UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

WENDY YOHANA ARROYO PEREZ

HISTORICAL BIOGEOGRAPHY OF THE GENUS *Dolichothele* (ARANEAE: THERAPHOSIDAE)

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Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree Doctor in Genetics and Molecular Biology, in the area of Animal Genetics and Evolution.

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Genética e Biologia Molecular, na área de Genética Animal e Evolução.

Orientador: Profa. DRA. VERA NISAKA SOLFERINI

ESTE ARQUIVO DIGITAL CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA WENDY YOHANA ARROYO PEREZ, E ORIENTADA PELA PROFA. DRA VERA NISAKA SOLFERINI.

CAMPINAS
2021

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

Ar69h
Arroyo Perez, Wendy Yohana, 1986-
Historical biogeography of the genus *Dolichothele* (Araneae:Theraphosidae) / Wendy Yohana Arroyo Perez. – Campinas, SP : [s.n.], 2021.

Orientador: Vera Nisaka Solferini.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Aranha. 2. Diagonal seca. 3. Paleontologia - Mioceno. 4. Análise filogenética. I. Solferini, Vera Nisaka, 1957-. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Biogeografia histórica do gênero *Dolichothele* (Araneae:Theraphosidae)
Palavras-chave em inglês:
Spiders
Dry diagonal
Paleontology - Miocene
Phylogenetic analysis
Área de concentração: Genética Animal e Evolução
Titulação: Doutora em Genética e Biologia Molecular
Banca examinadora:
Vera Nisaka Solferini [Orientador]
Ana Maria Lima de Azeredo Espin
Louis Bernard Klaczko
Fernando de Faria Franco
Evandro Marsola de Moraes
Data de defesa: 20-09-2021
Programa de Pós-Graduação: Genética e Biologia Molecular

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000- 0001-8915-082
- Currículo Lattes do autor: http://lattes.cnpq.br/3166031141532125

Campinas, 20 de setembro de 2021

COMISSÃO EXAMINADORA

Profa. Dra. Vera Nisaka Solferini

Profa. Dra. Ana Maria Lima de Azeredo Espin

Prof. Dr. Louis Bernard Klaczko

Prof. Dr. Fernando Faria Franco

Prof. Dr. Evandro Marsola de Moraes

Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós-Graduação em Genética y Biologia Molecular do Instituto de Biologia da Universidade Estadual de Campinas.

DEDICATION

For my son, Thiago

ACKNOWLEDGMENT

I especially thank to my family. My parents, Maria and Eloy and my brothers, Lorena and Didier even stay close despite to the distance. I always felt their affections and strengths to walk this path.

To my husband, Deivys Alvarez-Garcia, for teaching me to be passionate about science and research.

To my advisor, Vera, for the opportunity and confidence to carry out this work. I admire her way of teaching science, especially her approaches about evolution.

To the professor's doctors: PhD. Elen Arroyo Peres, PhD. Ana Maria Lima de Azeredo Espin and PhD. Carlos Alberto Almeida, for their important contributions to the improvement of the thesis.

To the professor's doctors: Ana Maria Lima de Azeredo Espin, Louis Bernard Klaczko, Fernando Faria Franco and Evandro Marsola de Morais for accepting our invitation to join the defense board.

To Ministerio de Ciencia, Tecnologia e Innovación – MinCiencias (before, Colciencias) from Colombia for the scholarship granted.

To FAPESP (São Paulo State Research Support Foundation) for the partial funding of this research project - grant #18/03428-5 Dimensions US-BIOTA-Sao Paulo.

To the Genetics and Molecular Biology program for funding participation in international events and sequencing of part of the samples.

To the museum curators from the following institutions (giving acronym and curator): CAD, Coleção Aracnologica Diamantina, J.P.L. Guadanucci; IBSP, Instituto Butantã da Universidade de São Paulo, A.D.Brescovit; MZSP, Museu de Zoologia da Universidade de São Paulo, R. Pinto-da-Rocha; UFMG, University Federal of Minas Gerais, A. J. Santos and UNB - University of Brasilia, P. Motta, for providing access to their institutions and/or loan of

important specimens.

