Threatened Biodiversity; see details in Table 2), the focus of A.1. is on the Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) categories. The Red List assessment for amphibians and reptiles in Mozambique has 18 species assessed and their respective statuses can be found in Appendix S1. During the sampling for this study, the only species collected that has been assessed as threatened (Endangered), was Cordylus meculae at Location 4, at 65 km West from Mecula Mount, the only previously known location for the distribution of the species (Branch et al., 2005). Since this species is assessed as Endangered and its range is restricted to a single mountain, Location 4 would trigger KBA status according to A.1 a), e) and B1. In addition, we also found a putatively new species of *Geocalamus* sp., in this case, the site would also qualify for B2 since there are more than one species in this particular site.

Criteria B (Geographically restricted biodiversity), B1: "Individual geographically restricted species" can be applied to Locations 5 and 6 and trigger KBA status because a putatively new species was found on those locations, *Elasmodactylus* sp. nov. Until further evidence is produced, its known distribution is restricted to those locations.
The remaining locations did not meet any of the criteria for a KBA status.

a. Indices' scores

In order to compare the results from the IUCN's KBA, for each location we calculated SR, PD, WE, PE, ED, the EDGE score and the WEGE index.
For amphibians (Fig. 4), locations with higher SR scored higher in all indices calculated, being location 3 the one with highest scores. No threatened amphibians were collected in this study, hence we expect WEGE index to rank all the location in the same way as WE.

For reptiles (Fig. 4), although SR and PD were relatively constant across locations, WE, PE, EDGE and WEGE ranked locations differently. The WE and PE indices selected locations 5 and 6 as highest and location 4 in third place unlike WEGE and EDGE which selected location 4 as top priority followed by locations 5 and 6.

When comparing the WEGE scores between taxa, despite the value being highest in amphibians in location 3, it still scored 10.5 times lower when compared to location 4 for reptiles (0.037 – 0.389) (Fig.4). Showcasing the fact that location 3 for amphibians, by not having any narrow ranged or threatened species, had low conservation importance. The same pattern emerged for the EDGE index, but a lesser extent, 7.7 times lower for amphibians.

WEGE versus EDGE

In order to test the behavior of WEGE and EDGE we made slight changes in our datasets. When we remove the geckos from location 5 and 6, WEGE stops ranking locations 5 and 6 as conservation priorities while EDGE still kept one of them (Fig. 5: B). When we add the terrapin to location 3, WEGE still ranks location 4 higher than location 3 because it considers that an area

which has both an endangered species and range restricted has more conservation importance than an area with a widespread equally endangered but phylogenetically more distinct species. Unlike EDGE which started to rank Location 3 as the top priority (Fig. 5: C).
Using the WE index in the formula (rather than ED) guarantees that rare species will have an equal contribution to the ranking of a location, independently from the range of their sister species, unlike the EDGE score.

DISCUSSION

IUCN's KBA and Biodiversity indices

This is the first study that attempts to compare IUCN's KBA site selection process with biodiversity metrics, so for this purpose we included four of the most widely known metrics, SR, PD, WE, PE and EDGE.

The analyzed dataset is relatively small but it is typical of many empirical situations for land owners or governments e.g. decision where to allow mining or logging. It also has the benefit that all areas are assessed by the same methods and experts meaning that we do not have to deal with issues of variation in knowledge as a function of different skills between biodiversity assessors (Ahrends et al., 2011).

The IUCN's KBA uses a set of guidelines to check whether a particular site triggers a KBA status, unlike biodiversity metrics which attempt to quantify different spectrums of biodiversity. Hence, different biodiversity metrics are expected to weight sites differently. The biodiversity of specific sites should arguably not be assessed by just summing the number of species existing in each location, but also taking into account other factors such as genetic diversity, distribution ranges or conservation status (Magurran, 1988; Barthlott et al., 1999). Otherwise, the presence of many widespread species producing a high SR would mask the importance of vulnerable or endangered micro-endemic taxa (restricted to very few sites). The fact that SR and PD indices are known to be highly correlated with sampling effort (Bunge & Fitzpatrick, 1993; A. Rodrigues et al., 2005; A. S. Rodrigues et al., 2011; Tucker & Cadotte, 2013) advocates against their use in inconsistently and poorly sampled regions, compared to dense sampling which will in most cases show higher species diversity. In addition, SR and PD completely disregard the information on species range in their score, which is a strong predictor of extinction risks for species (Purvis et al., 2000) and one of the fundamental aspects of conservation prioritization and management of natural resources (Anderson, 1994; Myers et al., 2000; Roberts et al., 2002; Slatyer et al., 2007).

