Universidade de São Paulo - USP
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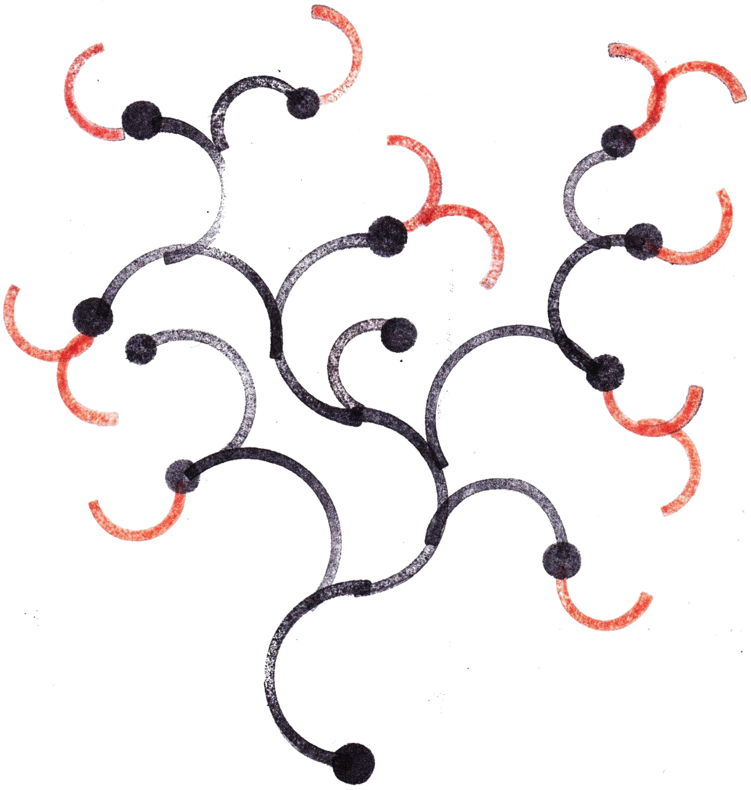
Phylogenetic Diversity, Richness and Conservation of Dipsadid snakes in cis-andean South America

Diversidade Filogenética, Riqueza e Conservação de Serpentes Dipsadidae na América do Sul cis-andina

Filipe Cabreirinha Serrano



São Paulo
2022

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências, na Área de Ecologia.

Orientador: Cristiano de Campos Nogueira

São Paulo
2022

Ficha Catalográfica

Filipe, C. Serrano
Phylogenetic Diversity, Richness and Conservation of Dipsadid snakes in cis-andean South
America / Filipe C. Serrano ; orientador Cristiano de Campos Nogueira -- São Paulo, 2023.
135 p. + anexo

Tese (Doutorado) -- Instituto de Biociências da Universidade de São Paulo. Ciências Biológicas (Ecologia).

1. Biogeography. 2. Neotropical. 3. Phylogenetics. 4. Herpetology. 5. Vicariance. I. de Campos Nogueira, Cristiano, orient. Título.

Comissão Julgadora:

Prof(a). Dr(a).	Prof(a). Dr(a).
Prof(a). Dr(a).	Prof. Dr. Cristiano Nogueira

A Rosa,
Aos seus olhos
pelos quais aprendi a ver o mundo

ACKNOWLEDGMENTS

I thank Cristiano Nogueira for welcoming me in Brazil, the exciting discussions on snakes, biogeography and the Cerrado, for supportive encouragement and financial support for national and international experiences and mostly for supporting my independence.

I thank my friends and co-authors Laura Alencar and Ricardo Sawaya for the emotional and academic support. Both were keystone for my first steps on Brazilian waters (pun intended) and a lifeline during the process. Furthermore, I thank my co-authors Felipe Grazziotin, Matheus Ponte-Nogueira and Richard Grenyer for how much I have learned from them.

I thank Marcio Martins for his trust in me, all the valuable knowledge during but not limited to our fieldwork in the Cerrado and for the example he sets.

I thank Juan Camilo Díaz-Ricaurte for the companionship and joint learning. We made lemonade without lemons, and that will forever be one, if not the greatest, takeaway from my PhD.

I thank my colleagues from LabVert: João ‘JP’ Vieira-Alencar, Ricardo ‘Richie’ Luría-Manzano, Rosana Paschoalino, Gabriella Leal, Luciana Furtado, Bruna Gomes, Eletra de Souza and Isabela Gil Ramos.

I thank many colleagues and friends from the Ecology Programme: Júlia Barreto (my partner in crime), Andrés Rojas, João Menezes, Susana Caracena, Isabella Romitelli, Bruna Cassetari, Sebastian Alvarado, Caro Montalegre-Talero, Catalina Z, Matheus Januário, Solimary Garcia-Hernandez; as well as Glauco Machado, Paulo Inácio Padro and Vera Lima - the heart and soul of this programme, which dedication and empathy are a prime example of what Brazil does best.

I thank my flatmates Clarice Borges Matos, Mário do Val and Francisco d'Albertas for the safe haven on our 'Potato Plaza'. With all the good and the bad, our home was my open window to São Paulo.

I thank my family in São Paulo (and hopefully out of São Paulo too), the Farhat family - Ana, Ivan, Leonardo, Raquel, Mariana, Bruno, Cristiane, Paulo, Vó Cila. Words are not enough to express how grateful I am for how well I was received and for how much I have learned from you.

I thank Marisa 'Beta' Rodrigues - one of the major culprits of my Brazilian adventure. Our friendship has endured much and has passed the test of time. We started from the bottom, now we here.

I thank my Portuguese friends: Bé, Sengo, Silva, Zorro, David Mateus, Pedro Gaudêncio, Bernardo Bagulho (the author of my beautiful cover), Inês Maltez, João Amaral, Noca, Inês Palolo, Santos, Susana Canelas, Bacalhau. They had to be patient for my return and I had to be patient to listen every couple of weeks: "Have you finished your thesis? We want you back in Portugal.". To their lack of luck, I was even more patient with writing my thesis. However, they remained my vital connection to what is important to me, and I hope they continue to do so.

I thank Carolina Farhat. For making me see beyond the obvious, for making me believe in the simple things, for teaching me how to enjoy the 'easiness' of life. I am honoured to be able to learn so much from you and to build a life together. All this would have been impossible without you.

I thank my family - Clara, Avelino, Inês, my two soon-to-be-born nephews, the Serranos (Rosa, Balé, Hugo, Paulo, Gilda, Sofia, Ana, Miguel, Marita, João Filipe, Nuno, among many) and the Cabreirinhas (Céu, Fatinha, Luísa, Vasco, Pedro, Ermelinda, Franklin and Ofélia). For giving me wings to fly but never failing to giving me a place to land. I am proud of my humble but strong roots. Now that I have seen the world, I know that I am lucky to have been gifted such an unique and ‘organic’ perspective of life. Without my parents, who almost believe in me as much as I do, no part of this would make sense. Their support was a constant and essential factor in a process where I often felt nothing made sense.

I thank Brasil (with a ‘s’). No other place on Earth challenged me as much but then again who wouldn’t want to be challenged? (me, I would have liked to be slightly less challenged!).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

I deeply thank you all. This would not have been possible with each of your contributions. This thesis was written in a five year period, with a now broken laptop, working two simultaneous hour-intense jobs after the scholarship ended and two COVID-19 infections (one of which as I write this, in the final weeks before submitting). It was the loneliest period of my life but you were there to hold my hand through it all.

Finally, I thank my past self for being there for myself, for never giving up even during several moments of self-doubt and for having the strength, curiosity and audacity necessary to finish a Ph.D.

SUMMARY

ABSTRACT

Phylogenetic Diversity, Richness and Conservation of Dipsadid snakes in cis-andean South America

The Neotropical realm is a climatically and geologically diverse biogeographical region, encompassing a wide range of habitats, from the lush rainforests of the Amazon and Central America to the snow-covered peaks of the Andes. These also reflect its biodiversity and its distribution patterns. One of its most spectacular faunas is the Dipsadidae family, the richest snake clade in the world. Herein, I show how its origin, evolution and distribution have been strongly shaped by biogeographical events such as barrier-driven vicariance and dispersal, as well as ecological processes such as niche conservatism. These highlight how its two main Neotropical subfamilies (Xenodontinae and Dipsadinae) have different biogeographical trajectories which are likely reflected by their current patterns: Xenodontinae is a generalist clade that dispersal once and earlier from Central America to South America, while Dipsadinae is a forest specialist clade that dispersed later and several times thus leading to strong vicariant speciation in Central America. My results are an important baseline from the understanding of the evolution and biogeography of Neotropical biota, as of conservation for Neotropical snakes.

Keywords: Biogeography, Distribution, Neotropical, Phylogenetics, Serpentes

RESUMO

Diversidade Filogenética, Riqueza e Conservação de Serpentes Dipsadidae na América do Sul cis-andina

A região Neotropical é um região biogeográfica climaticamente e geologicamente diversa, englobando uma grande gama de habitats, desde as exuberantes florestas tropicais da Amazónia e América Central aos picos cobertos de neve dos Andes. Isto refleta também a biodiversidade e os seus padrões de distribuição. Uma das faunas mais espetacular é a família Dipsadidae, o mais rico clade de serpentes do mundo. Neste trabalho, mostro como a sua origem, evolução e distribuição foram fortemente moldadas por eventos biogeográficos como vicariância imposta por barreiras e dispersão, além de processos ecológicos e evolutivos como o conservatismo de nicho. Estes eventos e processos reforçam como as duas principais famílias Neotropicais (Xenodontinae e Dipsadinae) têm diferentes trajetórias biogeográficas que são refletidas pelos seus padrões atuais: Xenodontinae é um clado generalista que dispersou da América Central para a América do Sul uma vez e há mais tempo e Dipsadinae é um clado especialista em floresta que dispersou depois e múltiplas vezes levando a forte especiação vicariante principalmente na América Central. Os meus resultados são um importante avanço para entender a evolução e biogeografia da biota Neotropical e para a conservação de serpentes Neotropicais.

Palavras-chave: Biogeografia, Distribuiçãon, Filogenética, Neotropical, Serpentes

1. INTRODUCTION

Biogeography is the study of how and why life is distributed across space (Dansereau 1957; Wen et al., 2013). It builds on many other aspects of the life sciences such as geology, ecology, climatology but mainly and obviously geography. While it may now seem a relatively intuitive field of study, its main proponents and precursors such as Alexander von Humboldt and Alfred Russell Wallace, to name a few, were beginning to do so only less than 250 years ago (Wallace, 1876; Humboldt 1807; Linder et al., 2019). Their findings changed the way we view the world today and have inspired many, myself included, to do brave things such as enrolling in a Ph.D. program and writing a Ph.D. thesis. However, I do not believe their intention was born out of grandioseness or of a will to change the world but out of passion, curiosity and mainly innocence. With the hope that my own innocence and naivety might someday, somehow, <u>somewhere</u> inadvertently inspire someone, I will now briefly introduce not only my thesis and its goals, but also how my personal and professional life is intertwined with them.

Space is a fundamental aspect of life on earth (Croizat, 1964; Patterson, 1981). Not only is it the plane (in three dimensions + time) across which species <u>are</u>, but it is simultaneously responsible for subtle (and often not-so-subtle) changes that mold life – an intimate relationship between geology and biology. Or more succinctly, “earth and life evolve together” as postulated by Léon Croizat (Croizat, 1964), an Italian-born Venezuelan biogeographer. In my case, it was also space and travels through it that strongly influenced what I focused on in my Ph.D. thesis. After finishing my Bachelor’s in Animal Science at University of Évora (Portugal) – and after deciding that my days of working with cattle, ostriches, pigs among others were over – I started to look into studying herpetofauna (reptiles and amphibians) which had always been a passion of mine. In my Master’s degree, at University of Lisbon (Portugal) I had the chance to do so by studying the Spatial Ecology of the European pond turtle (*Emys orbicularis*) which is a vulnerable species in Portugal. This project entailed following radio-tagged terrapins alone remotely in the field for two months, which taught me patience and perseverance and ultimately led me to start feeling that research was the right path for me – culminating in my first Research Article (Serrano et al., 2019). This was further confirmed when I had the opportunity to join Miguel B. Araújo's Ecophysiology project in El Ventorrillo Research Station (Spain) which focused on how lizards of different population throughout the Iberian Peninsula responded to changing climate conditions. Once again, space was a fundamental factor, as now I was incorporating inter-population geographical differences and its associated abiotic factors into my growing "ideabook". Both these previous experiences led me to participate in a project studying the Spatial Ecology of King Cobra (*Ophiophagus hannah*), cobras (*Naja kaouthia* and *N. siamensisi*) and Green Tree Vipers (*Trimeresurus macrops* and *T. vogeli)* in Sakaerat Environmental Research Station (Thailand) which confirmed that snakes were not only a much better group to study the use of space than turtles (after all, I often chased moving King Cobras by motorbike) but also that it was the group that raised me the most questions and that I wanted to work with.

The next step in my journey was therefore, obviously, one of the few places in the world (ten countries in total) where not a single species of terrestrial snake occurs. 'Why did I come here?' I asked, followed by 'Wait, why are there no snakes in New Zealand?' – a hint of what soon would come. Then, after a not-so-quick half-year journey as a beekeeper in New Zealand to raise funds, I started looking into a Ph.D. and wondering what I really was interested on, what I was invested in knowing more of. I knew I had to overcompensate for the lack of "snake-ing" in my recent past and thus I began pondering which biodiverse countries could quench this tropical snake thirst while also providing good higher education. When an offer came from my beloved Thailand to study the Spatial Ecology of Green cat snakes (*Boiga cyanea*), I reluctantly realized that I would not be satisfied with studying a single species in a single place and that my scientific horizon was now much broader, both spatially and taxonomically. I turned my head eastward and almost carelessly decided on Brazil as my next destination since it had it all – snakes, highly-respected universities and a common language (which apparently was not enough to make me write this thesis in Portuguese). Reaching out to Brazilian scientists (Laura Alencar, Cristiano Nogueira, Ricardo Sawaya and Marcio Martins – now co-authors of chapters of this thesis and/or other important works), I discovered that what I wanted to study actually had a name (who would have thought, right?): **BIOGEOGRAPHY**. At the time and due to my non-biology background, I was (and still am but less so) green on many scientific terms of biogeography, evolution and ecology. I clearly remember how I dozed off to sleep in the campervan I had irregularly parked on my way to the Franz Josef Glacier (New Zealand) thinking of the newfound word 'vicariance'. 'Is that why there were no snakes in New Zealand?" I thought.

And there it was: vicariance – the biogeographical process in which a barrier divides the ancestral distribution of a given species which over time decreases gene flow and leads to allopatric speciation. This reflects that barriers are more recent than the ancestral species distributions, contrary to dispersal that implies a barrier that precedes the range of ancestral taxa. Dispersal is thus when a given species overcomes an already existing barrier to a previously unoccupied area (a valley, an island, a new continent), also representing allopatric speciation. The dispersal to Brazil was an explosion of smell (feijoada, caipirinha, caldo de cana!), sound (Brazilians are much louder than Portuguese people) but mostly of ideas. The more I read about Neotropical snakes, biogeography and conservation, the more I wanted to incorporate different ideas. Eventually, my supervisor Cristiano Nogueira and I decided on applying 'Phylogenetic Diversity' (a metric I ended up not directly using) to the 'Conservation' (which is now more a consequence rather than a starting point) of 'cis-Andean

Dipsadidae snakes' (which started as only the 'Xenodontinae subfamily' but soon "overcame" the Andes to become 'South American Dipsadidae' then 'Neotropical Dipsadidae' to include Central America and finally 'Dipsadidae' as a whole to encompass both North American and Asian species). Like dispersal, a central aspect of both Spatial Ecology and Biogeography, I guess a thesis rarely follows an obvious straight line. However, rather than an obstacle, this proved to be a major opportunity to learn and to think 'outside of the box'. You see, Dipsadidae is no small group: it comprises over 700 species (Uetz et al., 2020), over 60 genera distributed in four major clades: Xenodontinae (mainly South American and originally my study group), Dipsadinae (widespread in the Neotropical region), Carphophiinae (which occur in North America) and Asian relicts (the genera *Thermophis* and *Sticophanes*) (Grazziotin et al., 2012, Zaher et al., 2019). The species of this family have a wide range of functional traits such as body size (from ~20 cm *Apostolepis* sp. to over two-meter *Clelia clelia*), habitat use (semi-fossorial *Atractus* to aquatic *Helicops* to arboreal *Xenoxybelis* to terrestrial *Xenodon*), color (the dull-colored *Omoadiphas aurula* to the colorful *Erytholamprus dorsocorallinus*, with some species such as *Oxyrhopus rhombifer* being coral-mimics) and diet (from spider specialists such as *Philodryas agassizii* to generalists such as *Philodryas olfersii*). Thus, by not being restricted to "solely" a subfamily in a portion of the South America continent, I was allowed to let these differences guide how I would study what they have in common: the historical biogeographical and ecology processes that shaped their impressive current diversity. In fact, I was deeply inspired by John Cadle's works (Cadle, 1985) and mainly by a chapter by John Cadle and Harry Greene titled "Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages" (Cadle & Greene 1993).

These works explore how the two predominantly Neotropical subfamilies (Xenodontinae and Dipsadinae) represent two sister lineages which have markedly distinct distribution patterns (therefore previously respectively referred to as South American xenodontines and Central American xenodontines). One of the main aspects they focus on, and the one that initially grabbed my attention, is the subfamilies' asymmetric richness and distribution within the Neotropics: while Dipsadinae is distributed from southern North America to most of South America, Xenodontinae is nearly almost solely restricted to South America and the West Indies (with a few exceptions that occur in Central America). The authors go on to explore different aspects of their ecology and how they may explain these patterns, while also posing the hypothesis that their ancestral distribution could hold the key to explaining part of the (hi)story. The authors extensively propose that distribution (and associated ecological traits) is strongly linked to phylogenetic aspects, even though at the time phylogenies were still scarce and mostly based only on morphological traits, with John Cadle being a pioneer in trying to reconstruct this group's evolutionary history (Cadle, 1985). These ideas led me to test whether these richness and distribution patterns could be explained by **Phylogenetic Niche Conservatism** (Wiens & Graham, 2005). Phylogenetic Niche Conservatism (PNC) is the tendency for the niche, a multidimensional aspect of a species ecology via its relationship with biotic and abiotic conditions, to be similar throughout the evolutionary history of a group. It leads to species being similar to their ancestral lineage (and often to extant related species) and often not being able to transverse ecological barriers thus being geographical limited to favourable regions. If PNC was indeed an important factor in determining the current asymmetric richness and distribution patterns of Dipsadidae, I would expect for Dipsadinae to be a forest specialist and thus being richer in the forests of Central America and Amazon but being able to disperse to forested austral regions such as the Atlantic Forest. On the other hand, if Xenodontinae is an 'open area' specialist it would be mostly constrained to South America due to a major ecological barrier posed by the extensive forests in lower Central America. This is what I test in Chapter 2, titled "One clade, two histories: Phylogenetic niche conservatism drives distribution patterns of Dipsadidae, the richest Neotropical snake family". But why is it Chapter 2 if it was the initial idea that kickstarted my project?

Well, it turns out that one key element that is also necessary to understand the above predictions was... the origin of both subfamilies. For the patterns to be strongly upheld by PNC, species would have to have an ancestral lineage in an area in which the niche corresponded to that of the extant species and thus Dipsadinae and Xenodontinae would have to have originated separately in Central and South America, respectively. And this was still formally untested, even though (once again) John Cadle and Harry Greene's works (Cadle & Greene, 1993) had set up a great foundation. Hypothesis for Dipsadidae's ancestral origin ranged from an ancient Gondwanan origin, dispersal from Africa or the most well-supported Asia to North America origin (Cadle, 1984; Cadle, 1985). Yet, it was also unknown whether Dipsadinae and Xenodontinae, strongly supported as sister clades, had originated in Central (or South America) followed by a later dispersal to a different landmass or if this split was due to vicariance caused by one of the several important geomorphological events that occurred in the Neotropical region (Cadle, 1985; Cadle & Greene, 1993). Perhaps even more importantly, information on **WHEN** this split had happened was yet not been available (Cadle, 1985; Grazziotin et al., 2012; Zaher et al., 2019). So, we took a step back from the former Chaper 1 (formally now Chaper 2) and aided by valuable suggestions by Tiago Quental, Renata Pardini and Felipe Grazziotin (who were the evaluating committee of my Ph.D.'s qualification exam), I aimed to reconstruct Dipsadidae's historical biogeography by building a dated phylogeny for this group and looking at its species distribution from outside the Neotropical region. This, a great collaboration with many authors – especially Matheus Pontes-Nogueira and Felipe Grazziotin – resulted in Chapter 1 "There and back again: when and how Dipsadidae, the richest Neotropical snake clade, dispersed and speciated throughout the Americas”. This went to show that (and slightly also spoiling the results), as previously stated, both **dispersal** and writing a thesis do not often follow a straight arrow.

Now that we had answered from where (and when) this group had originated and if the distribution patterns of its two main Neotropical subfamilies fitted with PNC, we still had one important question: how much of the distribution of species – especially within each subfamily – was due to vicariance? While Chapter 1 had markedly tested vicariance and dispersal at a broader taxonomic, temporal and phylogenetic scales and Chapter 2 had looked into mainly ecology and habitat use, we wanted to understand how fine-scale biogeographical processes could lead to species co-occurring in specific areas and if this could reflect the previous historical and ecological events. To do so, we used bioregionalization – delimiting a specific region based on its biota – by testing if we could detect areas of co-occurring species (which we call biotic elements, hereby BE; Hausdorf & Hennig, 2003). of Dipsadidae and if we could recovered overlapping regions for both subfamilies and the family as whole. This approach has two important premises: i) “pattern and process” - the resulting pattern of co-occurring distributions must differ from those which would be recovered if species ranges were randomly assembled and ii) “vicariance” - closely related species must occur in different BE. While the former attempts to incorporate a bit of “uncertainty measure” in order to properly do hypothesis testing (which is no easy feat in biogeography; McDowall 2004; Crisp et al., 2011), the latter infers that, for vicariant speciation to have taken place, an ancestral distribution of a given taxa must have resulted in two taxa with non-overlapping distributions separated by a barrier (Hausdorf & Hennig, 2004). While this has been typically done by only superficially testing whether species from the same genus (Hausdorf & Hennig, 2003; Nogueira et al., 2011) are in the same BE, I felt that was an oversimplification of what vicariance could achieve. First off, what is a genus? While there are differing degrees of “relatedness” across the tree of life, a genus is a somewhat abstract concept of attempting to put things in boxes and is often subject of changes (Grene, 1974). Secondly, species within a genus are not “phylogenetically equidistant” i.e. all species are equal but some are more equal than others since species from different clades within a genus might occur in the same BE by having being simultaneously split from their respective sister species without it “disproving” vicariance. Thirdly, monospecific genus (those with a single species) would be excluded from the analysis even though they do have (as all should) a phylogenetic relationship with other species or clades. Therefore, one my main goals in this thesis (and one of my biggest achievements *cough cough*) was the attempt to formally (albeit still in a preliminary approach) incorporate phylogenetic information into the second premise of Biotic Elements regionalization, since it has been done for other analysis with different approaches (Daru et al., 2017; Ficetola et al., 2021).

This thesis weaves a seemingly organized narrative that incorporates historical biogeography since the family’s origin (Chapter 1) to how this has influenced how current patterns of habitat use (Chapter 2) together with how barriers (which might also be ecological in nature) might have shaped its the evolutionary history (Chapter 3) and distribution in the Neotropical region. Furthermore, I show two examples of a strong association of distribution and habitat (Chapter 4), and how this may inform and impact conservation actions (Chapter 5). However, this overly long and convoluted story of how this thesis shows that its conceptualization and writing has been a iterative process of non-stop piling of ideas, facts, thoughts and “inspiration”, fuelled by many different excellent articles and authors which, consciously and subconsciously, have planted seeds in me. With that being said, I wish you luck in the next pages and hope that this work here may also help to sow other fields.

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2. Chapter 1: There and back again: when and how the richest Neotropical snake clade (Dipsadidae) dispersed and speciated throughout the Americas

Abstract

Dipsadidae, a megadiverse Neotropical snake clade, has a widespread distribution and occurs in a large range of diverse habitats, thus being an excellent model to understand the diversification of Neotropical biota. Herein, we investigate the origin and historical biogeography of Dipsadidae and test if its two main Neotropical subfamilies, Xenodontinae and Dipsadinae, have different geographical origins. We generated a Bayesian time-calibrated phylogeny, including 287 species of Dipsadidae, using BEAST and subsequently estimated ancestral areas of distribution. We compared the fit of the following models using BioGeoBEARS: DEC (subset sympatry, narrow vicariance), DIVALIKE (narrow and wide vicariance), BAYAREALIKE (no vicariance and widespread sympatry), and these same models with an added jump dispersal parameter. The two best models show that Dipsadidae originated approximately 50 million years ago (mya) in Asia and that dispersal was a remarkable process in its historical biogeography. The DEC model with jump dispersal indicated that this family underwent a range extension from Asia and posterior vicariance of North and Central America ancestors. Both Xenodontinae and Dipsadinae likely originated in Central America and dispersed to South America during Middle Eocene, but did so to different regions (cis and trans-Andean South America, respectively) and with different timings of dispersal. Xenodontinae entered cis-Andean South America around 40 mya and jump dispersed to the West Indies around 35 mya, while Dipsadinae entered trans-Andean South America multiple times 20 – 25 mya. Our results show that Dipsadidae has an Asian origin and that the two main Neotropical subfamilies originated in Central America, later dispersing to South America in distinct events in different time periods. The current biogeographical patterns of the family Dipsadidae, the most species rich snake clade in the Neotropical region, have likely been shaped by complex evolutionary and geological processes such as Eocene land bridges, Andean uplift and the formation of the Panama isthmus.