To Professor Dr. José Paulo Guadanucci for introducing me to the spiders of the genus *Dolichothele* and showing me their potential as a study model, as well as for his help in the taxonomic identifications of the specimens.

To Celia, a laboratory technician, for her great willingness to help and good conversations.

To all colleagues at the LDG-laboratory., especially Millke and Rafa, for living in these doctoral years summarized in discussions about papers, collections, analyzes, conversations, coffee, lots of cakes and laughter. I hope these bonds of friendship will last forever.

To Jackeline, Érica, Flavia and Mauricio, for the friendship.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

“*Tell me and I´ll forget,*
teach me and I´ll remember,
involve me and I´ll learn”
Benjamin Franklin

RESUMO

A biodiversidade da região Neotropical está relacionada com uma sucessão de grandes eventos geológicos e climáticos que modificaram a paisagem e a própria evolução das espécies. Compreender como eventos do passado afetaram os biomas e a biodiversidade local é um dos grandes desafios da biogeografia Neotropical. Embora tradicionalmente focada na Amazônia e na Mata Atlântica, nos últimos anos a pesquisa na região neotropical mudou gradualmente para investigar também os processos de biodiversidade nos biomas de vegetação aberta, como a Caatinga, o Cerrado e o Chaco, que formam a região conhecida como “Diagonal Seca” (DD). Para investigar a relevância dos eventos geológicos e climáticos nos processos de diversificação da paisagem brasileira, conduzimos uma abordagem multilocos abrangente para o gênero *Dolichothele*, um grupo de aranhas endêmica, principalmente, dos habitats secos brasileiros. Nós realizamos uma análise filogenética bayesiana utilizando dados, por meio do sequenciamento Sanger, de três regiões nucleares e três regiões mitocondriais, além de realizarmos a datação das divergências entre as espécies. Os possíveis eventos envolvidos na diversificação de *Dolichothele* são discutidos com base nas principais hipóteses propostas para explicar a diversidade neotropical e formação da DD, levando em consideração a distribuição geográfica e a data de divergência das espécies. Nossa hipótese sobre as relações filogenéticas entre as espécies mostra o gênero *Dolichothele* como um grupo monofilético e as estimativas de divergência das espécies mostram que as grandes divisões de linhagens intraespecíficas ocorreram no Mioceno tardio, e outros eventos de diversificação apenas aconteceram no período Pleistoceno. Assim, nossos resultados adicionam evidências de que os eventos de orogenia do Neógeno foram cruciais para os principais eventos de diversificação na DD, enquanto à influência das flutuações climáticas do Pleistoceno, poderiam ter resultado na adaptação a novos nichos ambientais, promovendo mudanças de habitat e moldando os padrões de especiação na região Neotropical. Nossos resultados levam à hipótese de que a história evolutiva de *Dolichothele* inclui expansões no bioma do Cerrado e eventos independentes de colonização dos biomas adjacentes. Reforçamos o papel da diagonal seca sul-americana como um importante centro de diversificação.

ABSTRACT

The biodiversity of the Neotropical region is related to a succession of major geological and climatic events that have changed the landscape and the evolution of species. Understanding how events in the past affected biomes and local biodiversity is one of the great challenges of Neotropical biogeography. Although traditionally focused on the Amazon and the Atlantic Forest, in recent years research in the Neotropical region has gradually changed to also investigate the biodiversity processes in open vegetation biomes, such as the Caatinga, Cerrado and Chaco, known as "Dry Diagonal" (DD). To investigate the relevance of geological and climatic events in the diversification processes of the Brazilian landscape, we conducted a comprehensive multilocus approach for the genus *Dolichothele*, a group of spiders' endemics, mainly, from of Brazilian dry habitats. We performed a Bayesian phylogenetic analysis using data, by Sanger sequencing, of three nuclear regions and three mitochondrial regions, in addition to performing the dating of the divergences between species. The possible events involved in the diversification of *Dolichothele* are discussed based on the main hypotheses proposed to explain the Neotropical diversity and formation of the DD, considering the geographic distribution and the date of divergence of the species. Our hypothesis about the phylogenetic relationships between species shows the genus *Dolichothele* as a monophyletic group and the divergence estimates of the species show that the great divisions of intraspecific lineages occurred in the Late Miocene, and other diversification events only happened in the Pleistocene period. Thus, our results add evidence that the Neogene's orogeny events were crucial for the main diversification events in the DD, while the influence of Pleistocene climatic fluctuations, could have resulted in adaptation to new environmental niches, promoting changes in habitat and shaping speciation patterns in the Neotropical region. Biogeographic reconstruction analyses indicated the Cerrado biome as the ancestral range of the genus *Dolichothele*. Our findings allow us to hypothesize that the evolutionary history of *Dolichothele* comprises expansions within the Cerrado biome and independent colonization events to the neighboring biomes. We highlight the South American open diagonal as a significant diversification center.