WE and PE are also expected to correlate with sampling effort, since new sets of records can only consolidate or increase the score but never decrease it (Lande, 1996; Nipperess, 2016), although this correlation seems to exist at lesser extent than in SR and PD (Soria-Auza & Kessler, 2008; Oliveira et al., 2016). But besides the sampling effort issue, the use of WE and PE in conservation policies might encounter additional problems. A benefit of PE is that for two recently diverged taxa, the vast amount of their evolutionary history is shared and it therefore matters very little if they are treated as separate species or not. This is critical for groups with large genera, which often comprise both widespread and range-restricted species as a result of species radiations. One example is the widely distributed skink of the genus *Cryptoblepharus*, which if analyzed through WE would score considerably higher compared with an analysis using PE. *Cryptoblepharus* is very widespread, with some species occurring from the eastern fringes of the Indo-Australian archipelago, Australia and Oceania, to the islands of the far Western Indian Ocean and adjacent parts of the African coast (Rocha et al., 2006). The WE index, in contrast, gives a weight of 1 for every species, which makes the index more vulnerable to taxonomic changes but guarantees the equal contribution of species inside large genera.

Regarding our analysis, in a dataset consisting only of widespread species belonging to the same threat as for amphibians, we obtained a similar pattern between the indices, while for the reptiles, with both widespread and range restricted species, we obtained contradictory values depending on the index. As expected, our results show that different indices rank sites differently.

Suitability of the WEGE Index

While WEGE and EDGE produced very similar ranking of locations in the empirical dataset, this is not a guaranteed output of the two measures. In our empirical dataset we only have one endangered reptile while all other reptiles are least concern, and since the extinction risk for endangered species are so much higher than least concern, both indices are governed solely by the IUCN status part of the index. The two measures will however respond differently if multiple species with the same IUCN score are present.

The IUCN's KBA attributed a KBA status to different locations when compared to the best performing locations for the SR, PD, WE and PE indices. In contrast, the new index proposed here (WEGE) is capable of ranking locations in a continuous scale and matching the KBA status triggered by IUCN's KBA. The WEGE index adds the component of conservation status of each species to the WE index. The internal logic of this metric is to combine conservation scoring of each species with a measure of the relative importance of the site in question for each species. This could also be achieved by combining a conservation score which incorporating evolutionary history such as e.g. PE rather than WE, but since KBA by design weigh all species equally irrespective of their evolutionary uniqueness we chose to select a measure with the same lack of taxonomic weighing. By incorporating WE in the EDGE score formula and creating the WEGE index, we obtained an index in line with the IUCN KBAs standards criteria compared to the SR, PD, PE, WE and EDGE. Additionally, the WEGE index allows the use of species which although being published haven't yet been assessed by IUCN. This is achieved by the attribution of the UA (under assessement) category to the species which in turn will be converted to the ER component in the formula. In the case of Mozambique, there are 226 species of reptiles (Uetz et al., 2019) of which only 90 have been assessed (IUCN 2019).
The WEGE index can be used either to find suitable candidates' areas to be considered as KBA's or as a mechanism of weighting the contribution to conservation of particular KBA's as well as areas outside KBA's. Additionally, it uses a simpler methodology by employing only two metrics instead of a set of seven conditions (A1a - e and B1 and B2). Finally, WEGE can act as a complement in the process, by which, sites selected using IUCN's KBA can now be ranked objectively according to their conservation value.

Avoiding the categorical ranking of locations can bring great advantages when prioritizing efforts with limited resources. IUCN's criteria lack this aspect by attributing a binary system where one particular site either triggers KBA status or not. By using WEGE, we rank sites within the same category and enabling the decision-making process to be objective and transparent as possible. This methodology can highlight areas which even though they trigger KBA status, their score is low and might be on the cusp of losing their KBA status.
KBA sites which are driven either by the presence of one threatened or range restricted species, will change if species become non-threatened or get their range considerably expanded. Consequently, lower performing WEGE sites have higher odds of losing their KBA status. One example that illustrates this scenario is the species *Cryptoblepharus ahli* Mertens, 1928, described by Mertens (1928), synonymized to the widespread species *Cryptoblepharus africanus*

by Brygoo (1986) to later, based on a morphological examination of the species to be elevated to full species by (Horner & Adams, 2007). This species by itself meets the requirements for the Mozambican Island to trigger KBA status, regardless of its IUCN status, since it is at the moment an accepted species confined to a single small island. Further analysis of the genetics of this particular species will have an impact on the KBA status of this island.

Within the current IUCN ranking system, a site can have either one or many endemic species and they would trigger a KBA status and be treated the same, using WEGE, we are able to objectively demonstration that areas with more endemic species have higher conservation value.