Keywords: ancestral area, dispersal, diversification, historical biogeography, Serpentes, vicariance

2.1. Introduction

The Neotropical realm is a climatically and geologically diverse biogeographical region, encompassing a wide range of habitats, from the lush rainforests of the Amazon and Central America to the snow-covered peaks of the Andes. This diversity of habitats is the result of a rich and complex paleogeographical history between and within two continental landmasses — Central and South America — and associated island systems (e.g., Galapagos, West Indies; Clapperton, 1993; Pennington et al., 2004; Rull, 2011; Hughes et al., 2013). Even though major geological events such as the Gondwana breakup and the formation of volcanic hotspots happened during the Mesozoic era (Jokat et al., 2003; Wilf et al., 2013), many geomorphological events relevant to modern-day Neotropical region occurred in the Cenozoic. These include mountain uplift in Central America and the Andes, the formation of the West Indies island system, a potential short-lived land-bridge connecting South America to the West Indies (the Greater Antilles and Aves Ridge, GAARlandia; Iturralde-Vinent & MacPhee, 1999; but see Ali & Hedges, 2021) and formation of the Isthmus of Panama, a contiguous landmass connecting Central and South America whilst separating the Atlantic and Pacific oceans (Graham 2009; Hoorn et al. 2010).

These geomorphological events and their abiotic and biotic consequences widely shaped the evolutionary history of the Neotropical biota, contributing for the Neotropics to be today the world's most biodiverse region (Antonelli & Sanmartin 2011; Rull, 2011). Therefore, Neotropical faunal assemblages reflect several distinct biogeographical histories. While some clades likely originated by mid-Cretaceous vicariant event between South America and Africa (e.g. boid snakes: Noonan & Chippindale, 2006; Iguanian and Scleroglossan lizards: Albino & Brizuela, 2014), others later overwater dispersed from Africa (e.g. Epictine threadsnakes: Adalsteinsson et al., 2009; Platyrrhine monkeys and Caviomorph rodents: Defler 2019; South American Amphisbaenidae: Graboski et al., 2022) or from Asia, via North America (viperid snakes: Wüster et al., 2008; turtles: Lichtig et al., 2019). Furthermore, the more recent Great American Biotic Interchange (GABI) promoted dispersal and faunal admixture between Central and South American fauna — mainly mammals and birds (Bacon et al., 2015; Defler 2019; South American Amphisbaenidae: Graboski et al., 2022) — despite some evidence of pre-GABI dispersal (Heinicke et al., 2007; Agnolin et al., 2019). Other groups, such as reptiles, are thought to have been less directly involved in GABI, mostly diversifying in Central America with later dispersal to South America with few groups doing the reverse path (Vanzolini & Heyer 1985).

Widely distributed taxa represent ideal models to study biogeographic processes in the Neotropics (Colston et al., 2013; Torres-Carvajal, et al., 2019; Azevedo et al., 2020). Snakes are exceptionally diverse in the Neotropical realm, where roughly one-third of all species occur (Guedes et al., 2017; Roll et al., 2017; Nogueira et al., 2019). Dipsadidae (Bonaparte, 1838) is the richest snake family in the Neotropics with over 700 known species, which are diverse in diet, habitat use, and morphology (Cadle & Greene, 1993; Serrano et al., in prep.). This high level of biological variation among dipsadids is reflected on the distributional patterns and the phylogenetic relationships within the family, making it a promising but scarcely explored model to evaluate biogeographic hypotheses of diversification (Grazziotin et al. 2012, Zaher et al., 2019). It comprises four well-known groups: the monophyletic and highly diverse subfamilies Dipsadinae and Xenodontinae, which are widespread in the Neotropical realm (Cadle & Greene, 1993); plus two relict groups, one distributed in North America that includes the subfamily Carphophiinae and the genera *Heterodon* and *Farancia* (Pinou et al. 2004), and another exclusively distributed in Asia composed of the genera *Thermophis* and *Stichophanes* (Huang et al., 2009; Grazziotin et al. 2012; Zaher et al., 2019).

Despite the uncertainty around the family's geographical origin, hypotheses of ancestral distribution have ranged from a Gondwanan distribution (Cadle, 1985), an Asian origin followed by a dispersal from Asia via North America (Cadle, 1985) and an African origin followed by a trans-Atlantic dispersal to South America (Cadle, 1984), possibly followed by a dispersal to North America (Duellman, 1979). Recent phylogenetic studies have supported an Asian-North American dispersal event based on the interpretation of the successive sister-group relationship between the Asian genera Thermophis and Stichophanes and the clade composed by American dipsadids (Grazziotin et al. 2012; Zaher et al., 2019). This Asian-North American dispersal event has been supposed even before the studies positioning of *Thermophis* and *Stichophanes* (Cadle, 1985), and it is frequently associated with the formation of the Beringian Bridge during the Miocene, around 16–10 mya. The same hypothesis is presented as the general biogeographical explanation for the presence of other snake families, such as Colubridae and Natricidae in the New World (Vidal et al., 2000, Pinou et al., 2004).

However, in recent studies, the estimated divergence between American and Asian dipsadids is older than the Miocene. Zaher et al. (2018; 2019) estimated this divergence between 22 mya and 27 mya, around the transition between the Oligocene and Miocene. Other studies have suggested older dates, pointing to a divergence between Asian and American dipsadids dated in the transition between the Eocene and Oligocene

(Entiauspe-Neto, et al. *in press*). An alternative hypothesis supporting pre-Miocene divergence times is related to cladogenic events as the opening of the Greenland corridor approximately 48 mya.

Within the diversity of dipsadids, some studies restricted to small groups of species (e.g. Leptodeira: Daza et al., 2009; Imantodini: Mulcahy, 2007; Thermophis: Huang et al., 2009) have only reconstructed recent biogeographical patterns and attained some estimates of divergence times but achieved inconclusive results regarding the ancestral range distribution and biogeographic processes of the main groups. Since the classical studies of Cadle (1984a, 1984b, 1984c), the evolutionary history of the two major dipsadid subfamilies has been understood as reflecting independent origins and processes of diversification. Following Cadle's hypothesis, Dipsadinae originated in Central America, where the subfamily diversified and further dispersed to South America. Xenodontinae, on the other hand, would have originated and diversified in South America, and from there, dispersed to Central America. Although Duellman (1979) suggested a different scenario—a common South American origin for Dipsadidae and further dispersal to Central and North America — the hypothesis provided by Cadle was well accepted by the herpetological community, and it has been supported by further studies (Cadle & Greene, 1993; Vidal et al., 2000; Zaher et al., 2009; Hedges et al., 2009; Vidal et al., 2010; Grazziotin et al., 2012; Zaher et al., 2018; Zaher et al., 2019). Cadle also suggested that the divergence between both subfamilies had happened during the late Palaeocene–Eocene separation of Central and South America, around 40–60 mya (Cadle, 1985). However, recent studies have estimated divergence times between Dipsadinae and Xenodontinae varying around 19 mya and 24 mya, during the Late Miocene (Zaher et al. 2018; 2019).

Zaher et al. (2019) also suggested that the sister group affinities retrieved between Diaphorolepini (an exclusive South American tribe) and the remaining Dipsadinae, on the one hand, and Conophiini (an exclusive Central American tribe) and the remaining Xenodontinae, on the other hand, points to a complex historical scenario of origin and diversification of the two main Central- and South-American dipsadid lineages than previously thought (Cadle, 1985; Cadle & Greene, 1993). Therefore, both the family's origin and its overall biogeographical history, such as timing and route of dispersal between Central and South America, remains uncertain.

Historical biogeography (Posadas, Crisci & Katinas, 2006) is an essential tool to understand the origin and composition of current Neotropical biotas such as snake assemblages since biogeographical processes such as dispersal, vicariance, and extinction strongly influence local and regional biodiversity through time (Ricklefs, 1987, Moritz et al., 2000, Crisci, 2001). However, comprehensive studies on Neotropical historical biogeography have been severely hampered by the lack of detailed phylogenetic hypotheses and distributional data (Bagley & Johnson, 2014) as well as analytical limitations (Landis et al., 2013; Matzke, 2013). Despite information available on the distribution, richness and phylogenetics of diverse groups such as snakes has increased e (López-Aguirre et al., 2018; Nogueira et al., 2019, Azevedo et al., 2020), their historical biogeography is complex and still poorly understood.

Here, we generate and use a comprehensive time-calibrated phylogeny and a Bayesian estimation of the ancestral geographical ranges aiming to: (1) infer the most likely distribution of ancestral lineages of Dipsadidae, (2) reconstruct the historical biogeography of dipsadid snakes in the Neotropical region; and (3) complement the current knowledge of paleogeographical scenarios related to the diversification and current patterns of distribution of dipsadids in Central and South America. Specifically, we tested the hypotheses that: i) Dipsadidae had an Asian origin with dispersal via North America; and ii) Dipsadinae and Xenodontinae — the two Neotropical subfamilies — have different geographical origins (Central and South American, respectively).

2.2. Materials and Methods

2.2.1. Phylogenetic tree

We based our phylogenetic analysis on the molecular dataset from Zaher et al. (2018), the most complete and up-to-date available dataset considering the diversity of Dipsadidae. The concatenated matrix included DNA sequences of six genes (12S, 16S, cytb, bdnf, c-mos, and nt-3) for 344 species representing the families Dipsadidae, Pseudoxenodontidae, Colubridae, Calamariidae, Sibynophiidae, Grayiidae, Natricidae, Viperidae, Pareatidae, and the superfamily Elapoidea. The boids *Eryx conicus* and *Boa constrictor* were included to root the phylogenetic tree. The dataset is largely biased towards Dipsadidae (287 species, 83.4% of species in the phylogeny), with 283 New World species (84 genera), of which 10 (five genera) belong to the subfamily Carphophiinae, 167 (54 genera) to Xenodontinae, and 106 (23 genera) to Dipsadinae. The Asian incertae sedis Dipsadidae genera *Thermophis* (three species) and *Sticophanes* (one species) are also included in the molecular dataset to allow the estimation of the origin and early evolution of South American dipsadids. Overall, our sample of Dipsadidae represents nearly a third of all valid species for this family (Uetz et al., 2020).

To determine the optimal partitioning scheme and nucleotide substitution models of DNA, we used PartitionFinder v2.1.1 (Lanfear et al., 2012). We previously partitioned our concatenated matrix based on gene fragments and we tested all models implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) through Bayesian Information Criterion (BIC), while using the 'greedy' algorithm (Lanfear, 2012).

We performed a time-calibrated Bayesian inference analysis to estimate divergence time within Dipsadidae in MrBayes 3.1.2. We defined a set of topological constraints based on the topology presented by Zaher et al. (2018) to reduce the tree space and decrease the running time of our analysis. The set of topological constraints is listed in the nexus file. Node calibration points were defined based on the fossil record and we used similar ages and fossil interpretations as described by Zaher et al. (2018) and Zaher et al. (2019).

We set the branch length prior as a birth-death clock model (Yang and Rannala, 1997), with speciation and extinction probabilities set to exponential (lambda = 10) and beta (alpha = 1 and beta = 1) distributions, respectively. We divided the total number of terminals in our molecular matrix by the approximate total number of extant alethinophidians (Uetz et al., 2020) and we set the sample probability to 0.109. For the model of variation of the clock rate across lineages, we used the independent gamma rates (IGR) model (Ronquist et al. 2012) with the parameter IGRvar — the amount of rate variance across branches — set to the exponential (lambda = 10). To set the clock rate, we followed Pyron (2017), and we used a log normal distribution with a mean corresponding to the log of the average number of substitutions per site from root to tips estimated from the tree provided by Zaher et al. (2018), divided by the mean root age (-3.295561). The standard deviation for the log normal distribution was set as the exponent of the mean (1.037742).

We implemented this analysis in two independent runs with eight Markov Chains Monte Carlo (MCMC, one cold and seven incrementally heated) and 50 million generations. To generate the 50% majority rule consensus tree, a conservative burn-in of 25% was applied after checking the log-likelihood scores and the split-frequencies of the runs, and all sampled trees prior to reaching these generations were discarded.

Clades with support values ≥ 0.85 were considered well-supported. We combined the resulting trees from the two runs using the sumt command in MrBayes, and eventual polytomies were randomly solved by adding small branch-lengths (0.0001) using functions from the 'ape' package (Paradis & Schliep, 2019) in R 3.5.2 (R Core Team, 2019). The complete time-calibrated Bayesian tree was pruned to Dipsadidae to implement further historical biogeographical analysis.

2.2.2. *Biogeographical analysis and ancestral range estimation*

We used previously-defined biogeographical regions to delimit areas and considered the ‘Mexican transition zone’ as the limit between North and Central America since it separates the Nearctic and Neotropical regions (Morrone, 2010; Morrone et al., 2017), and northern Nicaragua as the limit to Central America because it represents the austral border of the Mesoamerican Dominion (Morrone et al., 2014) and its southern portion (Panama, Costa Rica and southern Nicaragua) is much younger than its northern portion due to their different geological histories (Bacon et al., 2015; O’Dea et al., 2016;). Since we aimed to understand only major biotic exchanges between insular and continental landmasses, the West Indies were treated as a single area to decrease the number of biogeographical units and consequently the models’ running time.

We considered six biogeographical units (Fig. 1a), assigning each species distribution to one or more than one of them: (A) Asia, (B) North America (American continent north of the Trans-Mexican Volcanic Belt), (C) Central America (from the Trans-Mexican Volcanic Belt to northern Nicaragua), (D) the West Indies, (E) Trans-Andean South America (from western slopes of the Andes to the Pacific Ocean shore) and (F) cis-Andean South America (from eastern slopes of the Andes to the Atlantic Ocean shores). We constrained the maximum number of occupied units to three, since none of the extant species occurs in more than three areas.

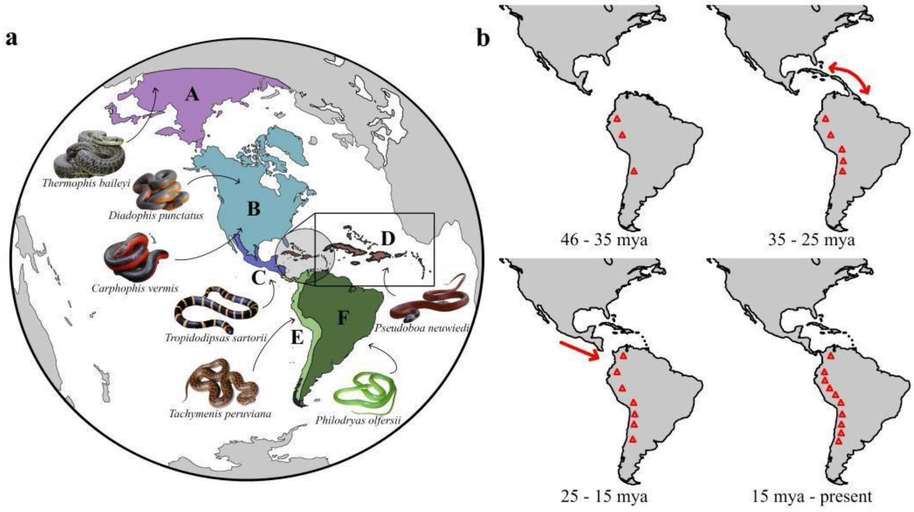

Figure 1 - a) Biogeographical units considered in this study and their representative species; A - Asia, B - North America, C - Central America, D - West Indies, E - trans-Andean South America and F - cis-Andean South America. b) Relevant geomorphological events in the

Neotropical region since the Eocene Epoch (56 to 33.9 million years ago - mya). Red arrows represent land connections and red triangles represent increasing elevation in the Andes.

We estimated the ancestral ranges for Dipsadidae using the *BioGeoBEARS* package (Matzke, 2013) in R 3.5.2 (R Core Team, 2019), using variations of the likelihood models DEC (Dispersal-Extinction-Cladogenesis; Ree & Smith, 2008), DIVA-like (Dispersal-Vicariance Analysis; Ronquist, 1997) and BayArea-like (Bayesian Inference of Historical Biogeography for Discrete Areas; Landis et al., 2013). The DEC (Dispersal-Extinction Cladogenesis — Ree & Smith, 2008; Matzke, 2013) model assumes that derived lineages following cladogenesis can only inherit a single range area, which is a subset of their ancestor's range; DIVAlike (Ronquist & Sanmartin, 2011) which allows vicariant events, but does not allow for sympatric-subset speciation by derived lineages. BAYAREAlike (Landis et al., 2013), on the other hand, assumes that no range evolution occurs at cladogenesis, and derived lineages inherit the same range of the ancestral state, making it a heavily dispersalist model.

Although we tested all models implemented in *BioGeoBEARS*, we acknowledge that statistical comparison among models without incorporating subjective biological knowledge can favor models that, despite increasing the data likelihood, do not necessarily incorporate the most probable historical scenario (Sanmartín 2021). We assume that for an old (probably more than 40 my old) wide dispersed taxa (four continents) like Dipsadidae, evolution by vicariance needs to be considered in biogeographical models, even if it occurs at a low rate. Therefore, we maintained BAYAREAlike models in our analysis only to test the relative importance of scenarios mainly driven by dispersal (see results below), but we base our main discussion on the best models that allow vicariant processes.

We furthermore compared the above models with the added +j parameter, which allows founder-event speciation and was added due to its potential importance in reconstructing insular historical biogeography (Klaus and Matzke; 2020; Matzke, 2022; but see Ree and Sanmartín; 2018). To each model we also added a time-stratified matrix with dispersal probabilities between pairs of areas specified based on geological events occurring in each period (Fig. 1b), varying between 0.1 (unlikely), 0.5 (probable) and 1 (likely). For this matrix we considered potentially relevant events (Figure 1b) at 46 mya [million years ago] (origin of the clade), 35 mya (potential uplift of GAARlandia or stepping stone islands; Iturralde-Vinent & MacPhee,1999), 30 mya (disappearance of GAARlandia or stepping stone islands; Iturralde-Vinent & MacPhee,1999), 25 mya (approximation of the Central American and South American tectonic plates; Montes et al., 2012) and 15 mya (complete formation of the Panama Ishtmus; Bacon et al., 2015 but see O'Dea et al., 2016). All models were implemented in the Maximum Likelihood framework of BioGeoBEARS, (Matzke, 2013). In total, we implemented six Maximum Likelihood models which were compared via Akaike information Criterion – AIC (Akaike, 1974; Wang, 2006).

2.3. Results

2.3.1. Phylogeny and Divergence time estimation

Our phylogeny (Fig. 2) suggests a crown age of Colubroidea of 56.6 my (49.2-63.7 my 95% HPD), with the main split between Dipsadidae — strongly supported as monophyletic — and the remaining Colubroidea occurring in Mid Eocene approximately 49.1 mya (44.1-55.4 mya 95% HPD). The split between Asian and American Dipsadidae occurred at 44.9 mya (40.1-50.2 mya 95% HPD), with the more species-rich Neotropical Dipsadidae splitting from the North American Carphophiinae clade at 43.1 mya (38.2-47.3 mya 95% HPD). Both Xenodontinae and Dipsadinae were strongly recovered as monophyletic, while Carphophiinae was recovered as polyphyletic. While most clades within Xenodontinae were well resolved (bar the *Erythrolamprus* and *Helicops* genera and the Tachymenini tribe, for instance), several clades within Dipsadinae showed low to moderate support with the most noticeable being the Dipsadini tribe and the *Atractus* + *Geophis* clade.

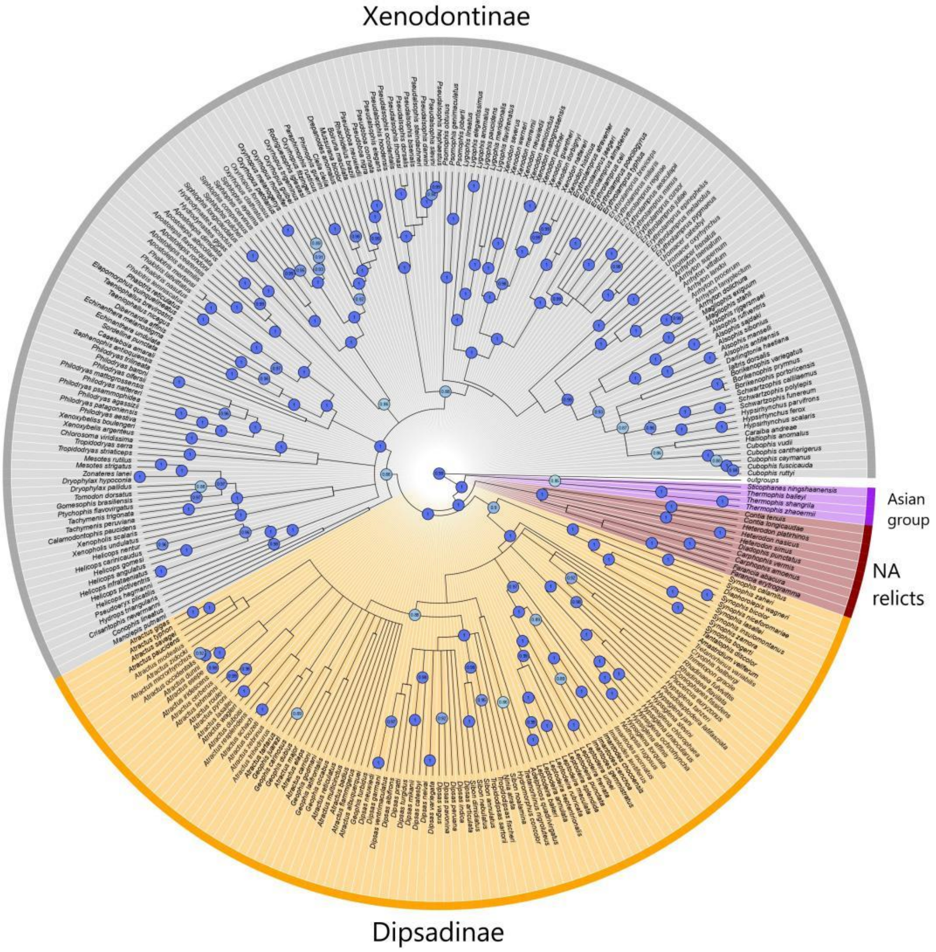

Figure 2 - Time-calibrated Bayesian consensus phylogeny of Dipsadidae, with major groups represented: Xenodontinae (grey), Dipsadinae (orange), Carphophiinae (North American relicts, dark red) and Asian dipsadids (purple). Blue circles indicate statistical support for nodes > 85%.

3.1.2. Ancestral range estimation

The best fitted model was BAYAREALIKE +j (AICc = 619.9), followed by DEC +j (AICc = 647.2). The best model being BAYAREALIKE highlights the importance of dispersal for this snake clade, especially since the founder-event parameter was present in the three best models. It also highlights that the anagenetic processes and range heritage were more important in the evolution of the dipsadids than the cladogenetic processes. However, as stated before, since BAYAREALIKE does not considers cladogenetic processes such as vicariance, we illustrate the historical biogeography of Dipsadidae with DEC +j. The most recent ancestor of Dipsadidae likely occurred in Asia, splitting from its sister groups (herein represented by *Pseudoxenodon macrops*) during the Early Eocene. The clade's extension of distribution to the New World (current North America and Central America) was then followed by a vicariant event between the Asian dipsadids and the American clade around 44.6 mya (40.1 – 50.2 mya 95% HPD) (Fig. 3). In the Mid Eocene, around 42.8 mya (37.6 – 48.6 mya 95% HPD), there was another vicariant event splitting the Carphophiinae subfamily in North America and the ancestor of the speciose Neotropical dipsadids in Central America. From then, around 42 mya, the two current major Neotropical subfamilies underwent distinct biogeographical processes. For Xenodontinae, a small lineage remained in Central America (Conophini), while the ancestor of the subfamilly dispersed into cis-Andean South America via jump dispersal. The ancestor lineage of Dipsadinae remained in Central America, with a further jump dispersal by the ancestor of the small lineage Diaphorolepidini to trans-Andean South America around the Eocene - Oligocene transition. Thereafter, Xenodontinae mainly maintained a cis-Andean distribution, except for the Alsophini clade, which underwent a major jump dispersal event to the West Indies during the early Oligocene, around 33.0 mya. The subfamily Dipsadinae, on the other hand, underwent many relevant biogeographical changes, especially since 30.7 mya, where the *Hypsiglena* + *Pseudoleptodeira* clade majorly reverted its distribution to North America. Compared to Xenodontinae, the occupation of South America by previously Central American dipsadines occurred much later, during the Oligo-Miocene transition, and by several jump dispersal events: at around 25.4 mya for the tribe Dipsadini and at around 22.3 mya for the genus Atractus. Overall, range extensions (e.g. range extension of a trans-Andean species to Central America) occurred at more recent times during Late Miocene and mainly within the subfamily Dipsadinae. Major events are summarized in Fig. 4.

Table 2 - The best-fitted models of ancestral range estimation of Dipsadidae with BioGeoBEARS, all including a transition matrix. Model comparison based on log-likelihood (LnL), the corrected Akaike information criterion; n, number of parameters; d, rate of dispersal; e, rate of extinction; j, relative probability of founder-event speciation. The best model is shown in bold.