Keywords

Spider, Dry Diagonal, Late Miocene, phylogenetic relationship

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INTRODUCTION

Neotropical Biogeography

The Neotropical region comprises most of South America, Central America, southern and central Mexico, and the Antilles (Morrone 2017). The Neotropics exhibits a complex topography, ranging from sea level to above 5000 a.m.s.l and encompasses a varied of biomes, from dry deserts to the humid forests (Rull 2020). Central America and Northern of South America are characterized by the presence of humid tropical forests (Amazon). Another large area of tropical forest is the Atlantic Forest, originally occupying most of the Brazilian coast. These tropical forests are characterized by high rainfall, high productivity, and dense vegetation (Kricher 1997; Begon *et al.* 2006). Between these two regions of tropical forests there are a set of open formations collectively called the "Dry Diagonal" (DD). These open formations have seasonal periods of drought and their vegetation coadapted to these conditions (Werneck 2011).

Different complex events influenced the diversification of biomes and organisms in Neotropical region throughout of their geological history. Many hypotheses were raised to explain this diversity, usually focused on the diversity of the Amazon region (Wesselingh *et al.* 2010; Antonelli *et al.* 2015) or including the neotropics in analyzes about the latitudinal gradient of diversity (Mittelbach *et al.* 2007; Brown 2014). The principal's hypotheses have been associated with major biogeographic events. In the Tertiary, several orogenic events had an impact on the diversification of the Neotropical region, with emphasis on the rise of the Andes (Wesselingh *et al.* 2010). Beginning in the Eocene, the Andes experienced its most intense uplift in the last 10 million years (Thouret *et al.* 2007). This increase caused an intense change in the geographical configuration of South America. Still in the Eocene, along with this uplift, and partly influenced by it, there was the compartmentalization of forests in South America, with the differentiation of tropical forests to the north and south. Savannas and open vegetation to the east and south (Colli *et al.* 2005).

More recently, along with the uplift of the central Andes region, the Pebas system and the fragmentation of the Amazon around this lake have emerged. The uplifting of the northern region of the Andes caused the gradual restructuring and disappearance of this lake system and formed the current rainfall pattern, with the rivers of the Amazon basin running east (Hoorn *et al.* 2010). The formation of the Andes has also deeply affected the region's climate, as it is the only barrier to air circulation in the southern hemisphere (Gregory-Wodzicki 2000; Insel *et al.* 2010). All this process had a profound impact on the diversification of the Neotropical groups, having already been related to increases in their diversification rates (Antonelli *et al.* 2009;

Matos-Maraví *et al.* 2013). In addition to the changes in geography, climate, and fluviology, the ascent of the mountains created new opportunities for speciation, separated previously existing linages, created barriers to migration, and is credited with being responsible for the extreme diversity of the region (Gentry 1982; Hughes & Eastwood 2006; Winterton *et al.* 2014).

Because of the reordering of the tectonic plates with the rise of the Andes, the Isthmus of Panama was closed, and the great American exchange took place (Webb 1991). Despite having less attention, this event was of great importance in the biogeography of the Americas since it marked the end of the isolation between the two blocks and allowed the migration of organisms between them. The isthmus was fully formed between 5 and 2.8 million years ago, but the beginning of biota migration and exchange precedes this date by tens of millions of years (Bacon *et al.* 2015; O'Dea *et al.* 2016). The complexity of environments allows to neotropics to support one of the greatest biodiversity of the planet with six of the 25 world's biodiversity hotspots (Mittermeier *et al.* 1998; Myers *et al.* 2000; Antonelli & Sanmartín 2011).