Limitations and challenges of the WEGE index

The two measures, EDGE and WEGE combine two clearly different and unrelated metrics, whilst WEGE makes use of species distribution and it's IUCN status just as in the IUCN's KBA. These two criteria are not independent since range size is one of the criteria for IUCN status. Importantly, however, the two criteria in WEGE clearly still measure distinct processes which for instance can be seen by the existence of widespread but endangered species like the already mentioned Bluefin tuna or highly restricted and least concern as the Mount Gorongosa Pygmy Chameleon. By combining the two we show that we get a better measure than solely relying on IUCN criteria or solely on WE.

Despite ranking locations according to the KBA's guidelines, the WEGE index does not incorporate all the KBA's criteria. We expect poorly sampled areas to only have limited data on species distribution, and thus information such as Ecological Integrity (criteria C), Biological Processes (Criteria D) and Irreplaceability Through Quantitative Analysis (Criteria E) are likely to not have been assessed for the area in question.

The last step of before proposing a particular site as a KBA requires an analysis of the manageability of the site in regards to its physical attributes such as forest cover limits or rivers and anthropogenic factors such as roads and existence of human settlements. The WEGE index in itself is not aimed at replacing this process, we believe this step to be of crucial importance and should be done case by case and involving local authorities. The aim of the WEGE index is to highlight and rank sites which should in the following step be scrutinized at a local level as in the KBA process or rank already existing KBAs.

Final remarks

The prioritization of areas in regard to biodiversity is complex. Different indices prioritize different areas. IUCN KBAs do not contemplate biodiversity indices in the decision-making process. However, for the case of amphibians and reptiles in northern Mozambique, we found a correlation between the areas that would in theory be considered as future protected areas and the WEGE Index.

Mozambique is a developing country that struggles to conciliate its rich biodiversity with the for the mining industry, and the high potential economic gain that could follow. The country also has one of the highest corruption levels in the world, and unbiased methods to quantify biodiversity are a crucial parameter for a transparent decision-making process in conservation. WEGE is able to rank locations according to the IUCN's KBAs while giving a continuous value to each site, thus facilitating their ranking and prioritization in real-world conservation.

SUPPORTING INFORMATION

Methods used for calculating indices, r packages used, KBA guidelines and raw data (Appendix S1), is available online.

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Figure-Legend Page

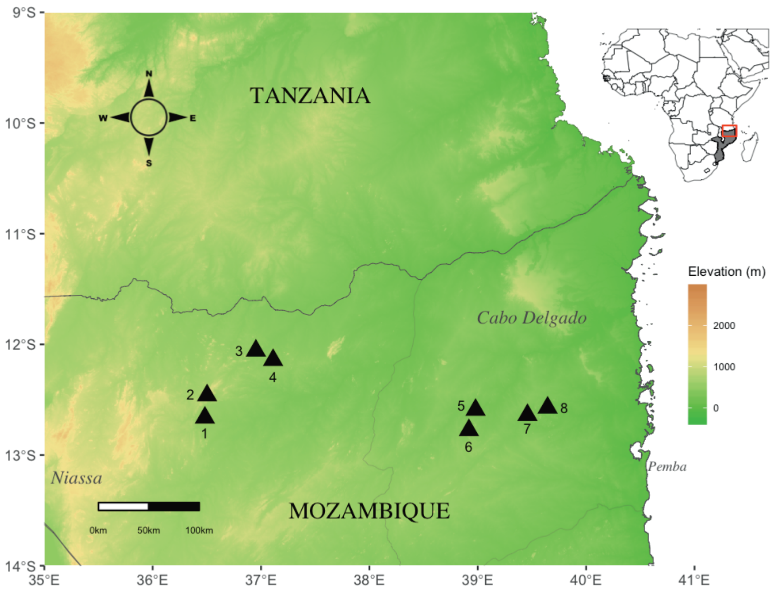

Figure 1: Map of Northern Mozambique with administrative borders showing sampled locations in Northern Mozambique. The eight sites sampled (indicated by triangles) correspond to largely isolated inselbergs amidst a savanna landscape.

Figure 2: View from location 5, showing the inselberg emerging from the surrounding savanna. The rocky outcrops host a rich diversity of lizards and amphibians.