Model	LnL	n	d	e	j	AICc
BAYAREALIKE +j	**-306.9**	**3**	**0.0035**	**0.0003**	**0.039**	**619.9**
DEC +j	-320.6	3	0.0055	1.00E-12	0.03	647.2
DIVALIKE +j	-332.6	3	0.006	1.00E-12	0.033	671.3

DEC	-340.6	2	0.0079	1.00E-12	0	685.3
DIVALIKE	-350.2	2	0.0095	1.00E-12	0	704.4
BAYAREALIKE	-370.6	2	0.0046	0.016	0	745.2

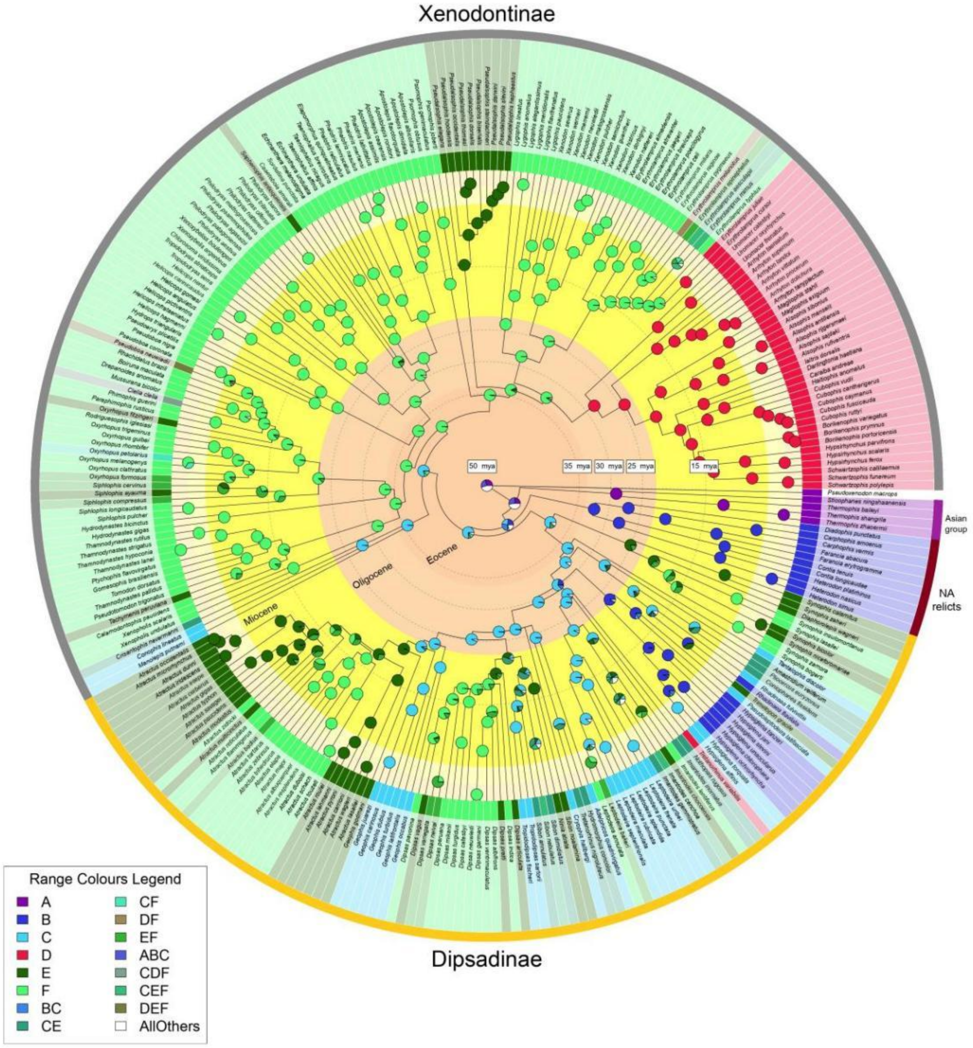

Figure 3 - Ancestral area estimations from the DEC+j model implemented in BIOGEOBEARS. The most probable ancestral areas are mapped by pie charts at each node and the actual occurrence of each specie is colour coded next to the species name (see legend). Orange and yellow-ish circles inside the phylogeny indicate geological epochs (Miocene, Oligocene and Eocene are named). Dashed circles represent the time divisions present on the time-stratified matrix, with time in millions of years ago (mya) indicated at the white boxes.

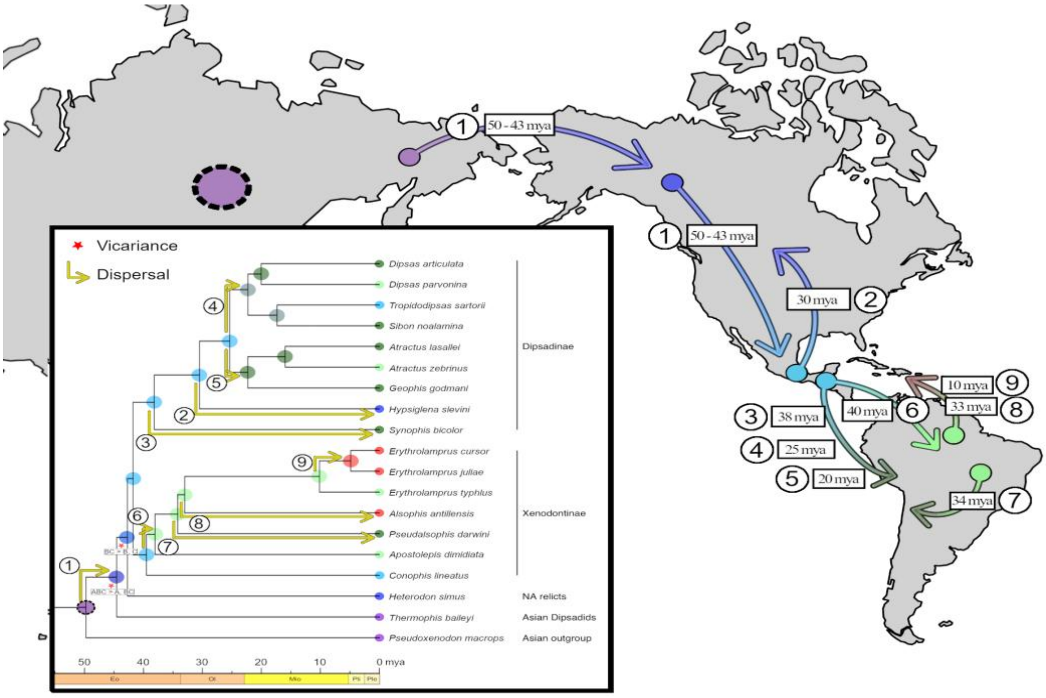

Fig. 4 - Summary of major biogeographical events of Dipsadidae. The purple circle represents the likely origin of the family, while arrows represent dispersal within the family at different time periods between regions. Inset: summarized phylogeny for representative taxa with numbered relevant dispersal and vicariant events.

2.4. Discussion

Overall, we reconstruct the complex biogeographical history of the family Dipsadidae, the most species rich clade of Neotropical snakes and an important component of Neotropical biodiversity. Our results show that Dipsadidae has an Asian origin, corroborating our first hypothesis, and that the two main Neotropical subfamilies likely originated in Central America, contrary to our second hypothesis.

2.4.1. Origin of New world dipsadids

Our findings strongly corroborate an Asian origin for dipsadids, as previously suggested (Cadle, 1984c; Grazziotin et al., 2012), and thus challenge studies that suggested that the Dipsadidae could have an African or Gondwanan origin (Cadle, 1985) or that Dipsadidae could have dispersed from South America to North America during its early diversification (Duellman, 1979). This origin is also consistent with the current distribution of its closest clades, including the Asian Pseudoxenodontidae and Natricidae. In spite of being almost globally widespread (i.e. occurring in the Paleartic, Neartic and Afrotropical regions) (Uetz et al., 2020), Natricidae is mostly absent from the Neotropical region and its ancestral distribution is Asian (Deepak et al., 2022). This validates our first hypothesis of an Asian origin for the Dipsadidae and subsequent dispersal to North America, possibly via the Beringia Land Bridge. This land bridge is estimated to have connected the Paleartic and Neartic realms during the Eocene (33-55 mya; Wolfe, 1975; Baskin & Baskin, 2016), being covered by warmer boreotropical forests which would have been suitable for ectotherms (Sanmartín et al., 2001; Townsend et al., 2011; Baskin & Baskin, 2016; Graham, 2018). This dispersal pattern is coeval with other squamate taxa (Dibamid lizards: Townsend et al., 2011) and similar, albeit earlier than, coral snakes (Kelly et al., 2009), lampropeltine rat snakes and crotaline vipers (Wüster et al., 2008; Burbrink et al., 2012). Alternatively, Dipsadidae could have reached North America from Asia via North Atlantic Land Bridges, especially the Thulean bridge, which were also present at the time of their origin (Tiffney, 1985; Jian et al., 2019). The Thulean land bridge connected southern Europe to Greenland, which in turn was connected to eastern North America and was available throughout the Early Tertiary until its submersion approximately 50 mya (Tiffney, 1985; Jian et al., 2019). Both plants and vertebrates have been suggested to have migrated via climatically suitable forest-covered North Atlantic Land Bridges (Sanmartín et al., 2001; Jian et al., 2019). However, dispersal via the Thulean bridge would imply that Dipsadidae once occupied and then went extinct in most of the Eurasian continent. While fossils associated with Dipsadidae (Paleoheterodon and Heterodon) have been described from southern Europe and North America, these are dated to Miocene/early Pliocene and could likely be a posterior incursion of North American fauna into Europe via the North Atlantic Greenland-Faroes bridge. Therefore, while both dispersal routes are possible, it likely that the geographically closer Beringia bridge likely provided a more suitable intercontinental dispersal route, as also suggested for other reptiles (e.g. Chen et al., 2013; Townsend et al., 2011).

2.4.2. The distinct processes shaping the diversity of dipsadines and xenodontines

We show that the main cladogenetic event originating both Neotropical subfamilies of Dipsadidae (Dipsadinae and Xenodontinae) must have occurred in Central America, prior to their dispersal to South America, as hypothesized for different clades of the Neotropical herpetofauna (Vanzolini & Heyer, 1985). Thus, our results rejected the hypothesis of different geographical origins for Dipsadinae and Xenodontinae as suggested by Cadle & Greene (1993).

The DEC + j model shows that Dipsadidae has dispersed to South America several times during its diversification. Both subfamilies originated and begun to diversify in the Middle Eocene, when a major increase in temperature - the Middle Eocene Climatic Optimum or MECO - took place, which has been shown to have increase the diversity of plants and mammals (Woodburne et al., 2014; Fernandez et al., 2021). Numerous other significant intercontinental faunal dispersals have been documented for this period for many vertebrates (Beard et al., 1994; Chaimanee et al., 2012). Furthermore, both Neotropical subfamilies, despite first entering South America quasi-simultaneously around 40 mya, have different biogeographical histories, despite the common biogeographical origin. Xenodontinae likely incurred in a single colonization through jump dispersal to South America by a Central American ancestor in the Middle Eocene (~ 40 mya), that was followed by quasi-isolation of the group in the region (Simpson, 1980; Cadle 1985). The exceptions to this isolation are dispersing lineages that returned to Central America and/or dispersed to the West Indies, including the jump dispersal by the Alsophini clade (Fig 3). The Dipsadinae subfamily also showed a jump dispersal event to South America from a Central American ancestor, albeit at a later period (23 mya). The time frame of these dispersal events from Central to South America indicated by our results (between 35 and 45 mya) is not congruent with paleogeographical reconstructions of a contiguous connection of the two continents, which suggested a large seaway separating the two landmasses (Montes et al., 2012, but see Coates & Stallard, 2016). Although this seaway likely represented a major obstacle to biotic interchange, the migration rate between the two continental masses has already been shown to have significantly increased around 41 mya (Bacon et al., 2015). Long-distance rafting and over-water dispersal from continental landmasses could explain such dispersal events (O’Dea et al., 2016), especially stepping-stone dispersal via islands in the present-day Caribbean Sea, as suggested for other species (ants: Archibald et al., 2006; butterflies: Condamine et al., 2012; carnivorous plants: Ellison et al., 2012). Even though most islands of the West Indies were not above sea level before about 40 mya for Greater Antilles and 15 mya for Lesser Antilles (MacPhee & Iturralde-Vinent, 1994; Iturralde-Vinent 2006), it is still possible that other existing island chains facilitated dispersal (Iturralde-Vinent & MacPhee 1999). For instance, as it moved eastward, the Caribbean plate’s leading edge might have provided an island corridor — the proto-Greater Antilles — which allowed for dispersal (albeit probably limited) between Central America and South America during the Middle Eocene, approximately since 49-45 mya (Iturralde-Vinent and MacPhee 1999; Ali, 2012; Roncal et al., 2020). Additionally, other proposed paleogeographical scenarios such as ‘GrANoLA’ — a Greater Antilles-Northern Lesser Antilles intra-oceanic subaerial connection (Philippon et al., 2020) — might also have played a role in the dispersal of dipsadid snakes from Central to South America, via continental islands (Cornee et al., 2021). Despite these ephemeral landmasses not being present in our analyses due to their disappearance (Iturralde-Vinent & MacPhee, 1999) and consequent lack of dipsadid records, jump dispersal likely played a role in the biogeographical history of this group, as supported by the +j (founder event) parameter in the best models.

Most lineages from the subfamily Xenodontinae diversified outside Central America and in the last million years in cis-Andean South America. One example is the tribe Alsophini (Xenodontinae) which dispersed to and subsequently diversified in the West Indies during the Eocene-Oligocene transition (ca. 34 mya), which confirms that most of this insular extant fauna is of South American origin (Agnolin et al ., 2019; Crews & Esposito, 2020), as previously suggested for Alsophini (Hedges et al., 2009). This pattern and time frame are perfectly congruent with the GAARlandia scenario (Iturralde-Vinent & MacPhee, 1999). While the existence of GAARlandia has been increasingly questioned due to conflicting geological and paleo-oceanographic data (Ali, 2012; Ali & Hedges, 2021), several taxa with different dispersal abilities have been shown to have dispersed to the West Indies during this period such as giant sloths (Delsuc et al., 2019), arthropods (Crews & Esposito, 2020), and freshwater fishes (Říčan et al., 2013). However, in spite of the congruent temporal window, it is still possible that West Indian xenodontines were the result of successive dispersal across the non-contiguous Aves Ridge, as suggested by the jump dispersal model and other taxa with similar patterns (Crews & Esposito, 2020, but see Ali & Hedges, 2021). Over-water dispersal seems to also be the process responsible for the more recent (~ 10 mya) dispersal of *Erythrolamprus juliae* and *E. cursor* into the Lesser Antilles, since these islands are younger than 15 mya (Iturralde-Vinent, 2006), and thus long after GAARlandia had emerged and disappeared, as also shown for Corallus boids (Henderson & Hedges, 1995).

The timing of a contiguous land bridge, the Panama isthmus, between Central America and South America has been a hot debate topic among geologists, ecologists and biogeographers, with recent studies providing evidence that it likely occurred before the Late Miocene (~ 10 mya) — much earlier than previously thought (~ 3.5 mya; see Bacon et al., 2015; Buchs et al., 2019). While dipsadids entered South America before the earliest estimates of the formation of the Panama isthmus, there is evidence of recent expansion to and from Central America, coincident with other two significant increases in migration rate (Bacon et al., 2015). This expansion occurred mainly for dipsadines between 12 and 9 mya, with several genera (e.g. *Sibon* and *Imantodes*) reaching trans-Andean South America. This is also true for xenodontines (e.g. *Oxyrhopus* and *Erythrolamprus* genera at around 5 mya) which underwent the inverse path more recently — expanding from the cis-Andean region to the trans-Andean region and Central America, with occasional dispersal to the West Indies. Further studies might focus on the processes behind this pattern, especially if differences in phylogenetic niche conservatism for habitat or other ecological aspects might have played a role in this extension, as some species have marked habitat-associated distributions (Serrano et al., 2020).

Regarding the late incursions of dipsadines from Central America into South America, different processes may be involved, as show for two closely related clades in close temporal proximity: the tribe Dipsadini at around 25 mya, and the speciose genus *Atractus* at around 23 mya. The ancestor of both theses clades was Central American but our results suggest that the ancestor of Dipsadini first extended its distribution to South America and later underwent vicariance, while *Atractus* most likely jump dispersed. Even though there was no contiguous landmass connecting the two continents at that time, other proposed hypotheses might explain how these two clades entered present-day trans-Andean South America: stepping-stone dispersal by volcanic island chains and/or over-water dispersal, both facilitated by the collision of the Choco block with the South American continent (North Andean block; Bacon et al., 2015; Buchs et al., 2019) in the Early Miocene, at around 25-23 mya, corroborated by thermochronology and changes in geochemical profiles (Farris et al., 2011). Furthermore, this aligns with another significant increase in migration rates between the two continents (Bacon et al., 2015). While the exact timing for a contiguous terrestrial connection between Central America and South America is disputed (O'Dea et al., 2016, but see Jaramillo et al., 2017; Molnar, 2017), the formation of a land bridge is a complex and gradual process which might have allowed for over-water or stepping-stone dispersal into present-day trans-Andean South America over time, as suggested for other taxa (O'Dea et al., 2016), including dipsadid snakes of the genus *Leptodeira* (Daza et al., 2009).

The collision of the Choco and North Andean blocks in Early Miocene allowed for biotic dispersal between the two continental masses, and also triggered important geological changes in South America: increased Andean orogenesis and propagation of the Llanos basin (Farris et al., 2011; Mora et al., 2020). While exhumation of the Andes had been likely occurring in some portions since the Late Cretaceous (~ 100 mya; Avellaneda-Jiménez et al., 2020), uplift in its northernmost portions (e.g. the Central and Western Cordilleras) significantly accelerated in the Miocene, at ca. 23 mya (Hoorn et al. 2010; Chen et al. 2019). As a consequence, diversification increased for several plant and animal taxa and the Dipsadinae were no exception. Our results show that the early diversifications of the tribe Dipsadini and the genus *Atractus* are congruent with peak uplifts in early Miocene (~23 mya), similarly to Aromabatidae frogs (Boschman & Condamine, 2021) and clearwing butterflies (Elias et al., 2009), even though a large portion of the Andes was at half its present elevation (Gregory-Wodzicki, 2000). An increasing geographical and genetic isolation likely occurred for species with cross-Andean distributions imposed by Andean uplift that subsequently led to a pattern of coeval cis-Andean/trans-Andean vicariant events in Dipsadidae – within the *Atractus* genus at 11 mya, as previously suggested (Passos et al., 2008) – and in Xenondontinae, in the Siphlophis genus (~ 8 mya), as well as for Neotropical pitvipers (Pontes-Nogueira et al., 2021). The Andean uplift may have indirectly contributed to speciation by altering climate and environment in pan-Amazonia (Hoorn et al., 2010), as such events have been shown to be strong drivers of diversification in the region (Pinto-Ledezma et al., 2017; Rangel et al. 2018; Vasconcelos et al., 2020), especially for ectotherms (Santos et al., 2009; Esquerré et al., 2019; Meseguer et al., 2021). However, further intense pulses of Andean Mountain building in middle Miocene (~12 mya) and early Pliocene (~4.5 Ma) coincide with potential cis-Andean/trans-Andean dispersal in xenodontine clades (in the genera *Oxyrhopus, Siphlophis* and *Erythrolamprus*) as well as increased speciation in *Atractus*. These direct and indirect effects of mountain uplift corroborate the role of the Andes as a "species pump", increasing species diversification into surrounding environments such as the Amazon and the Choco (Rangel et al., 2018, Rahbek et al., 2019).

Our results show that current biogeographical patterns of the family Dipsadidae, the most species rich snake clade in the Neotropical region, have been shaped by complex evolutionary and geological processes. Our reconstructed model recovered an Asian origin for the Dipsadidae family and potential significant paleogeographical events such as Eocene land bridges, Andean uplift and the formation of the Panama isthmus. While both dipsadines and xenodontines originated in Central America, they showed different evolutionary and biogeographical trajectories since they have dispersed into South America at different time periods and in two different regions: trans-Andean and cis-Andean South America. This is likely responsible for not only their present distribution, co-occurrence and regionalization patterns but also for relevant differences in their ecology and richness and may help to explain why both these two Neotropical subfamilies are much richer than their Asian and North American counterparts (Cadle & Greene, 1983; Serrano et al., in prep). Our results allow for a better understanding of the historical biogeography of the Neotropical region and how important events have shaped its biota.

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3. Chapter 2: One clade, two histories: Phylogenetic niche conservatism drives distribution patterns of Dipsadidae, the richest Neotropical snake family

Abstract

Species niche can strongly affect their distribution and subsequently shape large-scale richness patterns. As taxa speciate, descendant species often tend to inhabit similar ecological niches, referred to as ‘Phylogenetic Niche Conservatism’. One of the most important niche components is habitat type and thus forest and open ecoregions may show different richness patterns. Herein we map richness and habitat use for Dipsadidae, the richest snake clade of the Neotropics, and its two main subfamilies Xenodontinae and Dipsadinae. We furthermore test the roles of phylogenetic niche conservatism on asymmetric diversity patterns between Central and South America. We hypothesize that a significant difference in habitat type between these two lineages, with Dipsadinae being a clade of forest specialists and Xenodontinae a clade of open area specialists. We mapped richness and distribution of 492 species across both subfamilies in a 0.25º x 0.25º km grid. We then mapped relative richness (percentage of Dipsadinae in total Dipsadidae richness) per grid cell. We classified calculated the percentage of forest habitats in each species range. We then estimated ancestral states of habitat use for Dipsadidae and both its subfamilies to test for phylogenetic niche conservatism by comparing the rates and parameters of macroevolution models. Both subfamilies attain higher absolute richness in forests, but regions such as Amazonia and the Atlantic Forest are relatively richer in Dipsadinae than adjacent open areas such as the semi-arid Caatinga or the Cerrado and Guianan savannas. Overall, the ancestral lineage of Dipsadidae was likely a habitat generalist. Dipsadinae is composed of mainly forest-specialist species, while Xenodontinae is mostly a generalist clade. Habitat has been a major driving force in the Dipsadidae family, but only for the Dipsadinae subfamily. The low richness of Xenodontinae is likely not caused by phylogenetic niche conservatism since this clade is a habitat generalist. Therefore, the current large-scale biogeographical patterns of the richest snake family in the Neotropical region likely arose from clade-specific responses to major patterns of landscape and habitat evolution.

Keywords: ancestral; dispersal; historical biogeography; habitat.

3.1. Introduction

The ecological niche of species – the n biotic and abiotic conditions in which they thrive (Hutchinson, 1957, but see Grinell, 1919; Elton, 1927) – is essential in explaining richness patterns. However, time and space also play a role since environmental conditions vary across spatial and temporal scales (Amarasekare & Nisbet, 2001; Wiens & Donoghue, 2004). Thus, the niche of species can strongly affect their distribution and subsequently shape large-scale richness patterns over time (MacArthur, 1965; Holt, 1996). As taxa speciate, descendant species often tend to inhabit geographical areas or ecological niches similar to their immediate ancestors (Wiens & Donoghue, 2004). This leads to slow ecological niche change such that closely related species tend to retain their ancestral niche-related traits over evolutionary time. This tendency is known as "Phylogenetic Niche Conservatism" (PNC) (Peterson et al. 1999; Ackerly, 2003; Wiens et al. 2010), which is characterized by slow rates of niche evolution (Ackerly, 2009). With PNC, closely related species can be affected by similar dispersal barriers (Wiens 2004), thus failing to disperse into regions with different climatic or ecological regimes, which leads to distinct patterns in the geographic distribution of lineages (Wiens & Graham 2005).

Several aspects of niche might be conserved throughout a clade's evolutionary history. For instance, phylogenetic niche conservatism has been shown for traits and taxa such as thermal tolerances in mammals and birds (Khaliq et al., 2015), elevation in salamanders (Kozak & Wiens, 2010) and diet and ecomorphology in fish (Buser et al., 2010). One of the most encompassing aspects is habitat type. For instance, species that feed on earthworms are almost exclusively found in forests whereas fossorial species are mainly found in open areas (Cadle & Greene, 1993). Additionally, forests are usually associated with stronger precipitation regimes and more constant temperatures than open areas which in turn tend to have more marked seasonality and higher maximum temperatures (Atagana et al., 2014). These aspects are important for ectotherms such as snakes, which have been extensively used as a model group for the study of diversity patterns and processes (Cadle & Greene, 1993; Parent et al. 2008; Burbrink et al. 2012; Roll et al. 2017). Since other habitat-related aspects of herpetofaunal ecology are phylogenetically conserved, such as diet (Durso et al., 2013), morphology (Alencar et al., 2017) and even thermal tolerances (Díaz-Ricaurte et al., 2020), it is therefore possible that habitat type might reflect phylogenetic niche conservatism.

The present study focuses on the diversity patterns of Dipsadidae, the richest snake family in the Neotropics. This speciose family (over 700 species) is ecologically diverse in diet, ecomorphology, and habitat use (e.g. arboreal, aquatic, cryptozoic, fossorial) thus making it a good model system for macrocological and biogeographical studies (Cadle & Greene, 1993; Grazziotin et al. 2012). Dipsadidae is composed of three subfamilies: Carphophiinae, Dipsadinae and Xenodontinae (Grazziotin et al. 2012), and two Asian genera (Thermophis and *Sticophanes*). While Carphophiinae is mostly distributed in North America, Dipsadinae and Xenodontinae are widespread in the Neotropical region. These two subfamilies dispersed from Central America to South America in different periods: Xenodontinae to cis-Andean South America once around 40 million years ago and Dipsadinae to trans-Andean South America several times, from 38 to 20 million years ago (Serrano et al., Chap. 1). Thus, their respective dispersal and consequent faunal interchange is complex and predates the formation of the Isthmus of Panama (Cadle & Greene, 1993). The two continental masses differ in several geophysical and ecological aspects: while Central America is narrow and mostly comprised of moderately elevated forests, South America is larger, more diverse in habitat types and more topographically complex (Olson et al. 2001; Poveda et al. 2006). Furthermore, these subfamilies are associated with different habitat types: arboreal and malacophagous/vermivorous species belong mainly to Dipsadinae (Peters, 1960), whereas Xenodontinae is mainly a terrestrial clade with a generalist diet (Cadle & Greene, 1993). Several studies (Savage, 1982; Vanzolini & Heyer, 1985; Cadle & Greene, 1993) showed that their distribution and corresponding richness patterns were asymmetrical, with Dipsadinae being widespread in South America but Xenodontinae being poorly represented in Central America. However, the is still unclear what cause this asymmetry in distribution between the two subfamilies, especially why Xenodontinae is poorly represented in Central America.