Since Humboldt and Wallace, the reasons for the DD diversity have been a central question in Biogeography. The pioneering biogeographic study in the Neotropics was done by Wallace (1852), who proposed that the rivers in the Amazon region would act as barriers to dispersion, promoting speciation on opposite sides. The patterns and processes that characterize and shape the region' biodiversity have been explored by several studies on a variety of groups. Initially based on morphological taxonomy, from the 1990s these studies were deeply influenced by the revolution caused by the popularization of molecular systematics and the creation of new methods for the analysis of these genetic data (Riddle *et al.* 2008). The combined analysis of multiple independent markers within probabilistic contexts increases the confidence in historical reconstructions by reconciling cases of discordance between gene tree and species tree, thus being able to contribute to solve taxonomic limits and analyze speciation modes (Werneck 2016).

The biomes and the organisms of the Neotropical region have been widely affected by quaternary climate cycles (Werneck 2011). The refuge hypothesis proposed in the middle of the century XX, suggests that during the driest and coldest periods of these cycles there was an expansion of open vegetation and a reduction of humid forests, promoting speciation by the isolation of populations (Haffer 1969). The role of the refuge hypothesis subject of intense controversy (Brown & Ab'Sáber 1979; Colinvaux *et al.* 2000; Carnaval *et al.* 2009). Today it has become clear that the emergence of large part of the current biodiversity predates the

Quaternary and was also influenced by Tertiary events (Rull 2008, 2011; Turchetto-Zolet *et al.* 2013).

Biogeography now incorporates many different fields including, but not limited to, physical geography, geology, botany and plant biology, zoology, general biology, and modeling. The introduction of event-based biogeography allows the integration of relevant processes (e.g., dispersal, extinction, vicariance, and founder-event speciation) by explicit models (Sanmartín *et al*. 2008; Matzke 2014). These event-based estimations specify both the ancestral distributions and associated causal events thus making it easier to compare alternative evolutionary/biogeographical scenarios (Sanmartín *et al*. 2008; Ronquist & Sanmartín 2011; Matzke 2014). The study of closely related taxa distributed across different Neotropical biomes offer good opportunities not only to infer their biogeographic history, but also to shed light on the processes underlying biological diversification of Neotropical biotas (Aleixo 2004; D'Horta *et al*. 2013; Fernandes *et al*. 2013).

The reconstruction of the ancestral area has enriched the area of biogeography and allowed several advances in understanding the history of different regions of the world (e.g., Manos & Stanford 2001; Teeling *et al.* 2005; Aduse-Poku *et al.* 2015; Rota *et al.* 2016). This method has recently been applied to groups that occur in the neotropical region (Ramírez *et al.* 2010; Matos-Maraví *et al.* 2013; Alfaro *et al.* 2015). However, although invertebrates represent most of the global diversity (Mora *et al.* 2011), they still receive less attention in biogeography studies. In addition, almost all biogeographic studies with invertebrates in the Neotropical region used insects as models (e.g., Ramírez *et al.* 2010; Ceccarelli & Zaldívar-Riverón 2013; Matos-Maraví *et al.* 2013). Other extremely diverse groups, such as arachnids, are even more rarely used (but see Ferretti *et al.* 2012). Applying this method to the hyperdiverse group of spiders is essential for a deeper understanding of how the diversity of the different neotropical biomes was established.