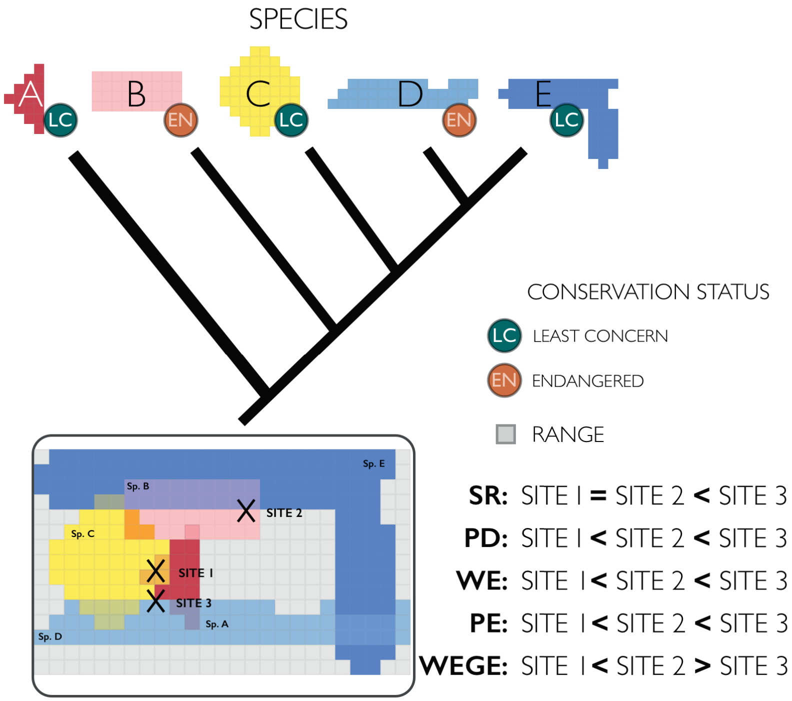

Figure 3:Infographic of how the WEGE compares with SR, PD, WE, and PE in three hypothetical scenarios.

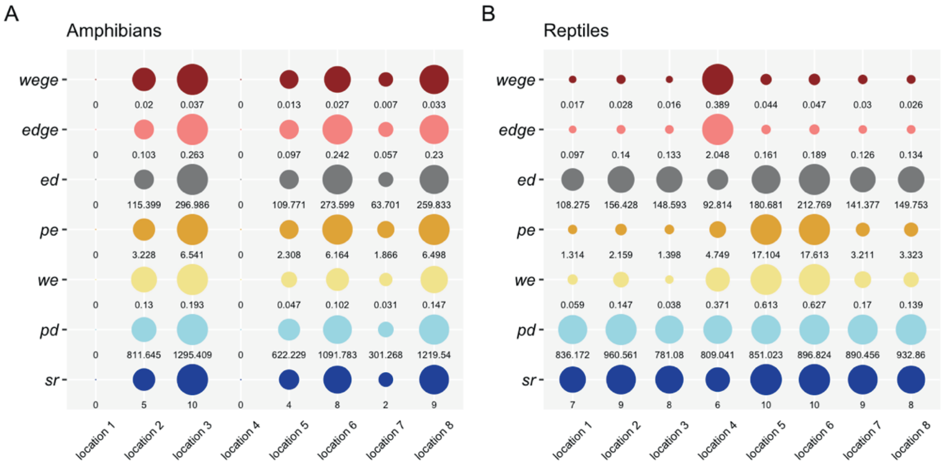

Figure 4: Comparison between indices calculated for each location for amphibians and reptiles. The size of the circle was transformed by dividing each value of the metric by the maximum value observed in each index

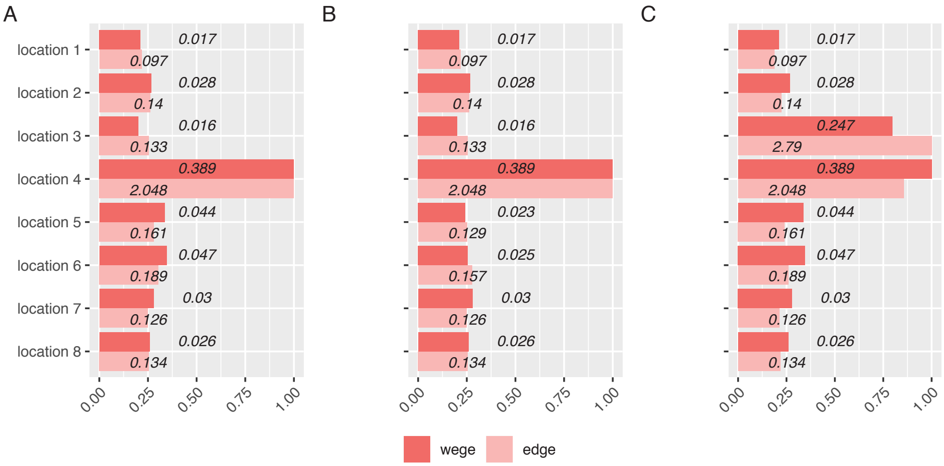

Figure 5: Comparison between the real dataset for reptiles (A) and the two hypothetical scnerios. B. Removal of *Elasmodactylus* from locations 5 and 6, C. Addition of record of *Cycloderma frenatum* to location 3. Bars have been squareroot transformed for visualization purposes but values are real.