Herein we hypothesize that the subfamily-level asymmetry in richness between the two regions is a reflection of niche conservatism in habitat type (forest vs. open areas) for Dipsadinae and Xenodontinae. If so, we expect that: i) there is a significant difference in phylogenetic conservatism for habitat type between these two lineages, with Dipsadinae being prevalent in forest-specialists while Xenodontinae is predominantly a clade of open area specialists (which would prevent it from attaining high richness in forest-dominated Central America), and therefore that ii) Dipsadinae attains higher richness in forest habitats while Xenodontinae does so in open habitats.

3.2. Materials and Methods

3.2.1. Species distribution and spatial analyses

We used a data set of available species distribution polygons (GARD 1.5; Roll et al. 2017) to map species range with an equal-area projection (South America Albers Equal Area Conic, Kimerling et al. 1995). For each species, we calculated percentage of habitat type by overlapping its range with the global ecoregions shapefile (Dinerstein et al., 2017) for one of two main biome classes: i) forested areas (temperate, and dry and moist broadleaf forests) or ii) open areas (deserts, savannas, shrublands and grasslands, and the Xeric Caatinga "forests"). We then mapped species richness in each subfamily and for the whole family using BIODIVERSE (Laffan et al., 2010). We calculated the spatial species richness patterns of within-family composition by dividing the number of species of the Dipsadinae subfamily in each raster cell (0.25º x 0.25º, ~27 km x 27 km at the equator) by the total number of species of the Dipsadidae family in that cell (hereafter 'relative richness'). Furthermore, in order to test if there are differences in species richness for both subfamilies in forested versus open habitats, we randomly placed 250 points in each habitat, assigning a minimum distance between points of 1º (111 km at the equator) to avoid spatial autocorrelation. Finally, for each point we extracted relative richness and absolute richness of each family, and compared them with t-tests to test our hypothesis ii) that Dipsadinae will attain higher richness in forest habitats and Xenodontinae will attain higher richness in open habitats.

3.2.2. Phylogenetic comparative analyses

We used the most complete available Dipsadidae phylogeny (Serrano et al. Chap1) and attributed species to one of the subfamilies Dipsadinae or Xenodontinae (Cadle & Greene, 1993; Grazziotin et al. 2012). The subfamily Carphophiinae and the Asian dipsadid species were not considered in our analyses since they are absent or poorly represented in the Neotropics. We removed from the phylogenetic tree species for which we did not have data. In order to investigate if PNC was determinant in the distribution patterns of Dipsadidae in the Neotropical region, we first determined ancestral states for habitat type in each subfamily using the packages 'ape' (Paradis et al. 2004), 'geiger' (Harmon et al. 2007) and 'phytools' (Levell, 2012) in the statistical software R, version 4.0.2 (R Core Team, 2020; available at http://cran.r-project.org). To do so, we used percent (%) of forest in each species range as a continuous trait. We used maximum likelihood to compare Brownian Motion (BM), Ornstein-Uhlenbeck (OU) and δ (delta) models of trait evolution (Cooper et al. 2010) for habitat type. While BM implies that traits vary proportionally with time, so that the phylogenetically closer two species are the more similar that trait is, OU models assume that a trait evolves toward an optimal value. Under OU models, trait evolution involves two aspects: a non-directional BM component and a directional component under which trait values are pulled to an optimal value (θ) with strength of attraction α. When $\alpha = 0$, OU converges to BM. On the other hand, δ fits the relative contributions of late versus early evolution in the tree to the covariance of species traits. When δ is greater than 1, there has been a relatively fast recent evolution; if δ is less than 1, recent evolution has been slower (Pagel, 1999). Evolutionary rate was measured using sigma squared (σ^2) – the rate at which a trait changes through time (Felsenstein 1973; Felsenstein 2004) – estimated from the fitted models (Harmon et al. 2008, Pennell et al. 2014). The best model of trait evolution was selected using the Akaike Information Criterion (AIC) (Akaike, 1974; Harmon et al., 2007). Using both the ancestral state of Neotropical Dipsadidae and the macroevolution models, we can assess how PNC has shaped current habitat type for each subfamily. To corroborate our hypothesis i) we would expect for the evolution of % of forest in species range to be constrained (non-BM models, higher α, low σ^2) and that Dipsadinae attains high θ values (high optimal value, forest specialists) while Xenodontinae attains low θ values (low optimal value, open area specialists).

We also tested for phylogenetic signal – statistical dependence among species' trait values due to their phylogenetic relatedness (Münkemüller et al., 2012) – for habitat type with Blomberg's K and Pagel's lambda (λ) (Blomberg et al., 2003), as implemented in the *phytools* R package (Revell, 2012). Despite both metrics assuming the classic Brownian motion (BM) evolutionary model, they vary in their interpretation. Blomberg's K compares the variance of a trait with that resulting from a Brownian Motion model ($K = 1$), with values of $K < 1$ representing less phylogenetic signal than expected (larger intra-clade variance) and values of $K > 1$ representing more phylogenetic signal than expected (larger inter-clade variance) (Münkemüller et al., 2012). On the other hand, Pagel's λ represents the transformation of the phylogeny that fits a Brownian Motion model. Thus, when $\lambda = 1$, the structure of the phylogeny alone can explain changes in traits and thus traits follow a pure Brownian Motion evolutionary model, indicating a high phylogenetic signal. Conversely, when $\lambda = 0$, the trait is evolving independently of the phylogeny and therefore the phylogeny alone does not explain trait evolution (Münkemüller et al., 2012). Thus, stronger deviations from zero indicate stronger relationships between trait values and the phylogeny such that a strong phylogenetic signal ($K > 1$ and high λ) allows us to assess that closely related species are similar in % of forest in their range. This further strengthens the role of PNC for each subfamily as per our hypothesis i) and it may contribute to distinct geographical patterns of richness of each subfamily in different habitats throughout South America.

3.3. Results

We gathered range maps for 726 species of Dipsadidae, of which 391 and 335 belonged to Dipsadinae and Xenodontinae, respectively. Both subfamilies are widespread and broadly sympatric (Fig. 1B) and communities with only one subfamily are located only at the edges of the Neotropics, such as northern Mexico and the Yucatán Peninsula (Dipsadinae only, Fig. 1C) or the lower portion of the South American continent, in Patagonian steppes (Xenodontinae only, Fig. 1D). Relative richness (% of Dipsadinae in local richness of species from the Dipsadidae family) decreased southwards (Fig. 1A), with values higher than 50% north of the isthmus of Panama (25 to 10º N) and then decreasing abruptly towards austral latitudes (from 25% to 0%). There were two slight increases of relative richness of Dipsadinae: one near the equator at the same overall latitude of the Amazon forest and another near 23º S in southeastern Brazil. The West Indies showed low relative richness, especially if compared to Central American communities in the same latitudinal ranges (see Fig 1B). In South America, the northern Andes had the most balanced composition, nearing 50% relative richness throughout most of its extension, contrasting sharply with southern Andes where Xenodontinae prevails (see Fig. 1B). The Amazon forest showed homogeneous relative richness, reaching up to 40%, higher than the adjacent regions to the north and east (see Fig. 1B).

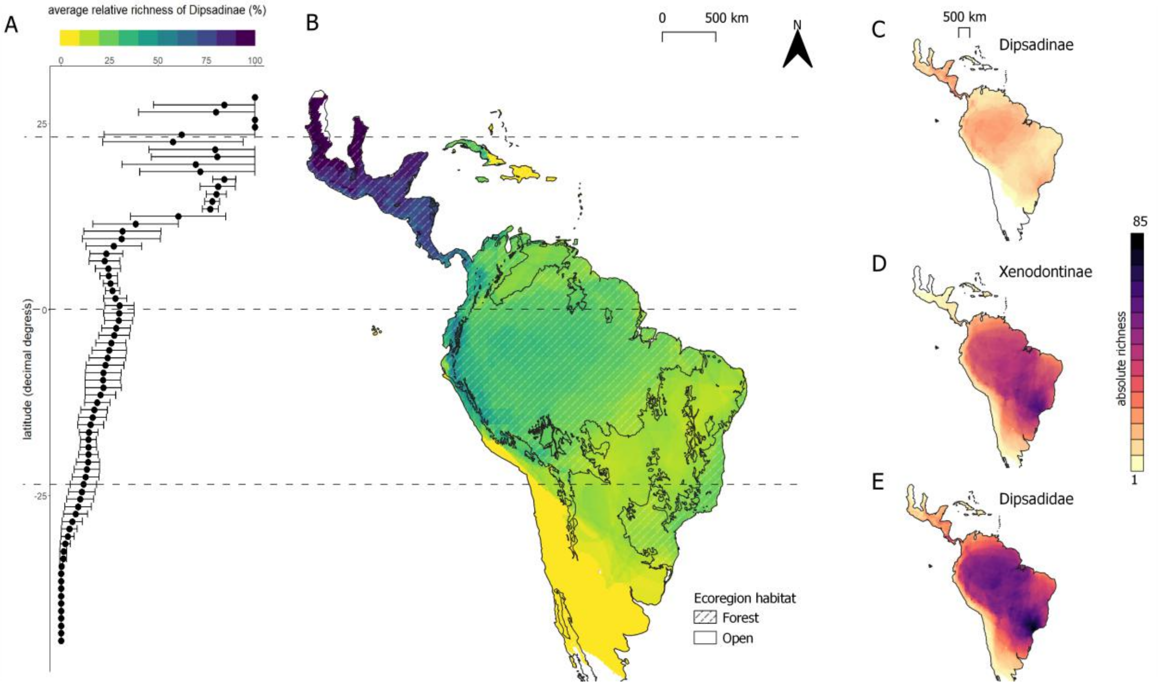

Figure 1. A) Average relative richness of Dipsadinae (% of Dipsadinae in local richness of species from the Dipsadidae family) per latitude, B) map of relative richness of Dipsadinae in the Neotropical region with forest and open ecoregions highlighted, C) absolute richness of Dipsadinae, D) absolute richness of Xenodontinae and E) absolute richness of Dipsadidae (Dipsadinae + Xenodontinae).

Absolute richness of Dipsadinae (Fig. 1c) was overall lower than that of Xenodontinae, attaining higher values in Central America, northern Andes and Amazonia, with a smaller, disjunct richness hotspot in Southeastern Brazil. Xenodontinae (Fig 1d) richness peaks in Southeastern Brazil, followed by adjacent areas in Central Brazil and then Amazonia. Absolute richness of the entire Dipsadidae family (Fig. 1e) peaks in Southeastern Brazil, followed by Central Brazil and Amazonia, forming a somewhat continuous and extensive richness hotspot from the Brazilian shield to Amazonian lowlands.

Comparing the richness of the two subfamilies in forest or open habitats (Fig. 2), there were significant differences for Dipsadinae, with forests attaining a significantly higher number of species compared to open habitats ($p < 0.001$), as proposed in our second hypothesis. In spite of significant differences in absolute Xenodontinae richness between the two habitats ($p < 0.001$), forests presented a higher number of species, contrary to our prediction that this subfamily would be richer in open areas. Xenodontinae also had higher variances in absolute richness for both forest and open areas.

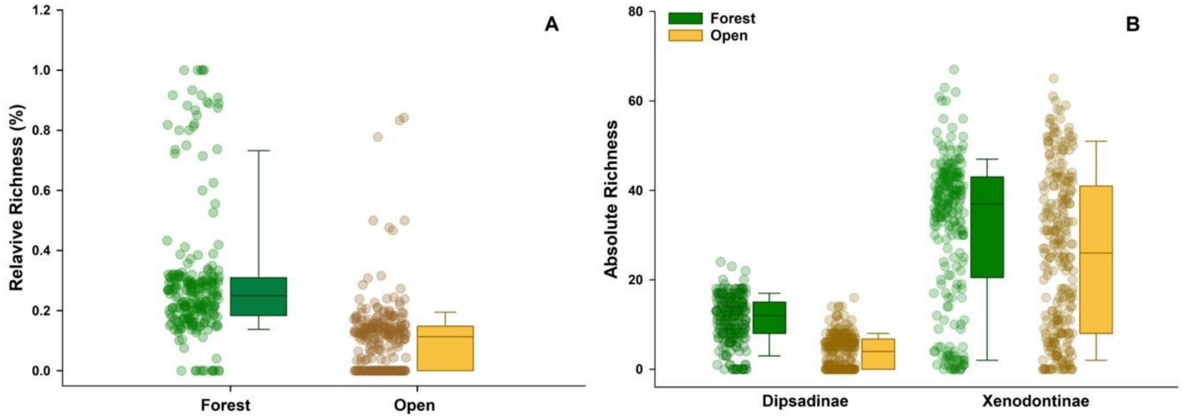

Figure 2. Boxplots of A) relative richness (% of species from the Dipsadinae family in total richness) in forest versus open habitats for the whole family and B) absolute richness of each subfamily in forest and open habitats.

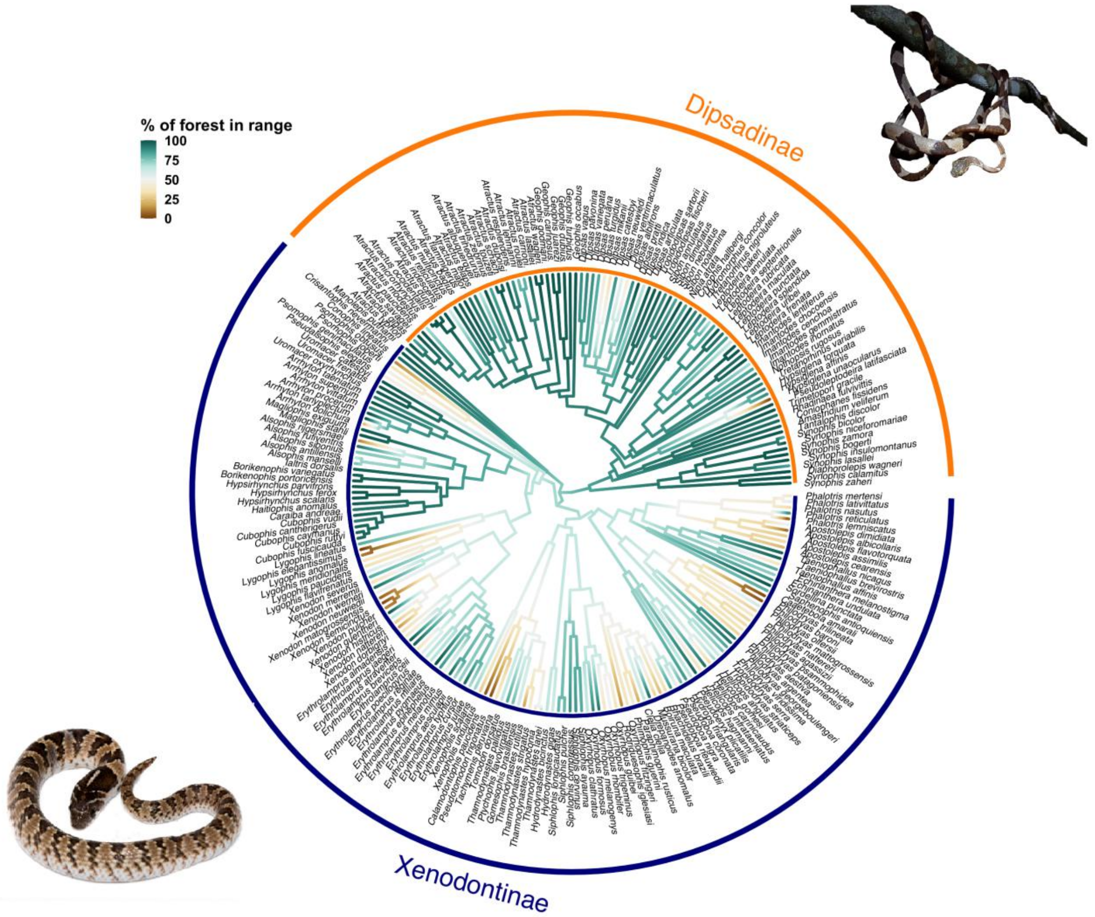

Figure 3. Ancestral reconstruction for habitat (% of forest in species range) for both subfamilies Xenodontinae (represented by *Xenodon nattereri*, lower left, photo by Juan C.

Díaz-Ricaurte) and Dipsadinae (represented by *Imantodes cenchoa*, upper right, photo by Filipe C. Serrano).

A total of 236 (32.5 % of total) species of 72 genera from Dipsadidae were included in the phylogeny, of which 90 (23.1 %) of 23 genera and 146 (43.6 %) of 51 genera belonged to Dipsadinae and Xenodontinae, respectively. The reconstruction of ancestral states for habitat (% of forest in range) for the two subfamilies showed that ancestral lineages of Dipsadinae had most of their distribution in forest habitats while only a few extant species are habitat generalists or occupy mainly open habitats (Fig. 3) There were marked differences in the habitat type between the two subfamilies, with Dipsadinae (90.3 ± 21.2 %) attaining higher values of average percent of forest in species range than Xenodontinae (60.6 ± 35.6 %). The ancestral lineages of Xenodontinae, however, were likely habitat generalists, with habitat shifts being common among its major lineages, which only recently became habitat specialists (either forest or open area).

Table 2. Trait evolution for both subfamilies and both traits.

Models	**Xenodontinae**					
	AIC	AICc	σ^2	θ	Other model parameters	ΔAICc	wAICc
BM	1415.75	1415.83	59.78	68.51	-	24.75	0.00
OU	1390.92	1391.09	115.58	64.12	0.060147 (α)	0.00	0.90
Delta	1395.41	1395.57	28.00	64.52	2.9999 (δ)	4.49	0.10

Models	**Dipsadinae**						
	AIC	AICc	σ^2	θ	Other model	ΔAICc	wAICc

					parameters		
BM	759.67	759.81	16.61	93.21	-	8.40	0.01
OU	751.50	751.78	33.39	90.72	0.055939 (α)	0.37	0.45
Delta	751.13	751.41	8.26	90.80	2.9999 (δ)	0.00	0.54

Models	**Dipsadidae (whole family)**						
	AIC	AICc	σ^2	θ	Other model parameters	ΔAICc	wAICc
BM	2212.50	2212.55	43.48	77.72	-	30.23	0.00
OU	2182.22	2182.32	72.25	74.14	0.043043 (α)	0.00	0.99
Delta	2190.87	2190.97	21.28	73.28	2.9999 (δ)	8.65	0.01

Table 3 - Phylogenetic signal for both subfamilies and the Dipsadidae family.

	Xenodontinae		**Dipsadinae**		**Dipsadidae**	
	phylosignal	p	phylosignal	p	phylosignal	p
Blomberg's K	0.46	0.001	0.54	0.009	0.54	0.001
Pagel's λ	0.68	3.53E-10	0.98	0.014	0.71	2.46E-24

Regarding habitat diversification, the Ornstein-Uhlenbeck model was the best model for both the Xenodontinae subfamily (wAICc = 0.90) and the whole family (wAICc = 0.99)

(Table 2). For Dipsadinae, both the OU (wAICc = 0.45) and Delta (wAICc = 0.54) models showed strong support. Comparing OU models for both subfamilies showed that Dipsadinae has simultaneously a much lower evolutionary rate and a higher optimal value (33.39 and 90.72, respectively) of percentage of range in forest than Xenodontinae (115.58 and 64.12, respectively), despite similar values of alpha (0.056 and 0.060 for Dipsadinae and Xenodontinae, respectively). For Dipsadinae, the high δ in the equally supported Delta model seems to indicate that longer branches contribute less to trait diversification, with recent events being more responsible for trait evolution than older events. Regarding the phylogenetic signal of habitat type, both the Blomberg's K and Pagel's λ indicate a non-random trait divergence between related species. Blomberg's K yielded similar results for Dipsadidae and separately for its two subfamilies, with a moderate significant value of K (0.46 - 0.54) (Table 3), which indicates that variance occurs mainly within clades. On the other hand, Pagel's λ differed between the Xenodontinae ($\lambda = 0.67$, $p < 0.001$) and Dipsadinae ($\lambda = 0.99$, $p = 0.013$) subfamilies, with the whole family ($\lambda = 0.71$, $p < 0.001$) being similar to Xenodontinae (Table 3). These values suggest that there is a strong relationship between percentage of range in forest and the phylogeny, especially for Dipsadinae.

3.4. Discussion

In order to understand global patterns of diversity, we need to understand how large-scale biogeographical patterns are influenced by ecology and how this is reflected in inter and intra-lineage distribution (Wiens & Donoghue, 2004). Our results show that historical processes are important contributors to the asymmetric patterns of diversity and distribution of Dipsadidae (Savage, 1982; Vanzolini & Heyer, 1985; Cadle & Greene, 1993). We partly corroborated both our hypotheses: Dipsadinae is a marked forest-specialist subfamily as per our hypothesis i) but Xenodontinae is mostly a generalist clade, and overall both subfamilies are richer in forest habitats, thus not corroborating our hypothesis ii) for the Xenodontinae subfamily.

Phylogenetic Niche Conservatism has been considered a strong explanation for the distribution of several taxa (Wiens & Graham, 2005; Losos, 2008; Pyron & Burbrink, 2009a). We found that PNC is an important factor driving diversity patterns of Dipsadidae but mainly for the Dipsadinae subfamily, closely associated with forest habitats as shown in the ancestral state reconstruction and richness comparisons between forest and open habitats. This might have played a role in this subfamily's dispersal from Central to South America (Serrano et al., Chap1). Indeed, the Dipsadinae genus *Leptodeira* has been suggested to extend much of its distribution throughout South America during forest expansion (Daza et al. 2009). This is congruent with the results of Cadle & Greene (1993) and with our first and second hypothesis for Dipsadinae. This habitat association in the Central American subfamily is evident with the higher relative richness in Central American forests, Amazonia and Atlantic Forest compared to nearby open habitats such as Caatinga and Cerrado (Fig. 1b). Relative richness of Dipsadidae (% of Dipsadinae in local richness of species from the Dipsadidae family) decreased with latitude as suggested by Cadle & Greene (1993), although not linearly, with some examples being the low relative richness in the Guiana shield savannas (near the isthmus of Panama and north of the Amazon forest), which is likely responsible for the abrupt decrease in relative richness around 5 – 10º N, and the higher relative richness in the Atlantic Forest. The balanced relative richness in the northern Pacific coast of South America and the northern Andes seems to corroborate the zoogeographic association of Central America and the Chocoan region (Vanzolini & Heyer, 1985). Additionally, relative richness in the West Indies is much lower than in Central America, despite their proximity and the prevalence of forest habitats. This further strengthens the evidence that snake communities in the West Indies resulted from dispersal of species from South America (Maglio, 1970; Zaher et al. 2009; Burbrink et al. 2012; Serrano et al., Chap1), as found for several other non-flying vertebrates: lizards (Hass et al. 1991), frogs (Hedges, 1992), and mammals (Woods, 1990).

Xenodontinae, on the other hand, was not prevalent in open area specialists nor was its absolute richness higher in open habitats, contrary to our predictions. Since this subfamily is mostly a generalist lineage (with some marked forest specialists) and that it attains high richness both in forest and open habitats (this work, Cadle & Greene, 1993), its sparse distribution and low richness in Central America is unlikely to be due to a forest-related ecological barrier. Indeed, large forest tracts of Amazonia harbour very rich Xenodontinae faunas (Nogueira et al., 2019). It is possible that this habitat homogeneity and low elevation in the Amazon basin could impose few ecological barriers to widespread populations and thus fewer opportunities to speciate by vicariance (Ricklefs, 2007; Kisel et al. 2011). By contrast, on topographically diverse Central America and in most of the Brazilian shield, where lowlands and upland areas are in close contact, more lineages could be affected by geomorphological isolation in riverine depressions or in isolated mountains or tabletops (Marshall, 2007; Nogueira et al. 2011). There may also be other geometric constraints at play since area can be a main determinant of clade size and its distribution (Hawkins et al. 2003; Ricklefs, 2006). Considering that north of the Panama isthmus the continental extent is severely narrower, this may have represented a constraint to the expansion of Xenodontinae into Central America, especially if niches were already occupied by Dipsadinae. Therefore, it is possible that diversity patterns might be shaped by geometric constraints and associated restricted lineage dispersal during diversification, as suggested by Eiserhardt et al (2012).

Our macroevolutionary results also illustrate how these two subfamilies differ on habitat type and how this is might reflect their evolutionary history. Our ancestral state reconstruction showed that the common ancestor of both subfamilies likely occurred mainly in forests, which is consistent with the habitat in Central America – its estimated ancestral area – at the time (Serrano et al., Chap 1). After the cladogenetic event that resulted in the two subfamilies around 40 mya, Xenodontinae dispersed to South America while Dipsadinae remained in Central America for a longer period, with only a few lineages dispersing southwards around 25 mya. Dipsadinae thus diversified in the extensive tropical forests of Central America, which likely favoured forest-associated traits transversal in this subfamily such as arboreal habits or a malacophagous diet (Serrano et al., unpublished data). Even upon dispersing into South America, widespread lineages of the Dipsadinae subfamily such as the speciose genus *Atractus* (over 100 species) or the Dipsadini tribe continued to mainly occur in forests such as the Amazon or the Atlantic Forests (Nogueira et al., 2019). Xenodontinae species, on the other hand, were likely subjected to a more complex biogeographical scenario of both forests and open areas (Werneck et al., 2012). This subfamily entered South America around 40 mya, after the Early Eocene Climate Optimum, while subtropical open-herbaceous savannas were expanding and forests thinning (Azevedo et al., 2020; Bellosi et al., 2021). Furthermore, it is possible that, having dispersed earlier into South America, Xenodontinae had a longer period of adaptation and diversification which likely contributed to higher opportunities to occur in both habitats. In fact, our ancestral state reconstruction showed that most transitions of Xenodontinae to open habitats occurred simultaneous for several lineages, which would be congruent with increasing availability of open areas such as savannas and with the strong association to open areas for several of its species (e.g. *Lygophis paucidens*, Serrano et al., 2020).