South American Dry Diagonal

South American Dry Diagonal is a corridor composed by three main biomes: the Caatinga, the largest Neotropical nucleus of the Seasonally Dry Tropical Forests (SDTFs), in the Brazilian northeast; the Cerrado, in the central part of the continent, comprising a mosaic of vegetation kinds; and the Chaco, in northern Argentina, Paraguay and Bolivia, with a remarkably high climatic seasonality. This Dry Diagonal is located between two tropical rain forests, the Amazon, and the Atlantic rainforests, and has been considered to limit species interchanges between them (Oliveira-Filho & Ratter 1995). However, the distribution of some taxa suggests past connections migration routes between the two rainforests (Oliveira-Filho & Ratter 1995; Costa 2003; Ratter *et al.* 1997; Sobral-Souza *et al.* 2015; Souza-Neto *et al.* 2016). Due to its more recent origin, species migration from rainforests may have shaped the evolution of the high diversity of the DD (Pennington *et al.* 2009; Souza-Neto *et al.* 2016).

In the Neotropical region, an increasing speciation trend occurred during the Neogene, what has been attributed to the formation of the Andes, a massive mountain range that affected the region's climate, hydric systems, and rainfall regime (Hoorn *et al.* 2010). Hughes *et al.* (2013) reinforced the Neogene paradigm, arguing that a cluster of geological and climatic events during this period coincided with the preponderance of Mid to Late Miocene crown ages of species-rich Neotropical clades. During the Late Eocene and Oligocene, the global episodes of cooling and dryness favored the expansion of grasslands in the southern and central regions of the continent (Flower & Kennett 1994; Morley 2000), which culminated in the formation of a diagonal belt of more open and drier biomes (known as "Dry Diagonal"; Prado & Gibbs 1993; Werneck 2011; Zanella 2011). Several studies show that the dry diagonal formation, also in the Neogene, has affected Neotropical biota evolution, isolating previously widespread forest taxa, and promoting lineage diversification (e.g., Por 1992; Costa 2003).

There has been an increase in the number of studies of the Cerrado savannas and Caatinga seasonally dry tropical forests (Oliveira & Marquis 2002; Werneck 2011; da Silva *et al*. 2017; Antonelli *et al*. 2018b). Besides their main phytophisionomies, the Cerrado and Caatinga or the "Brazilian Dry Diagonal" (BDD) also harbor numerous mesic vegetation types, such as montane/submontane forests (Melo Santos *et al*. 2007; Moro *et al*. 2014) and riparian forests (Oliveira-Filho & Ratter 1995; Oliveira-Filho & Fontes 2000; Bueno *et al*. 2018). These forests have allowed the dispersal and persistence of rainforest lineages within "open formation" biomes, suggesting an intimate history between moist and relatively drier biomes in South America (Ledo & Colli 2017; de Sá *et al*. 2019).

The biogeographical history of the Cerrado is a mixed history of geological events from the Paleogene/Neogene and Quaternary climatic fluctuations (Werneck 2011). Some general biogeographic patterns highlight the importance of geomorphological surfaces (e.g., Serra Geral de Goiás, Chapada dos Guimarães), the stratification of the landscape and the gallery forests in the diversification of the Cerrado biota (Silva 1997; Simon & Proença 2000; Cardoso Da Silva & Bates 2002; Nogueira *et al*. 2011; Ledo *et al*. 2020). Studies addressing the phylogenetic relationships of species distributed in the Cerrado and tropical forests provide evidence of connectivity during less favorable climatic periods, providing refuge for forest biota (Brown & Gifford 2002; Ledo *et al*. 2020).

The Brazilian Shield comprises a landscape of alternating ancient plateaus and younger inter-plateau depressions. The plateaus, that reach up to 1676 m (de Carvalho Júnior *et al*. 2015), are largely covered by savanna vegetation and are considered to have bad climatic stability through time (Werneck *et al*. 2012b; Burbridge *et al*. 2004). The inter-plateau depressions were carved by the headwaters of three large river basins—Tocantins-Araguaia, São Francisco, and Paraná—and are covered by a mosaic of savannas and forests, which might have shifted during Quaternary climatic cycles (Ab'Saber 1983; Silva 1996; Bueno *et al*. 2017). According to this hypothesis, the Cerrado lineages would be more related to the tropical forest's lineages than to the Caatinga or Chaco ones (Brown & Gifford 2002; Costa 2003; Aguiar & Melo 2007). Ledo *et al*. (2020) showed that older lineages are associated with Cerrado-dwelling organisms, while younger lineages are associated with forest-dwelling organisms, highlighting the importance of riparian forests in the gene flow among regions. Studies with the herpetofauna of the Cerrado showed a pattern of west-east diversification (Prado, Haddad & Zamudio 2012; Werneck *et al*. 2012a; Santos *et al*. 2014); in lizards of the genus *Vanzosaura*, this pattern was congruent with the morphological variation (Recoder *et al*. 2014). Studies with other taxonomic groups are still needed to test these patterns, which may have implications for the protection of endemic lineages in the region (Werneck *et al*. 2012b).