The macroevolutionary models of trait evolution also reflected these differences between the two subfamilies. While the Ornstein-Uhlenbeck model, suggestive of an evolutionary optimum, was a strongly supported model for both subfamilies, model parameters indicate a higher optimal value and lower evolutionary rate of forest occurrence for Dipsadinae species. This is congruent with PNC and suggests that this trait is more constrained while the higher mean shows that species of the Dipsadinae subfamily have most of their distribution in forest habitats. Conversely, Xenodontinae species had a moderate percentage of forest in their range coupled with a noticeably high variance, which further reinforces the generalist habitat type of this subfamily throughout its evolutionary history. The phylogenetic signal of forest in species range indicates that there are no clear differences between or within clades due to a moderate Blomberg’s K for both subfamilies. Yet, Pagel's λ was much higher for Dipsadinae than for Xenodontinae. The high λ indicates that phylogenetic structure alone adequately represents trait variation in this family, further reinforcing the strong Phylogenetic Niche Conservatism of forest for Dipsadinae, as per our i) hypothesis.

In this study, we show that distinct lineages within a widespread and dominant clade might respond differently to available habitat, leading to distinct evolutionary and biogeographical patterns. The current asymmetrical richness patterns in the two major subfamilies of the megadiverse Dipsadidae, that accounts for the majority of snake species the Neotropical region, appear to reflect two major biogeographical aspects: that the low richness of Xenodontinae in Central America is not limited by habitat and that Dipsadinae has strongly retained a close association with forest habitat throughout its evolutionary history. This association has allowed Dipsadinae to disperse and speciate along forested South America, but hindered its presence and dominance in open areas. In turn, Xenodontinae is represented in Central America by only a few species. Although the mixing of the two subfamilies of Dipsadidae is complex and older than the formation of the Isthmus of Panama in the Pliocene (Cadle, 1985; Cadle & Greene, 1993), there is evidence that snake dispersal between Central and South America has also occurred extensively in the last ~3 m.y.a. (Estes & Baez, 1985; Daza et al. 2009; Daza et al. 2010) which may have allowed Xenodontinae to only recently disperse to Central America. Our results show that the idiosyncratic association to habitat type by closely related species of a megadiverse clade might influence the overall higher taxon-level richness patterns, as demonstrated for snakes in Central America (Daza et al. 2010), global mammalian diversity (Buckley et al. 2010), Neotropical bats (Stevens, 2011), and Neotropical palms (Eiserhardt et al. 2012). Thus, we highlight the importance of considering historical processes in shaping current diversity patterns in order to provide insights into how large-scale biogeographical patterns arise from clade-specific responses to ecological constraints.

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4. Chapter 3: Snakes on a puzzle: regionalization of Neotropical dipsadids reflects intra-clade phylogenetic differences of biogeographical processes

Abstract

Biogeographical units represent unique assemblages that reflect historical events that have shaped current species distributions. Thus, detecting biogeographical units such as Biotic Elements (BE) allows to understand if taxa with overlapping distribution patterns are the result of common biogeographical processes. Herein, we propose new regions of significant regionalization in the Neotropics using Dipsadidae, the richest snake family in the world, and incorporating phylogenetics to test the premise of vicariant regionalization. Only the subfamily Dipsadinae showed a significant regionalization, mainly in Central America. Furthermore, phylogenetic analyses showed that closely related species tend not to occur in the same BE, likely the result of vicariance. We show that there may be important intra-family differences in the impact of biogeographical barriers and consequent regionalization. The co-distribution patterns of Dipsadinae have likely been strongly influenced by eco- and geomorphological events since the limits of most Biotic Elements coincide with important geographical and ecological barriers. Our results offer a baseline to understand how Neotropical biota could have been shaped by vicariance, also highlighting the novel use of phylogenetic information to better test vicariant regionalization.

Keywords: Biotic Elements, Central America, Dipsadidae, Dispersal, Herpetofauna, Phylogenetic signal, Vicariance.

4.1. Introduction

Biogeography aims to document and understand spatial biodiversity patterns and the ecological and evolutionary processes behind them (Myers & Giller, 2013). Even though the search for patterns of species co-distribution has been one of the main goals of biogeography since its inception (Wallace, 1876), only recently has the increasing availability of detailed range data allowed for analytical methods to detect natural biogeographical units (Hausdorf & Hennig, 2003). Biogeographical units represent unique assemblages and thus are useful for conservation priority-setting (de Klerk et al., 2002; Whittaker et al., 2005). Furthermore, they often reflect historical events that have shaped current species distributions and general biodiversity patterns (Hausdorf & Hennig, 2003). Thus, taxa sharing common distribution patterns are usually the result of the same biogeographical events and processes (Szumik & Goloboff, 2004). Detecting biogeographical units is therefore a fundamental step to understand historical and evolutionary relationships between areas since it allows for the understanding processes such as allopatric speciation, vicariance, post-speciation dispersal and diversification (Hausdorf, 2002; Hazzi et al., 2018).

The Neotropical region has long been recognized as a global zoogeographical region by the seminal works of early biogeographers (e.g. Wallace, 1876) and a number of recent studies are reinforcing its geographical limits and components (Holt et al., 2013; Morrone, 2017). This region ranges from northern Central America to southern South America and it is composed of many distinct domains, including several biodiversity hotspots such as the Atlantic Forest, the Cerrado savannas and Tropical Andes (Myers et al. 2000). Even though it is one of the world's richest zoogeographical regions for groups such as reptiles (Roll et al., 2017) and amphibians (Fritz & Rahbek, 2012), continental-scale regionalization of the Neotropics has been mainly inferred from insects (Morrone, 2006), birds (Prieto-Torres et al., 2019) and mammals (Escalante & Morrone, 2020), despite recent efforts to detect biogeographical units for reptiles and amphibians at the ecoregion scale (e.g. Nogueira et al., 2011; Guedes et al., 2014; Azevedo et al., 2016; Barbo et al., 2021).

Dipsadidae (Bonaparte, 1838) is the most diverse snake family in the Neotropical region (over 700 species) with a high diversity of traits such as diet, body size, and habitat use (Nogueira et al., 2019, Serrano et al., in prep.), thus making it a good model group to understand regionalization and general biogeographical patterns (Grazziotin et al. 2012, see also Cadle & Greene 1993). This family has two major subfamilies in the Neotropics:

Dipsadinae and Xenodontinae (Grazziotin et al. 2012; Serrano et al., in prep.). Even though both are speciose and widespread (Cadle & Greene, 1993), they have different biogeographical histories and patterns of habitat use: Xenodontinae is an early arriver in South America (~38 mya) and is poorly represented in Central America and it is mostly a habitat generalist while Dipsadinae is richer in Central America, with most of its clades only recently arrived in South America (~25 mya) and is composed mainly of forest specialists (Serrano et al., Chapter 1, Serrano et al., Chapter 2).

Herein, we propose a regionalization scheme based on the current distribution of Dipsadidae, the richest snake family in the Neotropical region, using Biotic Elements (BEs, Hausdorf and Hennig, 2003) and discuss how its distribution patterns have been shaped by the biogeographical history of the Neotropics. To do so, we use a phylogenetic approach to test the premise of vicariant regionalization that states that closely related species are not in the same Biotic Element (Hausdorf, 2002; Hausdorf and Hennig, 2003). Furthermore, we explicitly look for within-clade differences by also testing its main Neotropical subfamilies, Xenodontinae and Dipsadinae. We hypothesize that Dipsadidae and its subfamilies share non-random patterns of species co-occurrence caused by vicariance (resulting from ecological and/or geological barriers) thus, composing Biotic Elements that might also reflect phylogenetic processes. We expect that Biotic Elements of Dipsadinae will mostly overlap major forest regions such as the Amazon and Atlantic Forest, while Xenodontinae will have its Biotic Elements in both forest and open-area regions. Lastly, we hypothesize that Biotic Elements in Central America, where Dipsadidae have occurred for longer than in South America, will have less closely related species due to a longer history of dispersal and vicariance. We aim to propose new regions of significant regionalization in the Neotropics and highlight the importance of considering sister clades when testing regionalization. We expect that the two subfamilies will have a marked overlap of Biotic Elements, specially in forest habitats due to the Dipsadinae being forest specialists and Xenodontinae habitat generalists.

4.2. Materials and Methods

4.2.1. Study area

We focused our analyses on the Neotropical region. We considered the Trans-Mexican Volcanic Belt as the limit between the Nearctic and Neotropical regions (Mastretta-Yanes et al. 2015, Morrone et al., 2017). Therefore we eliminated all records pertaining to the 'Nearctic' region as defined by Morrone (Morrone et al., 2014), and considered only records in the 'Mexican transition zone', 'South American transition zone' and 'Neotropical' regions.

4.2.2. Data sources

Our database comprises the geographical range of 731 species of Dipsadidae (91.3% of the whole family), among which 399 (93.9% of the subfamily) belong to the subfamily Dipsadinae and 332 (96.2% of the subfamily) belong to the subfamily Xenodontinae. Species nomenclature and subfamily affinities are as proposed by Reptile Database (Uetz et al., 2020), Zaher et al. (2009) and Grazziotin et al. (2012). Species ranges were obtained from polygon maps from the Global Assessment of Reptile Distributions (GARD; Roll et al., 2017).

4.2.3. Analyses

We created a rectangular 2º x 2º grid (222 km x 222 km at the equator; area = 49,284 km^2) cell array based on the extent of the study area, clipped to the limits of the Neotropical region. This resolution is adequate for continental scales (Daru et al., 2020). Thereafter, we obtained a presence-absence matrix by the intersection of species ranges and the resulting 554 grid cells. To process distribution data and the study area we used QGIS 3.18 (QGIS Development Team, 2021), with an equal-area projection (ESRI:53009 Sphere Mollweide).

4.2.3.1. First vicariance prediction: Test for clustering of distribution data

A first and general prediction of the vicariance model is that past vicariance events should produce groups of species sharing significantly clustered distributions (Nogueira et al., 2011; Barbo et al., 2021). This non-random congruence of species ranges should emerge as a consequence of allopatric speciation caused by the emergence of biogeographical barriers splitting ancestral biotas (Hennig & Hausdorf, 2004). Significantly clustered distributions can be identified with analytical methods such as the 'Biotic Elements Analysis' which identifies groups of taxa with ranges significantly more similar to one another (Hennig & Hausdorf, 2004). Moreover, a second general prediction is that closely related taxa will tend to be segregated in distinct biotic elements, due to fact that pairs of sister taxa will tend to be restricted to opposite sides of barriers, as a result of vicariance (Hausdorf, 2002; Hausdorf and Hennig, 2003).

To perform the analyses we first created a dissimilarity matrix using the *Kulczynski* distance (1 – "Kulczynski unnamed 2" in Shi, 1993; as suggested by Hausdorf & Hennig 2003). We then implemented the Biotic Element Analysis with '*prabclus*' (Hausdorf & Hennig 2003) in R environment (R Core Team, 2021, available at http://cran.r-project.org). We performed a parametric bootstrap test for the non-random congruence of species distributions using the function '*prabtest*'. Null models were generated producing artificial ranges based on parameters (richness per cell, range size distributions, and patterns of spatial correlation and disjunction) obtained from the original dataset (Hennig & Hausdorf, 2004). The test-statistic T derives from the assumption that, if clusters of distribution ranges are present in the dataset, distances between original species ranges should be predominantly smaller than the distances of species ranges simulated at random. The test statistic is calculated as the ratio between the 25% smallest distances and the 25% largest distances, and it is expected to be small if ranges are clustered, and large for homogeneously distributed data (Hennig & Hausdorf, 2004). We then used Monte Carlo simulation (1,000 replicates) to approximate the distribution of the statistical test under null models. This step was performed for the whole Dipsadidae family and for both subfamilies, Xenodontinae and Dipsadinae, separately. As a statistically significant result is required to recognize non-spurious clusters of species ranges, we mapped only BEs resulting from datasets with statistically significant results ($p < 0.05$) among the three analyses above.

4.2.3.2. Mapping

We clustered our data using model-based Gaussian clustering (mixture method, function '*prabclust*') available in *prabclus*. This method estimates the number of meaningful clusters, with ranges that cannot be adequately assigned to any biotic elements treated as noise components (Hausdorf & Hennig, 2003). As suggested by Hausdorf & Hennig (2003), we used constant k = number of species/40, rounded up to the next integer, where k represents an initial estimate of the noise component (see Byers & Raftery, 1998 for detailed explanation). For the parameter of minimum and maximum number of clusters we tested ranges from one to 10 and one to 30 increasing by five in each clustering run. A table with the resulting number of BEs and noise components for each clustering run can be found in the supplementary content (Table S1). With the resulting species/cluster tables we pre-visualized the resulting BEs, as well as their component species and percentage of species per grid cell using the '*mapar*' function (developed by JPSV-A and available at https://github.com/joao-svalencar/mapar), implemented in R environment. We chose the parameter that returned the maximum number of BEs while maintaining spatial contiguity and the smallest number of species assigned to the noise component. We mapped Biotic Elements (distribution clusters) selecting the group of grid cells that contained at least one record of at least one of their respective component species. Grid cells with ≥ 70% of the component species were considered "core cells". Grid cells with less than 70% and more than or equal to 30% of the component species are regarded as "intermediate cells". Finally, grid cells with less than 30% of BEs component species are regarded as "marginal cells".

4.2.3.3. Second vicariance prediction: phylogenetic signal

According to the second major prediction of the vicariance model, closely related species should be distributed in different biogeographical units, as an effect of fragmentation of ancestral ranges by the emergence of biogeographical barriers (Hausdorf & Hennig, 2003). While this prediction is traditionally approached using a Pearson's Chi-squared test to assess if species from the same genus co-occur in Biotic Elements (Hausdorf & Hennig, 2003; Nogueira et al., 2011; Guedes et al., 2014; Azevedo et al., 2016), we believe it is an over-simplistic representation of vicariance since genus is an abstract concept and because it fails to consider important elements such as the phylogenetic relationships of species and clades, as well as age and number of species in each clade. We therefore used phylogenetic signal, which represents the tendency for closely related species to resemble each other more than less related taxa as the result of shared evolutionary history (Münkemüller et al., 2021). Phylogenetic signal was therefore used to evaluate if closely related species were present in the same Biotic Elements (used as a categorical trait of interest across the phylogeny). To corroborate the second prediction of the vicariance model, we expect low phylogenetic signal within BEs, which reveals a low tendency for phylogenetically related species to co-occur in the same BEs (overdispersed pattern, i. e. closely related species scattered among different BEs). On the other hand, a high phylogenetic signal might indicate the co-occurence of closely related species in the same BEs (clustered pattern), thus we could disregard vicariance as a major process behind the diversification of Dipsadidae snakes.

To calculate phylogenetic signal we used two metrics: Blomberg's K (Blomberg et al. 2003) and δ-statistic (Borges et al. 2019). Blomberg's K is frequently used for continuous traits, where values lower than 1 indicate that related species resemble each other less than expected under a Brownian motion model, while values higher than 1 indicates that closely related species are more similar than expected under a Brownian motion model i.e. a strong phylogenetic signal. The recently developed δ-statistic test uses entropy to specifically test for phylogenetic signal of categorical traits, with low values (usually lower than 6) representing low phylogenetic signal (Vidan et al., 2019). The δ value decreases when the trait evolves independently and can be any positive real number: the higher the δ-value, the higher the degree of phylogenetic signal between a given trait and the phylogeny. We used the R package 'phytools' (Revell, 2012) in R (R Core Team 2019) to calculate Blomberg's K and associated p-values. To calculate δ we used the R package 'ape' (Paradis & Schliep, 2018) and the code available in GitHub (https://github.com/mrborges23/delta_statistic; Borges et al., 2019).

Furthermore, in order to investigate the spatial patterns of phylogenetic structure of and within BEs, we calculated the Phylogenetic Mean Pairwise Distance (MPPD) of each of their grid cells with Biodiverse (Laffan et al., 2010). This metric is calculated by averaging the sum of the branches of a phylogeny between each pair of species present in a given grid cell (Webb et al., 2008). Thus, higher values represent cells with less closely related species or that diverged a long time ago. We hypothesize that grid cells of BEs in Central America will attain higher MPPD values as Central America is likely the ancestral area of both subfamilies (Serrano et al., Chapter 1), with Dipsadinae dispersing later to South America and thus being subjected to longer periods of diversification in Central America. To test this, we also used a linear regression to test the relationship between latitude and MPPD.

For all phylogenetic analyses, we used the most up-to-date comprehensive molecular phylogeny of Dipsadidae (Serrano et al., in prep), which comprised 203 species (77 genera), of which 167 species (54 genera) belonged to Xenodontinae, and 106 species (23 genera) belonged to Dipsadinae. This represents 25% of the total family diversity, with 48.4% of the Xenodontinae species and 24.9% of the Dipsadinae species. We removed from phylogenetic analyses all taxa not represented in the phylogenetic tree and all taxa that were classified as "noise" and thus not present in any BE.

4.3. Results

4.3.1. First vicariance prediction: Test for clustering of distribution data

Our analyses showed that only the subfamily Dipsadinae showed a significant regionalization spatial pattern, with co-distributed biotas regionalized in non-random assortments of ranges (T = 0.76, mean = 0.825, range = 0.743-0.886, p = 0.008), with both Xenodontinae (T = 0.557, mean = 0.535, range = 0.468-0.656, p = 0.842) and the whole Dipsadidae family being non-significant (Dipsadidae: T = 0.75, mean = 0.695, range = 0.624-0.767, p = 0.993). We therefore mapped and analyzed only the clustered species ranges recovered in the Dipsadinae subfamily.

4.3.2. Determination of Biotic Elements (BEs)

The parameters that returned more BEs while maintaining spatial contiguity and the lowest number of species assigned to the noise component were attained with the number of possible clusters ranging from one to 15. We detected 12 BEs for Dipsadinae (Fig. 1; Table 1) composed of 206 species (52% of the subfamily's dataset), while 189 species (48% of the subfamily dataset) were assigned to the noise component. The 206 species in BEs represented 93.1% (27 out of 29) of the genera in this subfamily.

Biotic Element 1 (BE 1; Fig. 1A) is composed of 10 genera with 41 species restricted to Central America except Sierra Madre Occidental with bordering grid cells representing a contiguous region comprising most Central America except Sierra Madre Occidental and it is therefore named: "Central America". Biotic Element 2 (BE 2; Fig. 1B) is composed of nine genera with 26 species restricted to the northwestern portion of Central America with grid bordering cells representing a contiguous region comprising the Sierra Madre Occidental and it is therefore named: "Sierra Madre Occidental". Biotic Element 3 (BE 3; Fig. 1D) is composed of five genera with 25 species restricted to the northwestern portion of South America with a single core cell in the Magdalena river valley and bordering cells comprising northwestern Colombia and it is therefore named: "Colombian Andes". Biotic Element 4 (BE 4; Fig. 1B) is composed of eight genera with 21 species restricted to the southeastern portion of Central America with a single core cell and bordering cells comprising the Panama Isthmus and Yucatan Peninsula and it is therefore named: "southern Central America". Biotic Element 5 (BE 5; Fig. 1D) is composed of five genera with 21 species restricted to Ecuador in the western portion of South America with a single core cell comprising the central Ecuadorian Andes and it is therefore named: "Ecuador". Biotic Element 6 (BE 6; Fig. 1C) is composed of six genera with 13 species restricted to lower Central America with core cells comprising Nicaragua and it is therefore named: “Nicaraguan depression”. Biotic Element 7 (BE 7; Fig. 1C) is composed of six genera with 12 species restricted to southern Mexico with a single core cell and a bordering cell comprising the Trans-Mexican Volcanic Belt and it is therefore named: “Trans-Mexican Volcanic Belt”. Biotic Element 8 (BE 8; Fig. 1C) is composed of four genera with 12 species restricted to southern Mexico with a single core cell and bordering cells comprising the Chiapas Highlands and it is therefore named: “Chiapas Highlands”. Biotic Element 9 (BE 9; Fig. 1E) is composed of three genera with 11 species restricted to the eastern portion of South America associated with the Atlantic Shield (Almeida et al., 1981) with core cells comprising the Serra do Mar region of the Atlantic Forest and it is therefore named: “Atlantic Forest”. Biotic Element 10 (BE 10; Fig. 1C) is composed of six genera with 10 species restricted to Panamá and Costa Rica in the southern portion of Central America with a single core cell comprising the Panama Isthmus and it is therefore named: “Panama Isthmus”. Biotic Element 11 (BE 11; Fig. 1E) is composed of four genera with seven species restricted to the northwestern portion of South America with core cells comprising the Amazon basin region of the Amazon Forest and it is therefore named: “Amazonia”. Biotic Element 12 (BE 12; Fig. 1D) is composed of three genera with six species restricted to a single core cell in southern Mexico comprising the “Sierra Madre del Sur” and it is therefore named: “Sierra Madre del Sul”. Most BEs match previously recovered biogeographical units in the regionalization of the Neotropical region (Morrone et al., 2022) (Table 1).

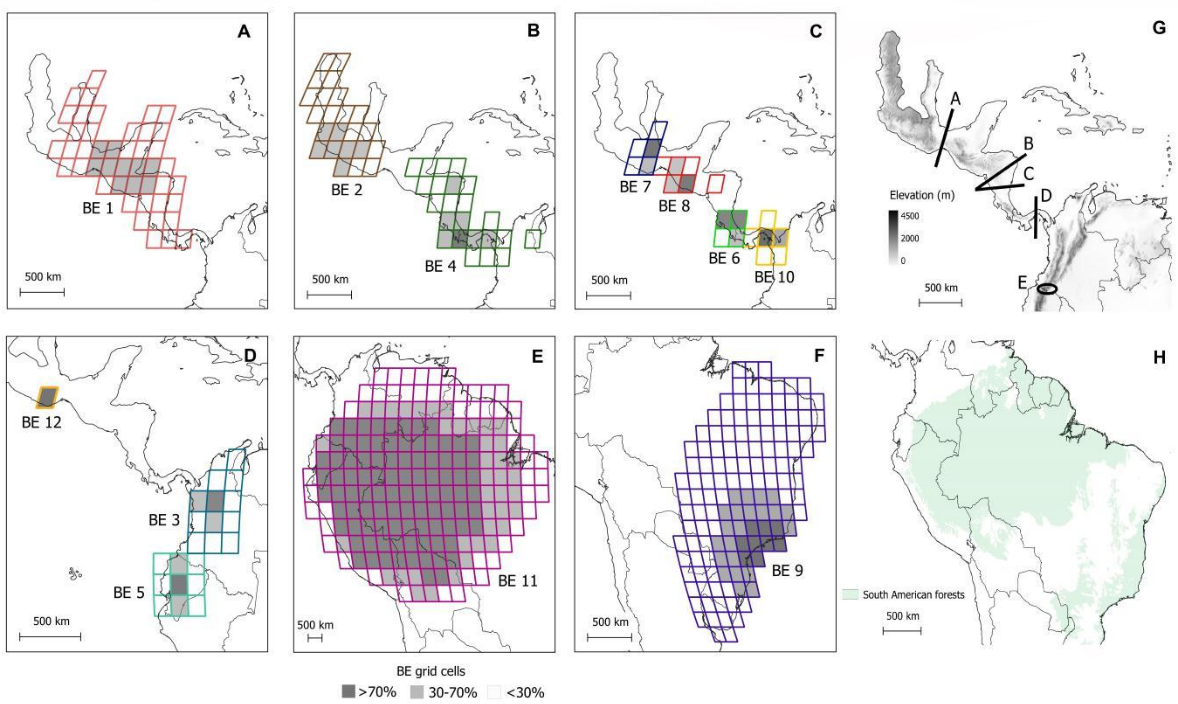

Fig. 1 - Biotic Elements for the Dipsadinae subfamily: A) BE 1 “Central America”; B) BE 2 “Sierra Madre Occidental” and BE 4 “southern Central America”; C) BE 6 “Nicaraguan depression”, BE7 “Trans-Mexican Volcanic Belt”, BE 8 “Chiapas Highlands” and BE 10 “Panama Isthmus”; D) BE 3 “Colombian Andes”, BE 5 “Ecuador” and BE 5 “Sierra Madre del Sul”; E) BE 11 “Amazonia” and F) BE 9 “Atlantic Forest”. G) represents major geomorphological features of Central America and northern South America: A - Panama isthmus, B - Tehuantepec isthmus, C - Guayape fault system, D - Nicaraguan depression and E - Huaca massif . H) represents major forest areas in South America.

Table 1 - BE and its corresponding number of genera and species, as well as equivalence to biogeographical units previous detected for the Neotropical region (Morrone et al., 2022). * denotes only core cells.

BE	Name	Nr of genera	Nr of species	BE in Morrone et al., 2022
1	Central America	10	41	
2	Sierra Madre Occidental	9	26	

3	Colombian Andes	5	25	Magdalena province*
4	southern Central America	8	21	Puntarenas-Chiriqui province
5	Ecuador	5	21	Western Ecuador
6	Nicaraguan depression	6	13	Guatuso-Talamanca province
7	Trans-Mexican Volcanic Belt	6	12	Trans-Mexican Volcanic Belt province*
8	Chiapas Highlands	4	12	Chiapas Highland province*
9	Atlantic Forest	3	11	Parana Dominion* and Chacoan Dominion
10	Panama Isthmus	6	10	Chocó-Darien province
11	Amazonia	4	7	
12	Sierra Madre del Sul	3	6	Sierra Madre Sul province

4.3.3. Second vicariance prediction: phylogenetic signal

From the 206 species of Dipsadinae in Biotic Elements, only 54 (26.2%) were represented in the phylogeny, albeit from 70.4% (19 out of 27) of the genera. Furthermore, four of the un-represented genera had only one species in BEs. Most species in Biotic Elements but missing from the phylogeny were from the speciose genera *Atractus* and *Geophis*.

According to the traditionally used Chi-squared test, species of the same genus co-occur in the same BE ($X^2 = 576.16$, df = 286, p < 0.001), not validating the second premise of vicariance. However, we found that both Blomberg's K (K = 0.464, p-value = 0.028, n = 50000 randomizations) and δ-statistic (δ = 1.845, p-value < 0.01, n = 1000 randomizations) supported a significant but low phylogenetic signal for the co-occurrence of phylogenetically related species in the same Biotic Element (Fig. 2), as per the second prediction of vicariance i.e. phylogenetically related species tend to not compose the same Biotic Element. This highlights the importance of considering phylogenetic information in assessing how related species might co-occur.

We furthermore confirmed our hypothesis that grid cells of BEs in higher latitudes (i.e. Central America) had significantly higher MPPD (Fig. 3A), albeit with a weak relationship between latitude and MPPD ($p < 0.001$, $R^2 = 0.163$, $F = 78.88$). This was confirmed by the spatial patterns of MPPD (Fig. 3B), with differences between and within BEs, as per our hypothesis. Cells attained the highest values in Western Sierra Madre, corresponding to BE 2, BE 7 and BE 12, followed by the Yucatan Peninsula, Ecuador and eastern Amazonia. On the other hand, BE 9 - Serra do Mar - had distinctly lower values of MPPD, especially in its central and northernmost portions.

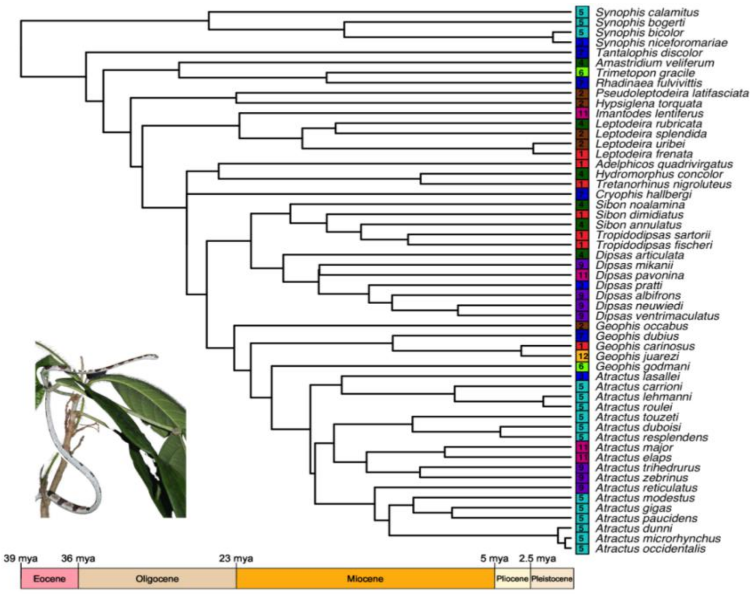

Fig. 2 - Dated phylogeny of Dipsadinae species with corresponding BE numbers and colors. Phylogenetic signal for presence in Biotic Elements of Dipsadinae ($K = 0.464$, p-value $= 0.028$; $\delta = 1.8498$, p-value < 0.01). Pictured is a *Imantodes cenchoa* from Xapuri, Acre, Brazil (photo credit: Filipe C. Serrano).

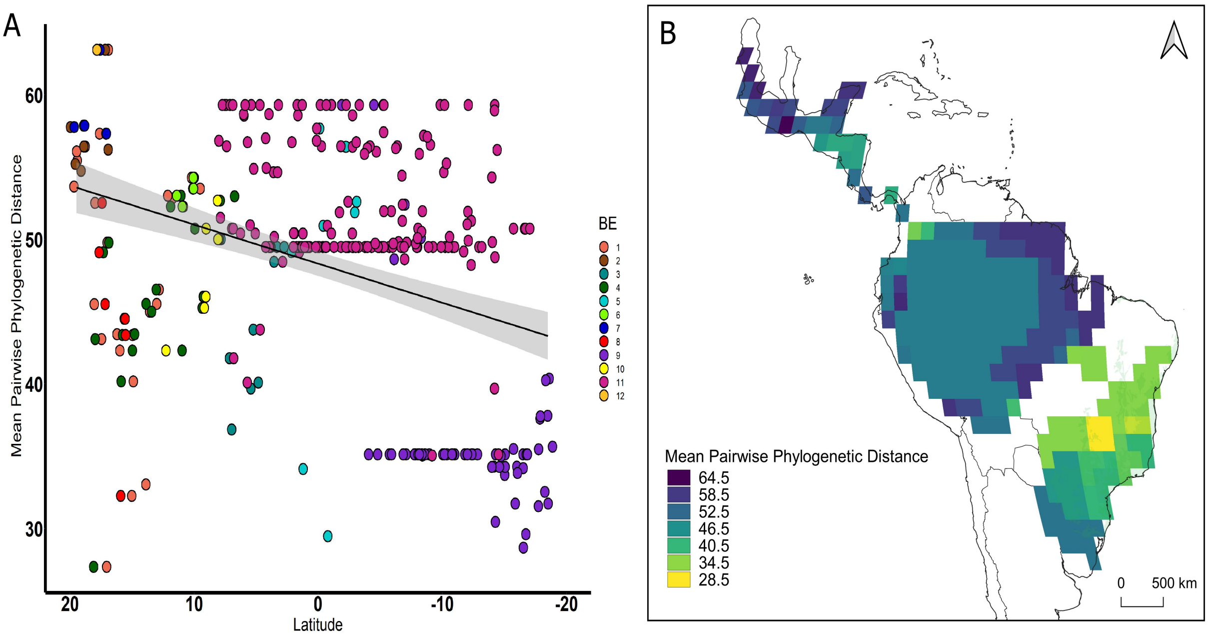

Fig. 3 - A) Relationship between Latitude and Mean Pairwise Phylogenetic Distance (MPPD) of the grid cells of each Biotic Element. Grid cells were colored according to their Biotic Element to aid visualization. B) Spatial patterns of MPPD in the grid cells of BEs in the Neotropical region.

4.4. Discussion

Our study shows that patterns of co-distribution of snakes from the Dipsadidae are, in general, not a result of vicariance, except for the Dipsadinae subfamily. In this subfamily, clusters of species ranges match previously recovered biogeographical units (Morrone et al., 2022), seemingly a result of vicariant processes, and were mainly located in Central America. Furthermore, closely related species of the Dipsadinae subfamily tended not to occur in the same Biotic Elements, shown by our phylogenetic approach and which validates our second hypothesis of vicariance. We also confirmed our hypothesis that, compared to South America, Biotic Elements in Central America have less closely related species thus a significantly higher mean phylogenetic distance between species. All these intra-family differences highlight how the complex geographical history of the Neotropics may affect clades differently and that different lineages react differently to biogeographical barriers (Azevedo et al., 2016; Myers et al., 2019). In fact, a previous study (Serrano et al., Chapter 1; Serrano et al., Chapter 2) had already identified that the diversity of Dipsadidae, and its subclades, could have been shaped by different evolutionary and historical processes.

Both the Xenodontinae subfamily and the whole Dipsadidae family failed to show significant regionalization patterns, with ranges indiscernible from a random assortment. Since there was a strong pattern of regionalization of Dipsadinae, the lack of significance for the entire family Dipsadidae might result from non-congruent patterns when both families are analysed, which further corroborates that distribution patterns of Xenodontinae is not strongly shaped by vicariance but by other spatial aspects such as geometric constraints (Serrano et al., Chapter 2). Even though Xenodontinae has been present in South America for at least 40 mya, being potentially affected by relevant biogeographical events (Serrano et al., Chapter 1), the diversity patterns of this subfamily were likely influenced by factors other than biome's intrinsic differences (Serrano et al., Chapter 2). In fact, Xenodontinae is an overall generalist clade, with several species occurring exclusively in either forest or open areas and others occurring equally in both (Serrano et al., Chapter 2). This may suggest that ecological barriers are not strong drivers of vicariance for this subfamily, while in smaller spatial scales, e.g. within ecoregions, geographical barriers seem to have a considerable role (Guedes et al., 2014; Azevedo et al., 2016; Barbo et al., 2021). Furthermore, Xenodontinae comprises more vagile species and with average larger body sizes (e.g. *Philodryas* spp.), more diverse in habitat use (aquatic species of the Hydropsini tribe and fossorial species of the Elapormorphini tribe) as well as less-marked diet specialists (Serrano et al., Chapter 4). While ecological factors such as habitat use and diet might contribute to intra-subfamily differences in distribution and subsequent impact of vicariance (Wiens, 2004; Naka & Pil, 2020), higher vagility and body size can lead to a higher chance of dispersal (Hausdorf, 2000; Kodandaramaiah, 2009) which could generate less congruent regionalization patterns (Wolf et al., 2001; Daru et al., 2017).

Contrary to Xenodontinae, Dipsadinae is mostly a forest specialist clade and attains its highest richness in Central America (Serrano et al., Chapter 2, see also Cadle & Greene 1993). Similarly, two thirds of its Biotic Elements are north of the Isthmus of Panama, in spite of its much smaller available area. This may be related to the interplay of diversification of this subfamily (Serrano et al., Chapter 1) with the complex ecological and geomorphological events of Central America (Marshall, 2007), which have likely induced vicariance along barriers. For example, BE 8 corresponds to a limit imposed by the Trans-Volcanic Belt while the southern limit to BE 2 and BE 7 and the northern limit to BE 4 is the Isthmus of Tehuantepec, which might have acted as barriers due to the orogenic activity in the Late Miocene—Early Pliocene (Barrier et al., 1998), as suggested for Dipsadinae snakes in the *Leptodeira* genus (Daza et al., 2009). In fact, besides *Leptodeira*, there are species from the same genus on both sides of the Isthmus (e.g. *Dipsas*, *Geophis*, and *Rhadinaea*), which suggests that vicariance likely played a role shaping snake biotas in the Neotropical region. Furthermore, the trans-Andean BE 3 and BE 5 are contiguous but do not overlap, unveiling the Huaca massif as a potential barrier for the genera *Atractus*, *Dipsas* and *Synophis*.

Previous studies have suggested that Pleistocenic climatic fluctuations in South America have been responsible for vicariant events in the correspondent areas of BE3 and BE5 for *Leptodeira* (Daza et al., 2009) - of which however most species were classified as “noise“ and thus not included in any South American Biotic Elements. This vicariance is congruent with our results of within-genus divergence for other genus. For example, *Synophis niceforomariae* (BE 3) diverged from its sister species *S. bicolor* (BE 5) in the Pleistocene, with a clade composed of two species diverging from *S. bogerti* (BE 5) in Middle Miocene (Serrano et al., Chapter 1). Another example is the Pleistocenic-diverged sister species *Atractus lasallei* and *A. lehmanni* belonging in different BEs (3 and 5, respectively) while both *Atractus carrioni* and *A. duboisi* compose BE5, despite belonging to separate clades that diverged in Early/Middle Miocene. Both BEs had already been recovered as Micro-Endemic Areas for the *Atractus* genus (Pomar-Goméz et al., 2021). Overall, there were potential differences in the nature of the limits of BEs between Central America and South America. While in Central America Biotic Elements were mainly limited by “hard” geomorphological barriers (e.g. Nicaraguan depression and Tehuantepec isthmus), the boundaries of South America BEs seem to be mainly “soft” ecological barriers linked to the transition of forest habitat to more mesic habitats. This transition is probably a result of the onset of savannas in South America during the Pliocene (Azevedo et al., 2020).

The phylogenetic metrics of BEs likely reflect this complex history of biogeographical events during the evolutionary history of Dipsadinae. The phylogenetic signal proved to be an efficient tool to discriminate how closely related species are distributed across Biotic Elements, which could not be detected by the traditional Pearson's Chi-square method using genera as proxy for closely related taxa (see Hausdorf & Hennig, 2003). Even though the latter method can provide insightful results, it might also overlook relevant patterns, especially for very speciose taxa (e.g. *Atractus*) in which ecological rather than historical drivers might be evoked to explain diversification (Pomar-Goméz et al., 2021; Serrano et al., Chapter 2). For example, sister clades *Pseudoleptodeira latifasciata* and *Hypsiglena torquata* belong to different genus but feature in BE 2 (Central America), thus allowing for better analysis of potential vicariance. On the other hand, BE 5 (Ecuador) is composed of 12 species of *Atractus* which could lead to the interpretation that "closely related species (regarded here as 'species from the same genus') are not homogeneously distributed across BEs". However, even though belonging to the same genus, these species are from two relatively distant clades, reinforcing the importance of detailed and comprehensive phylogenies to shed light on elusive biogeographical patterns (Crisp et al., 2011; Ronquist & Sanmartín, 2011).

The higher Mean Pairwise Phylogenetic Distance of grid cells from Biotic Elements in Central America confirms that South America is generally composed of more closely related species, at least for the Dipsadinae subfamily. This subfamily's dispersal into South America was less extensive than that of Xenodontinae's and on average later (Serrano et al., Chapter 1). Lower values of MPPD might indicate both recent events of speciation (common evolutionary history until present), or relevant interchange of closely related species through dispersal events (Laffan et al., 2010; Cadotte et al., 2013), with dispersal already being shown as a fundamental factor in the biogeographical history of Dipsadidae (Serrano et al., Chapter 1). This, added to the significant phylogenetic signal, suggests that closely related species are not present in the same BE but that this vicariant process has been stronger or that it occurred earlier in Central America. Additionally, it reflects that only a few lineages were able to successful diversify in South America, evidenced by Amazonia and Atlantic having the fewer genera despite being the largest BEs.

In conclusion, we show that there may be important intra-family differences in the impact of biogeographical barriers and consequent regionalization. The co-distribution patterns of Dipsadinae have likely been strongly influenced by eco- and geomorphological events in Central America since the limits of most Biotic Elements coincide with important geographical barriers. Additionally, divergence between closely related species confirms the timing and importance of limited dispersal likely caused by relevant geomorphological events. Furthermore, less pronounced regionalization patterns in South America - less Biotic Elements but with larger areas - might be due to its relatively recent history in South American forests due to their lower within-BE phylogenetic distances. Furthermore, South American Biotic Elements overlap prominent forest biomes such as the Amazon and the Atlantic Forest which suggests that the distribution of Dipsadinae is markedly impacted by ecological barriers related to habitat specialization. Our results offer a baseline to understand how Neotropical biota could have been shaped by vicariance, also highlighting the use of phylogenetic information to better test vicariant regionalization.

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5. Chapter 4: Mapping local and regional distribution of *Lygophis paucidens* Hoge, 1952 (Serpentes, Dipsadidae), an elusive snake from the sandy savannas of Brazil and Paraguay

PUBLISHED

Serrano, F. A. C., dos Santos Vieira-Alencar, J. P., Díaz-Ricaurte, J. C., & de Campos Nogueira, C. (2020). Mapping local and regional distribution of *Lygophis paucidens* Hoge, 1952 (Serpentes, Dipsadidae), an elusive snake from the sandy savannas of Brazil and Paraguay. Check List, 16(1), 75-81.

Abstract

Lygophis paucidens Hoge, 1952 is a rare Neotropical snake, previously mapped using only a few individuals in five localities. Herein we update and discuss the distribution and conservation status of *L. paucidens* within major Neotropical ecoregions, providing previously unavailable data on distribution and habitat use. We compiled and mapped point locality records from literature and museum specimens, complemented by field studies in three localities. We used those records to map the species Extent of Occurrence (EOO) and Area of Occupancy (AOO), two range-related metrics of central relevance for conservation. We recovered 52 records from 46 vouchered specimens and six field records, distributed in 35 individual point localities, all within the diagonal of open vegetation in South America, with most records in cerrado savannas with sandy soils. *Lygophis paucidens* is likely a psammophilous species, which might explain its occurrence in savanna enclaves in forested ecoregions and its potentially discontinuous distribution. This knowledge is, therefore, useful in aiding future conservation assessments.

Keywords: Biogeography, Cerrado, conservation, open grasslands, sandy soils, savanna, Xenodontinae.

5.1. Introduction

The Neotropical snake genus *Lygophis* Fitzinger, 1943 is distributed throughout South America, from Venezuela to Argentina and mostly associated with open areas of the Guyana shield or with savannas and drylands in the open diagonal of South America (Dixon 1989). This genus belongs to the tribe Xenodontini in the family Dipsadidae, the richest snake family in the Neotropical region (Grazziotin et al. 2012; Uetz et al. 2019). The genus *Lygophis* was recently resurrected by Zaher et al. (2009). It was supported as a distinct monophyletic group (Grazziotin et al. 2012; but see Curcio et al. 2009) and is comprised of eight species (Uetz et al. 2019).

Lygophis paucidens Hoge, 1952 is an elusive snake described from a specimen from Mato Verde, state of Mato Grosso, central Brazil. Since then, the most recent available map of its distribution was provided by Dixon (1989), using only five point localities. This species has not yet been assessed by the International Union for Conservation of Nature (IUCN 2019), and it is not included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) appendices (CITES 2017), although it is considered as “Least Concern” (LC) in the Brazilian List of Threatened Species of Fauna (Machado et al. 2008; ICMBIO 2018). Due to the low numbers of specimens, information on its natural history is still scarce. It preys mostly lizards of the family Teiidae (Hoge 1952; Dixon 1989) such as *Ameivula* cf. *ocellifera* Spix, 1825 (Michaud and Dixon 1989). *Lygophis paucidens* species is oviparous, diurnal, and terrestrial, actively hunting on the ground and occasionally found sheltered in leaf litter or under hollow logs (Pavan 2007; Rodrigues and Prudente 2011). Although *L. paucidens* is reported to occur only in open cerrado grasslands (Nogueira 2001), knowledge on its range and biogeographical aspects (such as elevation and ecoregions) are hampered by the lack of an updated map. Herein, we review the available reported localities and field records of *L. paucidens* and comment on its patterns of local and regional geographic distribution and conservation.

5.2. Materials and Methods

We reviewed deposited specimens at three herpetological collections in Brazil (CHUNB, Coleção Herpetológica of Universidade de Brasília; IBSP, Instituto Butantan; MZUSP, Museu de Zoologia da Universidade de São Paulo) (Table 1). Specimens were identified as *L. paucidens* by counting the number of maxillary teeth and ventral scales, coupled with a general analysis of colour pattern. We then compiled and reviewed the reported localities from the analysed vouchers and literature, and mapped them according to Brazilian biomes (IBGE 2019) and ecoregions outside Brazil (Dinerstein et al. 2017), and elevation using QGIS 3.4 (QGIS 2019). When exact coordinates were not available, we assigned records to the corresponding municipality using available online gazetteers (SpeciesLink 2019). Duplicate or uncertain records were not used. We also used additional data on field samples in three Cerrado localities (fieldwork data available in Nogueira 2001; Valdujo et al. 2009; Recoder et al. 2011) to provide data on activity and habitat use.

To further understand the distribution of *L. paucidens*, we used IUCN guidelines (IUCN 2019) and calculated EOO (extent of occurrence) and AOO (area of occupancy). EOO measures the general spatial range of a taxon, whereas AOO measures how much taxon-specific habitat is available and currently occupied by the taxon (IUCN Standards and Petitions Committee 2019). These metrics are analogous to the biogeographical scales proposed by de Candolle (1820): regional–station (EOO) and local–habitation (AOO). EOO was calculated using a minimum convex hull. For the more complex and data dependent AOO we analysed two contrasting and extreme scenarios: the first was obtained using only the available presence records; the second was obtained by mapping all areas with potential available habitat within the EOO polygon. In both cases (verified presence of potential presence in all areas of available habitat) we intersected point data with a 2 × 2 km grid cells (IUCN Standards and Petitions Committee 2019). To calculate available habitat we intersected these two layers: a land-use raster for remaining savanna patches in Cerrado (INPE 2013) and Caatinga (MMA 2011), as well as a soil type raster (Hengl et al. 2017), using areas with at least 60.5% of sand (the median value of % of sand in the soil of known presence records, see Results below). Both EOO and AOO were calculated using the GeoCAT Red List threat assessment support tool (Bachman et al. 2011).

5.3. Results

Lygophis paucidens Hoge, 1952

Figure 1A, B

Material examined. Table 1.

Table 1. Locality records for *Lygophis paucidens*. Collection codes for voucher numbers: CHNUB = Coleção Herpetológica da Universidade de Brasília; CHNUFPI = Coleção Herpetológica da Universidade Federal do Piauí; CZPLT = Para La Tierra Zoological Collection; CZDP = Coleção Zoológica Delta do Parnaíba; IBSP = Instituto Butantan; MNRJ = Museu Nacional, Rio de Janeiro; MPEG = Museu Paraense Emílio Goeldi; MZUFBA = Museu de Zoologia da Universidade Federal da Bahia; MZUSP =- Museu de Zoologia da Universidade de São Paulo; UFMT = Universidade Federal de Mato Grosso; URCA = Universidade Regional do Cariri. Source: 1 = field collected; 2 = literature; 3 = analysed specimens.

Voucher	**Country**	**Locality**	**State**	**Latitude**	**Longitude**	**Collected in**	**Source**	**Reference**
IBSP50343	Brazil	Alto Paraíso	GO	-14.08	-47.62	-	**1, 3**	
MPEG 17226	Brazil	Amarante do Maranhão	MA	-5.57	-46.74	1986	**2**	Prudente et al. 2018
CHUNB37262	Brazil	Arinos	MG	-15.91	-46.12	-	**3**	
MZUSP	Brazil	EE Uruçuí-Una, Baixa Grande do Ribeiro	PI	-8.88	-44.97	2000/2001	**2**	Dal Vecchio et al. 2013
CHUNB24448	Brazil	Núcleo Bandeirante, Brasília	DF	-15.78	-47.93	2001	**1, 3**	Nogueira 2001
MPEG 22791, 22802	Brazil	Castelo do Piauí	PI	-5.32	-41.55	2005-2007	**2**	Rodrigues and Prudente 2011
CHUNB3771, UFMT 675	Brazil	Chapada dos Guimarães	MT	-15.43	-55.75	-	**2, 3**	
CHUNB31335	Brazil	Cocalzinho	GO	-15.78	-48.77	-	**3**	
UFMT 2347	Brazil	Cuiabá	MT	-15.58	-56.08	-	**2**	
MZUSP9597	Brazil	Gentio do Ouro	BA	-11.43	-42.5	-	**2, 3**	Guedes 2012
MZUSP12702	Brazil	Guaraí	TO	-8.83	-48.5	-	**2, 3**	
IBSP10	Brazil	Ipameri	GO	-17.72	-48.16	1943	**2**	Hoge

448 - Paratype								1952
MNRJ 18656	Brazil	Jaborandi	BA	-13.62	-44.43	-	**2**	Guedes 2012
UFMT 34	Brazil	Jangada	MT	-15.33	-56.55	-	**2**	
MNRJ 15253	Brazil	Mateiros	TO	-10.74	-46.79		**2**	
IBSP12016 - Holotype	Brazil	Mato Verde, Luciara	MT	-11.22	-50.67	1948	**2**	Hoge 1952
CHUNB29666, MZUSP11106	Brazil	Niquelândia	GO	-14.05	-48.32	-	**3**	
CHUNB22070, IBSP64567, 65360, 65979	Brazil	Palmas	TO	-10.30	-48.35	-	**3**	
MZUSP14390	Brazil	Paranã	TO	-12.57	-47.88	2000	**2**	Pavan 2008
CZDP	Brazil	Ilha de Santa Isabel, Parnaiba	PI	-2.90	-41.78	-	**2**	Pereira and Guzzi 2015
CHUNB61141-44	Brazil	PN Sete Cidades, Piracuruca	PI	-4.11	-41.71	2010	**1, 3**	
MZUFBA1846, 1855	Brazil	Poções	BA	-14.54	-40.38	-	**2**	Guedes 2012
IBSP12832, IBSP12843 - Paratypes	Brazil	Rio São Domingos, Cocalinho	MT	-13.65	-51.15	1949	**2**	Hoge 1952
CZPLT-H122, H144	Paraguay	Laguna Blanca, San Pedro	San Pedro	-23.8	-56.29	2011	**2**	Cacciali et al. 2013
MZUSP10797	Brazil	Santa Rita do Araguaia	GO	-17.32	-53.2	-	**3**	
IBSP19959-60	Brazil	São Félix do Araguaia	MT	-11.62	-50.67	-	**3**	

IBSP51723	Brazil	São Francisco	MG	-15.95	-44.87	-	**3**	
IBSP1225-27 - Paratypes	Brazil	Teresina	PI	-5.08	-42.8	1917	**2**	Hoge 1952
CHNU FPI33	Brazil	Timon	MA	-5.36	-42.85	2005-2006	**2**	Silva et al. 2016
URCA-H5706	Brazil	Trairi	CE	-3.28	-39.27	2005-2013	**2**	Roberto and Loebmann 2016
CHUN B11558, 35356	Brazil	São Domingos	GO	-13.55	-46.35	-	**3**	
-	Brazil	José de Freitas	PI	-4.65	-42.35	2008	**2**	Cavalcante 2009
-	Brazil	Guarai	TO	-8.62	-48.32	2001	**2**	Pavan 2008
IBSP62696	Brazil	Assentamento Nascentes do Araguaia, Mineiros	GO	-17.67	-53.22	2000	**1, 3**	
MZUS P12874	Brazil	PN Grande Sertão Veredas, Formoso	MG	-15.25	-45.89	2001	**1, 3**	
-	Brazil	EE Serra Geral Tocantins, Mateiros	TO	-10.64	-46.65	2003	**1**	
-	Brazil	EE Serra Geral Tocantins, Mateiros	TO	-10.60	-46.81	2003	**1**	
-	Brazil	EE Serra Geral Tocantins, Mateiros	TO	-10.74	-46.79	2003	**1**	

Identification. *Lygophis paucidens* has a conspicuous pattern with three longitudinal dark lines on the back of the head (Fig. 1A. B), which quickly fade towards midbody. The hemipenis is clavate, with reduced lobes, reduced or absent interlobular sulcus and dorsal scale micro-ornamentation fasciculate (Moura-Leite 2001). It can be differentiated from other *Lygophis* species, such as *L. flavifrenatus* Cope, 1862, by the number of maxillary teeth (fewer than 13 in *L. paucidens*) and by its small optic foramen (Dixon 1989). Regarding pholidosis, *L. paucidens* presents the following counts: eight supralabials, 165–174 ventrals, 62–72 subcaudals, and 17-17-15 dorsal scale rows (Dixon 1989; Lema 1989; Cacciali et al. 2013).

Figure 1. *Lygophis paucidens*. **A.** *Lygophis paucidens* from Mineiros, GO, Brazil. **B.** *Lygophis paucidens* from Parque Nacional Grande Sertão Veredas, Formoso, MG, Brazil. **C.** Typical habitat (Serra Geral do Tocantins Ecological Station, TO, Brazil). Photos by Cristiano de Campos Nogueira.

Distribution. We compiled, reviewed, and mapped 35 point localities referring to 52 specimens of *L. paucidens* (Fig, 2), of which 46 were vouchered. Our map, thus, added 30 localities from the available literature and museum specimens to the point localities previously reported by Dixon (1989). This species was found to be widely distributed throughout several states from central and northeastern Brazil (Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Mato Grosso, Minas Gerais, Pernambuco, Piauí, and Tocantins) and in the San Pedro Department in eastern Paraguay (Table 1). Most point localities are within open and dry ecoregions, mostly within cerrado savannas (83.3%), with isolated records in semi-arid Caatinga. The two points outside these ecoregions are in transition zones with Atlantic Forest and Amazonia (Fig. 2).

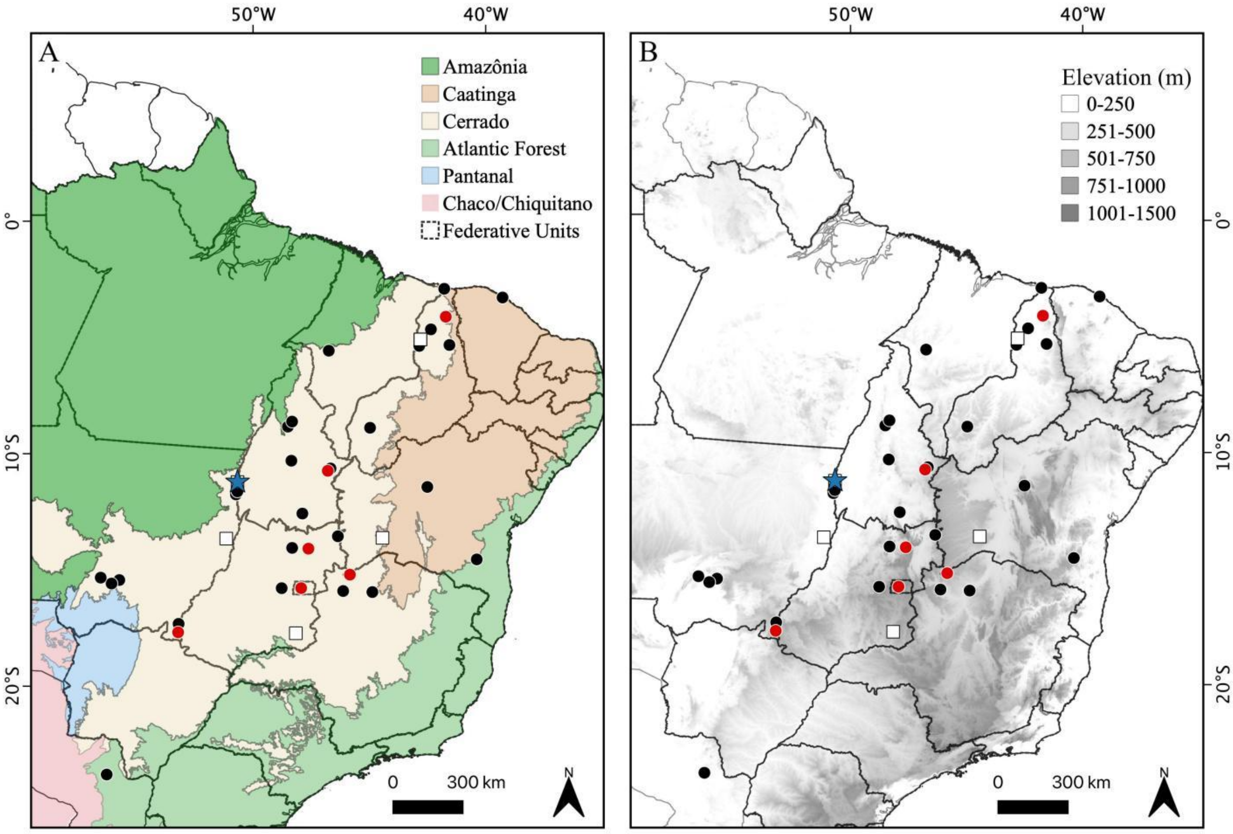

Figure 2. Geographical distribution of *Lygophis paucidens*. Map of distribution with **A.** ecoregions (adapted from IBGE 2019 and Dinerstein 2017) and **B**. elevation in South America. Localities previously mapped in Dixon (1989) are represented by white squares, of which ☆(blue star) denotes type locality. Circles represent localities previously not compiled and mapped: red circles represent field-confirmed records and black circles represent literature or museum records.

Habitat. *Lygophis paucidens* was found in three Cerrado localities: Emas National Park and surroundings, Mineiros, Goiás state; Grande Sertão Veredas National Park, Formoso, Minas Gerais state; and Serra Geral do Tocantins Ecological Station, Mateiros, Tocantins state. All specimens were active during the day in sandy soil cerrado savannas, such as campo sujo, campo cerrado or campo limpo formations (Fig. 1C). In the literature, this species was reported to occur on open cerrado (n = 5), forested cerrado (n = 2), palm marshes (n = 2), semi-open cerrado (n = 1), and coastal vegetation (n = 1) (Pavan 2007; Rodrigues and Prudente 2011; Cacciali et al. 2013; Dal Vecchio 2013; Roberto and Loebmann 2016; Silva et al. 2016). The median percentage of sand in the soil for the mapped localities was 60.5%. Moreover, *L. paucidens* is distributed over a broad altitudinal range, occurring from sea level to over 1200 m (Fig. 2B).

Conservation. Even though the estimated EOO of *L. paucidens* was wide, with over 2 million km^2 (2,300,322 km^2), AOO calculated from the 2 × 2 grid cells was much lower with only 148 km^2 (not mapped due to its small area). On the other hand, AOO calculated using the intersection of sand-rich soil areas (over 60.5 %, the median value of % sand in the soil for reported localities) and remaining savanna areas in Cerrado and Caatinga was nearly 1 million km^2 (946,176 km^2) (Fig. 3).

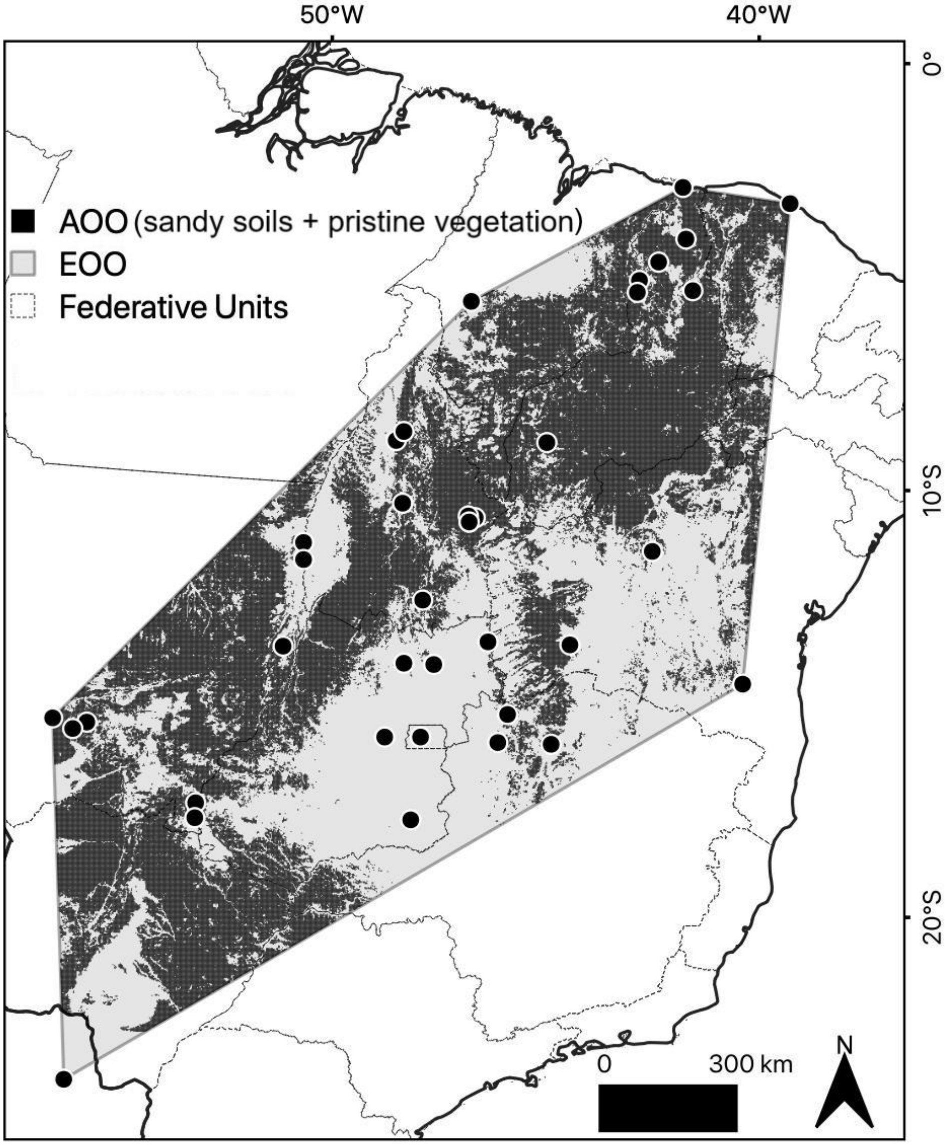

Figure 3. EOO (Extent of occurrence = 2,300,322 km^2) and upper bound of AOO (Area of occupancy = 946,176 km^2) of *Lygophis paucidens*. The upper bound of AOO is the total summed area of 2 × 2 km grid cells of remaining savanna patches on sandy soils in EOO.

5.4. Discussion

Lygophis paucidens was previously considered as endemic to the Cerrado, being restricted to central Brazilian savannas (Nogueira et al. 2010, 2011). Our new maps and new data reveal its presence outside the Cerrado ecoregion. However, most records are still within this ecoregion, or at least close to its contact areas, especially with Caatinga (e.g. Bahia: Gentio de Ouro and Jaborandi; Piauí: Parnaíba and Teresina). However, in large scale ecoregion maps these relatively small and isolated patches of savannas are not visible and mapped, due to a problem of scale. Thus, the presence outside the Cerrado core region may simply reflect this shortcoming of large scale ecoregion maps that fail to capture local habitat variation and, thus, may obscure detailed distribution and endemism patterns. We caution that the detection of ecoregion endemics must always take into consideration that boundaries between major vegetation units must be taken with care, as contact areas between ecoregions are much more complex than linear boundaries suggest. Thus, we hypothesize that the presence of *L. paucidens* outside the main limits of the Cerrado is associated with small Cerrado enclaves within other vegetation zones, which are impossible to map at the continental scale. As a clear example, the record in Paraguay, far outside the Cerrado limits, is reported from a typical open, cerrado area on sandy soils (Laguna Blanca; Atkinson et al. 2008). Moreover, Sete Cidades National Park (Piracuruca), mapped in the Caatinga region, is actually dominated by typical cerrado savannas with sandy soils (Castro et al. 2010).

Until 2001, *L. paucidens* had only been reported for six localities (Nogueira 2001), but recent reports have considerably extended its range, including the single locality outside Brazil, a cerrado area in Reserva Natural Laguna Blanca (Paraguay), which extended this species' distribution by more than 700 km southwest from the nearest record (although reported erroneously as 320 km by Cacciali et al. 2013). It is unclear whether *L. paucidens* occurs continuously, since there are noticeable gaps in its distribution. These gaps are especially evident within the Brazilian Cerrado and in relatively well-sampled localities (e.g. Emas National Park), where it was only found outside the park, in sandy soil savannas near the Araguaia headwaters, but not inside the park, dominated by tabletop plateau savannas with clay-rich soils (Valdujo et al. 2013). This species, thus, seems to occur mainly on patches of sandy soils (Cacciali et al. 2013; this work), which suggests a discontinuous distribution across central Brazil, or at least rarified distribution outside areas dominated by sandy soils. We argue that this strong psammophilous association might explain its rarity and disjunct distribution and support its existence in patchy open habitat enclaves in other ecoregions (Guedes 2012), such as transition zones with Caatinga, Atlantic Forest, and

Amazonia. This same distribution pattern and association with sandy soils is shared with other snake species such as *Bothrops lutzi* (Recoder et al. 2011) and *Psomophis joberti* (Nogueira et al. 2011).

Our study highlights the need to couple regional distribution and natural history data on local distribution to properly assess habitat, as proposed by early biogeographers such as de Candolle (1820). These two scales of study are complementary, and we can only understand ranges by adding detailed local scale information (as proposed by Wallace 1854) to large-scale mapping and revision of museum records, which might help to assess species conservation status. For *L. paucidens* the difference between the estimated lower and upper values of estimated AOO is remarkable. Using only known presence records (lower bound, see IUCN 2019) leads to classifying the species as "Endangered" , EN. On the other hand, using the much higher AOO estimated using all potential habitats, would classify the species as "Least Concern". These two extremes should be documented but avoided (see IUCN 2019), and new detailed mapping methods, coupled by expert opinion on range limits, could provide a more realistic estimate of AOO and a better assessment of threat, based on intermediate AOO values. This extreme variation in estimated AOO may be common in species with wide but discontinuous and complex ranges, and represents a challenge for biogeography and conservation assessments (e.g. see Cardoso et al. 2012; Maes et al. 2015). Species with relatively wide ranges, but with localized habitats and rarified ranges may be overlooked in threat assessments due to their large EOO values, and the wide potential variations in AOO.

We hypothesize that further studies on other localities with sandy soils—especially inside the EOO—may reveal additional records of this species, including sandy savanna enclaves outside the core Cerrado area in Brazil. Those records outside the Cerrado core area may reflect the presence of localized patches of savannas on sandy soils, indicating relictual savannas not may not visible in continental scale maps (Furley 1999). Moreover, we suggest that the range of *L. paucidens* is naturally discontinuous, given its strong association to a specific discontinuous habitat type: savannas with sandy soils. Finally, we suggest analysing in detail life-history aspects of species with scarce information (e.g. habitat preference), which can help to connect local and regional patterns of their distribution (de Candolle 1820; Blondel 1987), and aid in understanding relictual or disjunct ranges of other similarly distributed taxa.

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6. Chapter 5. The Wallacean Shortfall and the role of historical distribution records in the conservation assessment of an elusive Neotropical snake in a threatened landscape

PUBLISHED

Serrano, F. C., Vieira-Alencar, J. P., Díaz-Ricaurte, J. C., Valdujo, P. H., Martins, M. & de Campos Nogueira, C. (2023). The Wallacean Shortfall and the role of historical distribution records in the conservation assessment of an elusive Neotropical snake in a threatened landscape. Journal for Nature Conservation 72, 126350

Abstract

Documenting species distribution is essential to assess their conservation status and subsequent conservation actions. Historical records are thus essential to understand how species are distributed and how their range has changed throughout time. However, using historical records might contribute to overestimating the species current range and misrepresent their conservation status. Here, we illustrate the pitfalls of this approach using a widespread but elusive Neotropical snake species, *Philodryas livida* (Dipsadidae Bonaparte, 1838). We mapped occurrences of this species throughout time and calculated its Extent of Occurrence and Area of Habitat. Our results show that due to the intense, widespread anthropic land-use transformation since the discovery of *P. livida* in 1920, most historical localities are now likely unsuitable for its occurrence and both its current Extent of Occurrence and Area of Habitat become remarkably smaller (5.7% and 19.1% remaining, respectively) if only localities from the last 30 years are considered. Besides the natural elusiveness of the species, intense habitat loss and fragmentation may also explain the low number of recent sightings of *P. livida,* all concentrated within or near protected areas, indicating a putative decline in range relevant to its conservation status. We thus highlight how failing to consider the date of records and the associated land-use change throughout time might underestimate species range reduction and thus threat status. We strongly encourage the inclusion of the date of each occurrence record in conservation assessments, as suggested by the IUCN's mapping standards, such that historical records are carefully considered, especially in highly dynamic and threatened biomes such as the Cerrado savannas in Brazil.

Keywords: Area of Habitat, Extent of Occurrence, IUCN, Land-use range, rarity, threatened species

6.1. Introduction

Detailed knowledge on species' geographical distributions is fundamental to render accurate biogeographical interpretations and conservation strategies, especially in megadiverse and poorly sampled countries or regions (Ficetola et al., 2014, Nogueira et al., 2019). The need for accurate occurrence records dates from Wallace (Wallace, 1852) and the lack of detailed data on species distributions is named on his behalf as the "Wallacean Shortfall" (Lomolino, 2004). Every new distribution record increases our knowledge of species ranges and is likely a contribution to decreasing the Wallacean Shortfall. However, not every record is known to the scientific community, either because it is not made public through scientific publishing or because voucher specimens are not deposited in biological collections which, at least for some taxa, are required for a record to be recognized as valid. Despite that, the recent increase in digital availability of occurrence records from museums and herbaria is providing useful data for a primary understanding of many species' geographic distribution (e. g., GBIF - https://www.gbif.org; see Gaul et al., 2020). Nonetheless, the accuracy of such data has been questioned, and relevant limitations to the use of this information have become evident (Ficetola et al., 2014; Gaul et al., 2020; Zizka et al., 2020).

The range size of a species is one of the primary criteria considered to estimate its extinction risk (IUCN, 2001), and is widely used in species conservation assessments. However, occurrence data for a great number of species is still sparse, inaccurate or inexistent (Nogueira et al., 2019), resulting in information about species distributions that could be incomplete for many ecological and mechanistic applications (Gaul et al., 2020). Furthermore, even though habitat loss is the most important factor behind the current biodiversity crisis (Schipper et al., 2008; Powers & Jetz, 2019 ; Cox et al., 2022), its impacts are not homogeneously distributed around the globe. While long-occupied (e. g., Europe), inhospitable (e. g., the Great Australian Desert) or very remote regions (e. g., portions of the Amazon forest) have remained mostly unchanged in the last century (Sanderson et al., 2002; Kaplan et al., 2009; Myers et al., 2000; Moutinho et al., 2016; Williams et al., 2020, but see Mataveli et al., 2021), other landscapes are currently experiencing a high degree of human-induced transformation (e. g., the Cerrado savannas; Strassburg et al., 2017; Pacheco et al., 2021). Similarly, accounting for habitat loss on a finer scale reveals that particular areas are unequally prone to suffer from distinct aspects of land-use conversion (Strassburg et al., 2017; Grande et al., 2020; Pacheco et al., 2021). Recent rates of land-use conversion also led to rapid losses of landscape connectivity which hampers population viability, resulting in local extinctions (Thompson et al., 2017; Grande et al., 2020).

Although the complexity and time-wise dependency of geographical range limits have been long recognized, "time" has mostly been considered in a geological timescale framework (Upchurch & Hunn, 2002; Gaston, 2003). While small-scale local habitat aspects have been encouraged to be incorporated into conservation assessments (Brooks et al., 2019; Serrano et al., 2020), the changes of species distributions in ecological time remain largely unexplored. This is especially troublesome regarding rare (naturally scarce) and/or elusive (rarely detected) species (see Rabinowitz, 1981) that have wide distributions because new occasional or inaccurate records might significantly further increase the area of its range. Furthermore, the assessed area of distribution of elusive but widespread species might vary from a large continuous area to disjunct small patches, depending on how the current range is interpreted.

Similarly, older records without any recent confirmation by nearby faunal inventories may introduce a similar bias, potentially influencing its extinction risk assessments. Historical distribution records, for instance, often present inaccuracies about their geographical locations and are sometimes disregarded in fine-scale studies on species distribution modeling (Franklin, 2010). Additionally, if historical records are not supported by recent sightings of a species in the same general location – provided there was enough sampling effort to detect the species – they may inflate the estimated range . On the other hand, historical records are increasingly relevant since they provide a general overview of a species distribution, contributing to the understanding of large-scale biogeographical processes (Raxworthy et al., 2003). Thus, systematically identifying historical distribution records that may currently correspond to unsuitable areas for a given species with just a few known localities may improve our ability to properly assess its conservation status, as suggested by the IUCN (with parts of the species range being classified as Possibly Extinct) (IUCN, 2012), and by the recently proposed Area of Habitat (AoH) approach (Brooks et al., 2019). Herein, we use a potentially widespread but elusive Neotropical snake species, *Philodryas livida*, as a case study to highlight how using historical distributional information without accounting for the date of each record may lead to inaccuracies that are especially pervasive regarding extinction risk assessments.

6.2. Materials and Methods

6.2.1. Study species

Philodryas livida (Amaral, 1923) has a potentially wide distribution in Central South America, being considered endemic to the Cerrado savannas of South America (Nogueira et al 2011; Nogueira et al., 2019) in Brazil and northeastern Paraguay (Nogueira et al., 2019). Little is known about its natural history since it is rare in scientific collections, but it has been observed in the wild exclusively in grassland areas at intermediate to high elevations (133–928 m; N = 29 records in literature and museums; see Nogueira et al., 2019; Supp. Mat. 1).

6.2.2. Data collection and Mapping

We compiled distribution point locality records of *P. livida* from the literature (e. g., Thomas and Fernandes, 1996; Valdujo et al., 2009; Nogueira et al., 2019) and matched these records with the available collecting information at the herpetological collection of Instituto Butantan to complement our dataset with the approximate date of collection of each known specimen. Furthermore, we also gathered point occurrences from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org), using only vouchered records with collection date, coordinates and a location precision of less than 5 km, since non-filtered datasets might bias conservation assessments (Zizka et al., 2020). We mapped the evolution of its known range from the first collected specimen until the present by subsequently adding the records reported in the following decades (progressive approach). We used a Minimum Convex Polygon (MCP) formed by the species records (cf. Extent of occurrence, EOO; IUCN, 2001) to illustrate how knowledge on this species' range evolved throughout time. We then departed from the current known range of the species and subsequently removed the oldest records by decades (regressive approach). Importantly, if a given locality had specimens from different decades, we kept the records in the map up to the most recent decade when the species was captured in that locality.

Additionally, to assess if *P. livida* might have gone undetected in recent years due to sampling bias or low sampling effort, we reviewed published surveys both in localities where it had been previously reported and within its EOO formed by all known records. For the localities where the species has been previously detected, we searched for a combination of the terms "herpeto* OR [locality] OR inventory OR checklist OR snake OR reptile" in Google Scholar, where [locality] corresponded to the name of the municipality of known records. For localities within its reported range, we searched for "herpeto* AND cerrado AND inventory AND checklist AND snake AND reptile" and considered only surveys inside a buffer of 150 km around *P. livida*'s EOO, in order to minimize potential omission errors. For every survey, we recorded the last sampled year, duration of sampling, sampling effort (in days), sampling method, coordinates, type of habitat and whether *P. livida* or other species of the genus *Philodryas* had been recorded (Supp. Mat. 2).

6.2.3. Estimation of EOO and AoH

We used the MCP formed by the full dataset (i.e. with all known records) to estimate the current extent of occurrence (EOO), and the Area of Habitat (AoH, *sensu* Brooks et al., 2019) of *P. livida*. The EOO represents a measure of area by only considering the MCP and thus the overall extent of a species range (IUCN, 2012; Serrano et al., 2020), while AoH is an alternative to the estimation of AOO (area of occupancy) that takes into account the total area of the preferred habitat of the species, restricted to the elevational range where the species have been detected (Brooks et al., 2019). We calculated the AoH of *P. livida* as the total area occupied by grasslands (preferred habitat of the species; Nogueira et al., 2019; CCN and PHV *personal observation*) occurring between 200 and 900 m above the sea level, and restricted to the limits of the Brazilian Cerrado as proposed by Dinerstein et al. (2017). This elevation range takes into account the elevation of the most recent specimens collected (last three decades, see Results and Supp. Mat. 1) because old records are more likely to present inaccuracies than the most recent ones (e.g. Zizka et al., 2020). We used land-use data provided by the MapBiomas initiative for the year of 2020 (collection 6; the most recent release; MapBiomas, 2022), but disconsidered the 3.6% of the total EOO of *Philodryas livida* corresponding to Paraguayan records since data for the Cerrado is restricted to the political borders of Brazil. We used QGIS 3.24 (QGIS Development Team, 2017) to estimate the EOO, and Google Earth Engine (Gorelick et al., 2017) to estimate the AoH.

6.3. Results

Since its description in 1923, a total of 29 specimens of *Philodryas livida* have been collected in 14 localities (Fig. 1, Table 1). Georeferenced information was not available for two records (IB3681 and IB40953; Supp. Mat. 1). Most records were obtained before the late 1970s, with gaps of records in the 1960s, and 1980s. From the 1990s to the 2000s, the calculated EOO for *P. livida* increased from 72,918 (since the 1970s) to 215,901 km^2, when five individuals were collected at Emas National Park, Mineiros municipality, Goiás state, Brazil, between 1997 and 2001 (Valdujo et al., 2009, Table 2). Additionally, a new record revealed the persistence of the species in the municipality of Itirapina, state of São Paulo, in the same decade. Since then, the species has been recorded only twice, in 2011 and 2013, at the Reserva Natural Laguna Blanca in Paraguay (Smith et al., 2014), which expanded its range to the current known extent (Fig. 2, Table 2: “Full”) while also representing the first record outside Brazil. With our regressive approach, considering only records from the last three decades (n = 5 in three localities), the EOO and AoH of *P. livida* decreased 94.3% and 77.0%, respectively, (Fig. 3) in relation to those parameters for the full dataset. Remarkably, all recent records were reported from within or in the surroundings of protected areas. In the last three decades *P. livida* has been detected in only three out of the 14 localities where it had been known to occur. Remarkably, two of these records are from the last ten years while the remaining is spaced by at least 20 years from the present.

To serve as a counter-example, we also preliminarily use as comparison the locally-abundant and commonly-found *P. patagoniensis*, a phylogenetically-related species (Arredondo et al., 2020) that is sympatric with *P. livida* and widely distributed across open habitats in South America (López and Giraudo, 2008; Nogueira et al., 2019). We recorded 795 dated occurrences of *P. patagoniensis* in the same time period as reported for *P. livida* (out of 1594 total occurrences; 165 from the herpetological collection of the Instituto Butantan and 630 from GBIF), with 115 records (14.5% of total records) within *P. livida*’s range. Similarly to *P. livida*, the progressive approach also shows a steady increase in species range size throughout time (Fig. 2). Our regressive approach showed a constant decrease in range size only when regarding collection records after the 1960s. When considering only points from the last 30 years, there was a decrease of 66.3% of EOO.

Table 1: Number of individuals of *Philodryas livida* reported for each locality and each decade. * denotes a record which has been erroneously perpetuated in scientific literature. ** refers to a locality in Paraguay, the only record outside of Brazil. The original reported location is “Fazenda Santa Bárbara” with no details on municipality or state but several papers have wrongfully attributed this record to “Águas de Santa Bárbara, state of São Paulo”. This is another potentially pervasive consequence of historical records, as often their exact location is less accurate or uncertain.

Locality	**1920**	**1930**	**1950**	**1960**	**1970**	**1980**	**1990**	**2000**	**2010**
Corumbá	1							0	
Miranda	2								
São Carlos		3							
Itatinga		1							
Itirapina		2					1		0
Agudos			1		1				0
Fazenda Santa Bárbara*					1				
Brotas					1				
Campo Grande					1				
Lençóis Paulista					3				0
São Manuel					1				
Limeira					1				
Mineiros							5		
Santa Bárbara/PY**									2
Total	3	6	1	0	9	0	6	0	2

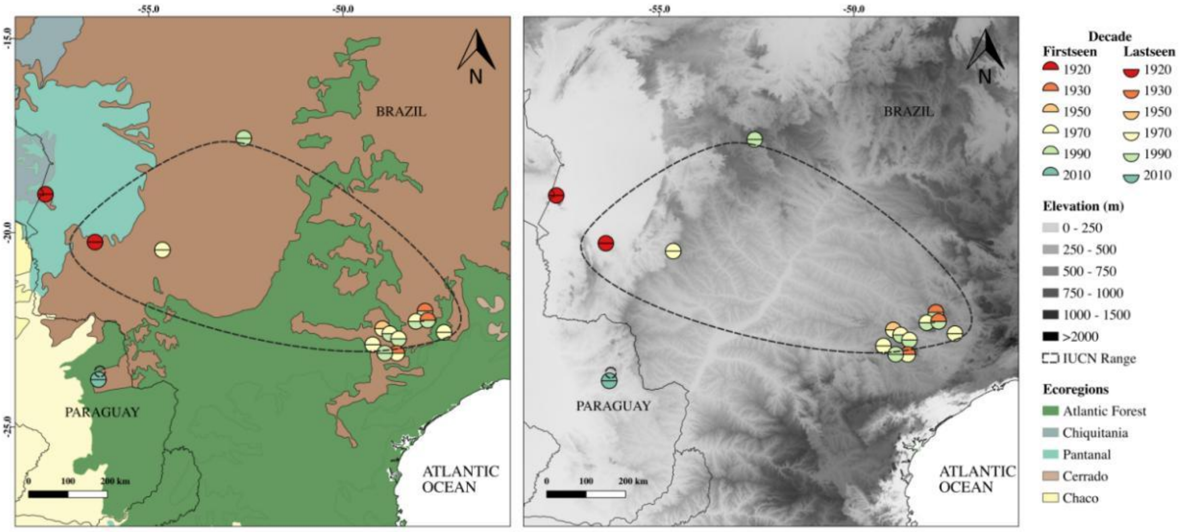

Figure 1. Distribution records (colored according to first and last seen decade) of *Philodryas livida*, with ecoregions (left) and elevational variation (right). The area within the dotted line corresponds to the species current IUCN's range.

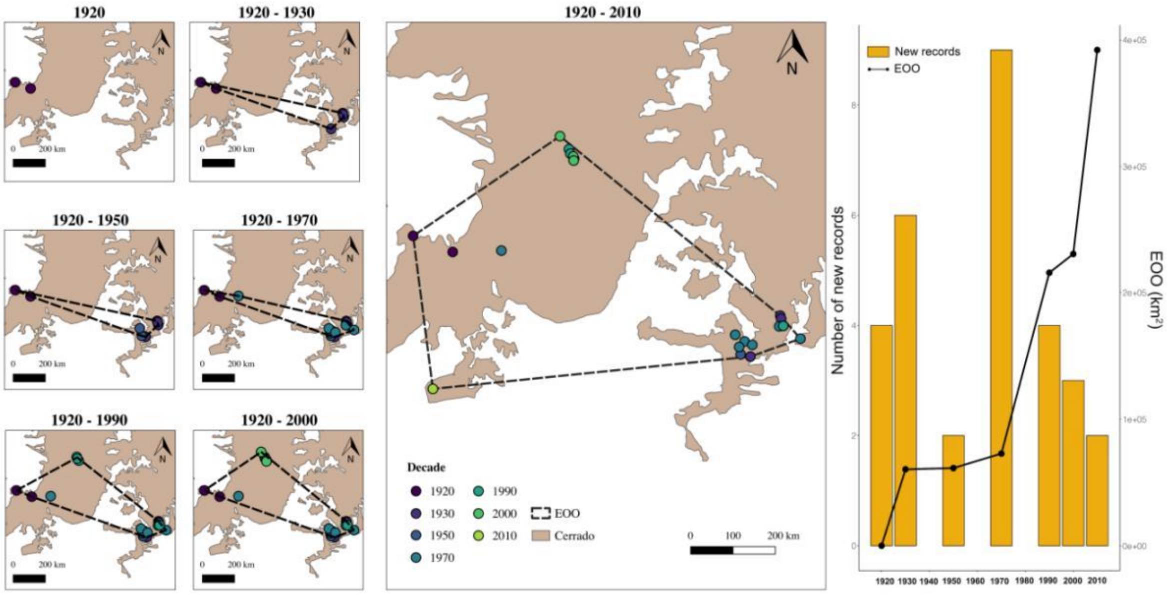

Figure 2. Progressive approach showing how *Philodryas livida*'s EOO evolved throughout time by iteratively adding localities from subsequent decades. The graph (left) shows how many new records were reported and the corresponding increase in EOO.

Table 2: Extent of occurrence (EOO) and Area of Habitat (AoH, *sensu* Brooks et al., 2019) of *Philodryas livida* in square kilometers (km^2) in two different scenarios (see main text for details). The EOOs and AoH are illustrated in Fig. 2 and Fig. 3 for the progressive and regressive approaches, respectively.

Progressive approach			**Regressive approach**			
Dataset	**EOO**	**AoH**	**Dataset**	**EOO**	**AoH**	**EOO %**
1920-1930	60,445	309	Full	392,075	2,295	100.0
1920-1950	61,422	326	1930-2010	310,352	2,109	79.2
1920-1970	72,918	377	1950-2010	300,246	2,077	76.6
1920-1990	215,901	1,439	1970-2010	292,768	2,060	74.7
1920-2000	230,724	1,693	1990-2010	270,927	1,978	69.1
Full	392,075	2,295	2000-2010	22,350	539	5.7

In our review we found three snake surveys in previously recorded localities of *P. livida*, with none recording the species' presence. The time elapsed between the last vouchered individual of the species and surveys in these localities ranged from 12 years in Itirapina (São Paulo, Brazil) to 83 years in Corumbá (Mato Grosso do Sul, Brazil). The total sampling effort was 544 days among studies (average of 181.3 ± 231.5 days), consisting of active search, search by car and pitfall traps, detecting *P. aestivus*, *P. olfersii* and *P. patagoniensis.* We also found nine snake surveys within the *P. livida* buffered MCP, most within or near protected areas, and with an average duration of 8.7 (± 6.9) months. The total sampling effort was 299 days (average of 42.7 ± 38.8 days), employing active search and pitfall traps. All these surveys failed to detect *P. livida* but five of them detected other species of the genus *Philodryas*: *P. agassizi* (n = 1), *P. mattogrossensis* (n = 2), *P. nattereri* (n = 2), *P. olfersii* (n = 3) and *P. patagoniensis* (n = 1).

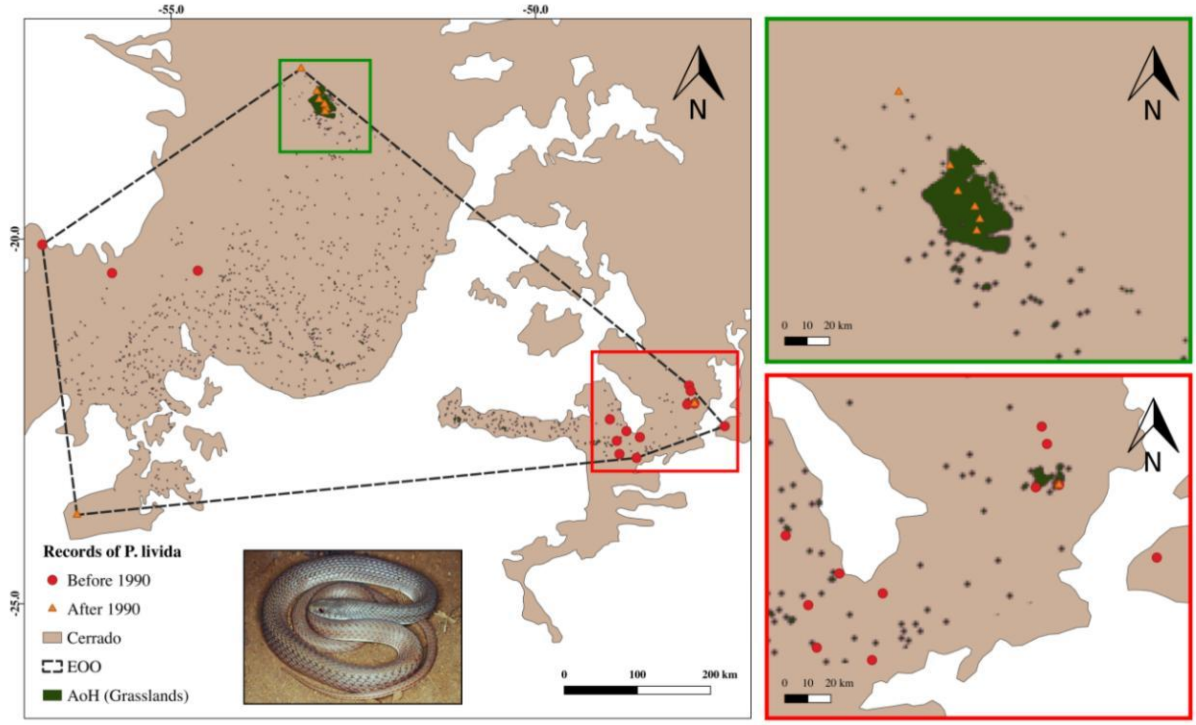

Figure 3. EOO and AoH of *P. livida* with records prior to and after 1990, with insets showing remaining areas of continuous suitable habitat (top right; Parque Nacional das Emas, Goiás state, Brazil) and areas under strong land-use change (bottom right; São Paulo state, Brazil).

6.4. Discussion

Understanding and measuring ranges is of paramount relevance for biodiversity science and conservation assessments (Mace et al., 2008; Rattis et al., 2018). Indeed, 83% of the categorizations of threatened snakes in the IUCN Red List (version 2021.1, IUCN, 2021) use the criterion B, which includes small range size as a mandatory subcriteria (IUCN, 2012). Our results show that historical records of an elusive species help to understand how distribution changed throughout time in a highly dynamic and altered landscape. However, this may lead to inadequate conservation assessments if all records (both historical and recent) are considered as evidence of species occurrence . Therefore, instead of carelessly using these records or fully disregarding them, they should be used with caution in appropriate data-informed contexts. Here we assess how the use of historical records can mislead the extinction risk assessment of a species if changes in land-use and suitable habitat are not considered.

Our results indicate that the distribution of *P. livida* has likely changed in recent decades. This leads to substantial decreases of EOO and AoH if only the last three decades are considered. Furthermore, the IUCN's most recent extinction risk assessment for the species (Scott et al., 2020), is based on a hand-drawn polygon which corresponds to an EOO of 410,216 km^2, which is 18.141 km^2 (4.62 %) larger than our most conservative estimate (progressive approach, Fig. 1) and 387,866 km^2 (1735.42 %) larger than our regressive approach. Although *P. livida* may have had a large range originally, it may be a rare species in two other aspects of rarity: it is specialized in an increasingly rare habitat (Cerrado grasslands) and may occur in low abundances (cf. Rabinowitz, 1981), at least in some localities (e. g. Reserva Natural Laguna Blanca). If this is the case, the sampling effort needed to detect it might be higher than that reported here for the recent studies carried out in areas where the species was previously found. However, if the opposite is true, it may have disappeared throughout most of its original range. Overall, the species has not been recorded in Brazil since the 1997–2001 survey by Valdujo et al. (2009), with its most recent records coming from a highly protected area of pristine habitat in Paraguay, where it took over 55 months of sampling to find two individuals (Smith et al., 2016), supporting that it could be the case that *P. livida* is naturally rare. However, other species of the genus *Philodryas* have been recorded in its range in the same timeframe while sharing some of its ecological attributes such as body size and period of activity (Feldman et al., 2015), although none of these species were found in large numbers (1–3 individuals). Conversely, in Parque Nacional das Emas – a large preserved protected area with extensive grasslands – *P. livida* had more than twice the abundance of other *Philodryas* species, which would indicate that this species might have high abundances and/or be easily detected in areas with suitable habitat (Valdujo et al., 2009).

Records of *P. livida* from the last 30 years have come exclusively from within or around protected areas. This may represent a spatial sampling bias because many snake inventories have been extensively conducted in protected areas (e. g., Ilha Solteira, Porto Primavera, Serra da Mesa). This highlights the importance of evaluating new sites for creating new protected areas, especially in the southern Cerrado savannas (Resende et al., 2021), considering that only 6.5% of the ecorregion's native vegetation is represented within protected areas (Françoso et al., 2015) and that the southern portion of the Cerrado is historically the most affected by land-use conversion (Strassburg et al., 2017). On the other hand, many older distribution records of Brazilian snakes come from third-party collaborators such as local residents and landowners, who used to send snakes to the Butantan Institute and other antivenom producing institutes by railway (Fernandes and Chaves, 2014). Indeed, most records of *P. livida* are from the 1970s, coinciding with the implementation of the "Pró-Álcool" program, which aimed to increase Brazil's internal production of sugarcane-based ethanol fuel (Rosillo-Calle & Cortez, 1998). This program extensively modified the species habitat but may have increased chance encounters of snakes, nonetheless leading to more than tenfold the number of reported individuals of other *Philodryas* species compared to *P. livida*. As the IUCN's extinction risk assessments may use range size as one of the subcriteria (under the widely used criterion B), overestimating a species range size by incorporating historical records not supported by recent data not only precludes our ability to assess its actual threat status, but also negatively impacts our efforts to analyze its distribution within an adequate framework of prioritization. Finally, the proximate cause of not taking into account historical distributional data is the fact that researchers generally do not have access to accurate date of collection of most records for many species, especially in large comprehensive distributions summaries (e. g., Nogueira et al., 2019; but see Serrano et al., 2020), and even in online based distribution records (e. g. only 49.8% of *P. patagoniensis* records were dated in GBIF).

It is about time to look into species distributions regarding short-term changes in their ranges without necessarily disregarding the relevance of historical records to understand historical patterns derived from geological time frames. These two pieces of information reflect different landscape processes and thus should be used in different contexts, which requires clear directives of which records should be used and in which approach. Information on habitat change and likely extinct populations can also be considered in novel approaches such as the Species Threat Abatement and Restoration" (STAR) metric, which evaluates the potential benefit of actions that aim to reduce threats and restore habitat for threatened species such as *P. livida* (Mair et al., 2021). We encourage researchers and conservation practitioners to adopt and stimulate among peers the habit of disclosing the detailed collection date of as many species distribution records as possible, especially in geographical distribution summaries (e. g., Serrano et al., 2020). This might increase our capacity of discussing a species conservation status while taking into account the factors involved in the decision of considering a species absent from a given historical occurrence site as well as considering the opinion of specialists on how different taxa are expected to be recorded.

We argue that a first step to better assess the contribution of historical records is to have access to the precise date of collection of distribution records. This should be followed by an expert evaluation of what in fact should be considered “historical” in a species or group specific context and how these records have been impacted by land-use changes that may reduce or lead to the disappearance of suitable habitat. This detailed information and expert knowledge can then be incorporated into IUCN’s standard methodology of classifying parts of a species range as ‘Possibly Extinct’ and to better evaluate the conservation status of the species. We also highlight the importance of thorough identification by taxon specialists, of depositing vouchers in scientific collections and especially of making this information public and widely available to use, in order to better understand how the distribution of species, in particular rare and/or elusive ones, might have changed over time. None of this can be achieved unless a considerable change of perspective is considered for both authors and journals dedicated to publishing, for example, distribution summaries. It is necessary to provide at least the basic information that compose a species distribution records: "What, Where and When" (Isaac & Pocock, 2015; Gaul et al., 2020) and to stimulate the publication of checklists even if they are derived from short-term, non-hypothesis driven studies, and especially when they are supported by vouchers deposited in public collections. These changes in the way we deal with distribution records and their use in conservation will help to reduce the Wallacean Shortfall.

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7. FINAL REMARKS

One of the main challenges of studying and preserving biodiversity is how to deal with data, or the lack of it (Zizka et al., 2020). Even though we are now entering the era of 'Big Data', many regions and its associated taxa still lack information on many aspects. Thus, biodiversity scientists must often work with incomplete and/or unrepresentative data which represent important gaps of knowledge or 'shortfalls' (Hortal et al., 2015). These shortfalls might be of species range (Wallacean shortfall), its phylogenetic relationships (Darwinian shortfall) or its abiotic tolerances such as habitat (Hutchinsonian shortfall). These gaps are especially evident for both reptiles (Etard et al., 2020) and the Neotropical region (Nogueira et al., 2019; Lees et al., 2020).

In my thesis, I aimed to understand the patterns and processes behind the distribution of Dipsadidae, the richest snake clade in the Neotropical region, using several approaches linked to their historical biogeography, evolution and niche. I showed that this clade has a complex evolutionary and biogeographical background which has influenced its current distribution patterns. Furthermore, I highlight that the relevance of looking into intra-family differences, with the Xenodontinae and Dipsadinae subfamilies differing in important aspects of vicariant speciation and habitat use. Below, I emphasize the main outcomes of this thesis and further directions that will help to better understand and generate data-driven strategies of conservation for this fascinating snake family.

Chapter 1: There and back again: when and how Dipsadidae, the richest Neotropical snake clade, dispersed and speciated throughout the Americas

Conclusions:

- Dipsadidae has an Asian origin;
- The two main Neotropical subfamilies originated in Central America, and dispersed to South America in distinct events in different time periods;
- The current biogeographical patterns of the family Dipsadidae have been shaped by complex evolutionary and geological processes such as Eocene land bridges, Andean uplift and the formation of the Panama isthmus.

Chapter 2: One clade, two histories: Phylogenetic niche conservatism drives distribution patterns of Dipsadidae, the richest Neotropical snake family

Conclusions:

- The current large-scale biogeographical patterns of the richest snake family in the Neotropical region likely arose from clade-specific responses to ecological constraints.
- In South America, Dipsadinae attain higher proportion of composition in forests
- Dipsadinae is a forest specialist and Xenodontinae is a habitat generalist.

Chapter 3: Snakes on a puzzle: regionalization of Neotropical dipsadids reflects intra-clade phylogenetic differences of biogeographical processes

Conclusions:

- There are important intra-family differences in the impact of biogeographical barriers and consequent regionalization
- Only Dipsadinae co-distribution patterns seem to be majorly caused by vicariance
- Less pronounced regionalization patterns in South America - less Biotic Elements but with larger areas, mainly in forest regions - distribution of Dipsadinae is markedly impacted by ecological barriers related to habitat specialization
- Vicariance can be inferred combining phylogenetic information with regionalization

Chapter 4: Mapping local and regional distribution of Lygophis paucidens Hoge, 1952 (Serpentes, Dipsadidae), an elusive snake from the sandy savannas of Brazil and Paraguay

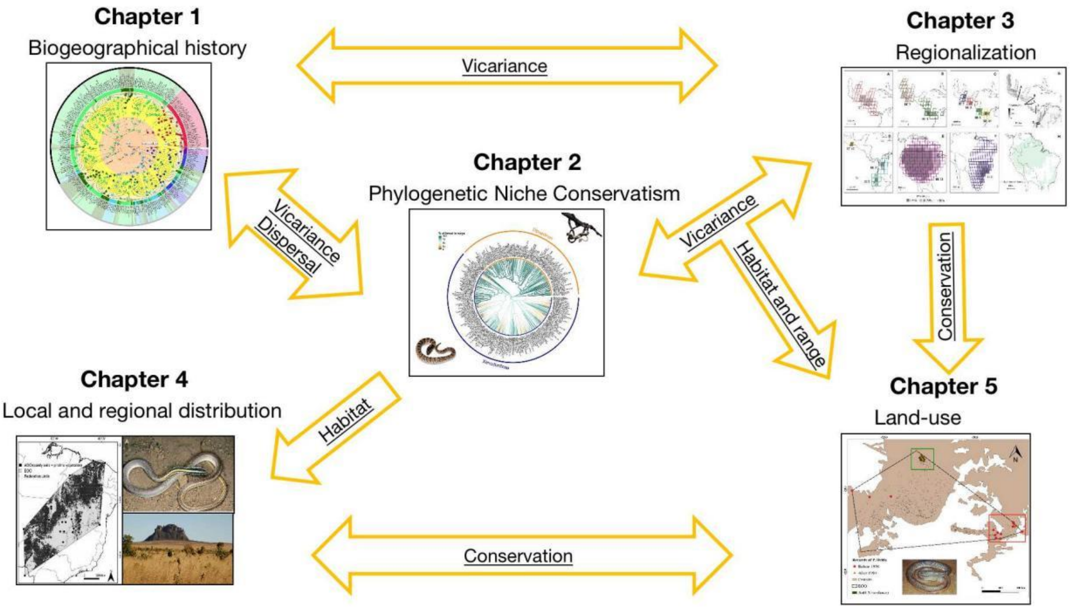
Conclusions:

- *Lygophis paucidens* is likely a psammophilous species, might explain its occurrence in savanna enclaves in forested ecoregions and its potentially discontinuous distribution.
- Our study highlights the need to couple regional distribution and natural history data on local distribution to properly assess habitat

Chapter 5: The Wallacean Shortfall and the role of historical distribution records in conservation assessments of an elusive Neotropical snake in a threatened landscape

Conclusions:

- Historical distribution records, albeit valuable, might underestimate and misrepresent the conservation status of elusive species;
- Not considering the date of records and the associated land-use change might underestimate species range decrease and thus threat status.


Chapter 1
Biogeographical history
Vicariance
Chapter 3
Regionalization
Chapter 2
Phylogenetic Niche Conservatism
Vicariance
Dispersal
Vicariance
Habitat and range
Conservation
Chapter 4
Local and regional distribution
Habitat
Chapter 5
Land-use
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List of published articles during the Ph.D.

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1. **Serrano, FC**; Díaz-Ricaurte, JC; Martins, M. (2022). Finding love in a hopeless place: a global database of misdirected amplexus in anurans. Ecology. 103(8): e3737.

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Conferences and Workshops:

1. 🌏 **Serrano, FC**; Marconi, V & McRae, L (2022). The untapped potential of non-English-language studies in the detection of species population trends: a case study for Brazilian vertebrates; British Ecological Society Annual Meeting 2022

2. 🌏 Segurado, P; Schmitt, W; Fernandes, MR; Mota-Ferreira, M; **Serrano, FC**; Beja, P (2022). On endangered turtles and "ghost ponds" and how to bring them back to life; Talk at 36th Congress of the International Society of Limnology in Berlin, Germany

3. 🌏 Segurado, P; Schmitt, W; Fernandes, MR; Mota-Ferreira, M; **Serrano, FC**; Beja, P (2022). Prioritizing pond restoration to recover the habitat connectivity of a pond-dwelling turtle; Poster 36th Congress of the International Society of Limnology in Berlin, Germany

4. 🌏 **Serrano, FC** & Nogueira, C. (2021). There and back again: when and how the richest Neotropical snake clade dispersed throughout the Americas; Virtual talk at IBS Early Career Conference in Amsterdam, the Netherlands

5. 🌏 **Serrano, FC**; Vieira-Alencar, JP & Nogueira, C. (2021). Snakes on a puzzle: regionalization patterns of the richest snake family in the Neotropical region; Virtual poster at IBS Early Career Conference in Amsterdam, the Netherlands

6. 🌏 **Serrano, FC** & Nogueira, C. (2019). One clade, two histories: Phylogenetic niche conservatism and area drive megadiversity patterns of Dipsadidae, the richest Neotropical snake family; Talk at International Biogeography Society Meeting in Quito, Ecuador

7. 🌏 Evolutionary Biogeography: Biodiversity Data from Field to Yield (2018); Workshop led by Alexandre Antonelli and Alexander Zizka in Natal, Rio Grande do Norte, Brazil

8. 🌏 Measuring and mapping evolutionary diversity for conservation planning (2017); Workshop led by Silvia B. Carvalho and Dan Rosauer in Vairão, Portugal