There are less studies on past climates and habitat conditions of Caatinga. Prado and Gibbs (1993), based on the distribution of tree species, proposed the inclusion of the Caatinga as a SDTFs that would had been part of the "Pleistocene Arc (PA)". The "Pleistocene Arc" hypothesis establishes that the current SDTFs fragments are remnants of an extensive and contiguous region across the American continent based on the geomorphological evidence (Ab' Sáber 1977, 1983) that there were physical links between the isolated fragments of the formation during the dry-cold and humid-warm climatic cycles of the Pleistocene. Within the PA, three 'nuclei' of distribution are originally recognized: 1- 'Caatingas nucleus', 2- 'Misiones nucleus' and 3- 'Sub-Andean Pedemonte nucleus', each one characterized by floristic aspects (existence of some endemic species) and substrate (geological, geomorphological differences, etc.) which would have reached a maximum distribution during the Last Glacial Maximum (LGM) (Prado & Gibbs 1993; Pennington, Prado & Pendry 2000; Prado 2000). Several studies with plants (Prado & Gibbs 1993; Pennington, Prado & Pendry 2000; Prado 2003; Queiroz 2006), bees (Zanella 2000, 2002) and lizards (Werneck & Colli 2006; Werneck *et al*. 2009) support this hypothesis. However, some studies show contradictory results. Mayle (2004, 2006) using paleoecological data and vegetation models suggests that dispersion is a better explanation for these patterns throughout the SDTFs, which would have established after the LGM. In addition, Werneck *et al*. (2011) propose an alternative scenario: a recent expansion of SDTF in the lower Pleistocene or Tertiary, followed by fragmentation in the LGM and secondary expansion in the Holocene. The Caatinga landscapes also includes plateaus restricted to inselbergs, covered by savanna or by montane and submontane mesic forests. In contrast, the depressions are covered by seasonally dry tropical forests and riparian forests (Prado 2003). Paleoenvironmental studies suggest moister conditions and great changes in the Caatinga during the Quaternary (De Oliveira *et al*. 1999; Pessenda *et al*. 2004; Pessenda *et al*. 2010), corroborated by the genetic structure of several depression and montane lineages (Carnaval & Bates 2007; Thomé *et al*. 2016; Gehara *et al*. 2017).

According to Werneck (2011), the dynamic landscapes of Cerrado and Caatinga could have resulted in a pattern of (1) older lineages (paleoendemics) on Brazilian Shield’s plateaus, characterized by high genetic structure, high demographic stability and high genetic diversity; and (2) younger lineages (neoendemics) confined to inter-plateau depressions, characterized by low genetic structure, lower genetic diversity, and signatures of demographic changes, hereafter called the “plateau/depression hypothesis”. Furthermore, riparian forests in Cerrado and Caatinga were likely connected during favorable periods in the Quaternary, allowing lineages interchanges across river basins and biomes (Oliveira-Filho & Ratter 1995; De Oliveira *et al*. 1999). Some studies suggest that the Brazilian Shield can also act as a barrier to gene flow for forest-dwelling organisms inhabiting inter-plateau depressions (Silva 1997; Cardoso Da Silva & Bates 2002; Werneck 2011) and altitude is an important predictor of anuran (Valdujo *et al*. 2013), bird (Silva 1996), and lizard (Nogueira *et al*. 2009) species turnover in Cerrado.

Spiders

Spiders (Araneae) are terrestrial arthropods widely distributed on the planet and constitute one of the most diverse animal groups (Mora *et al.* 2011). They are relatively easy to collect and identify and are one of few large arthropod orders to have a complete online taxonomic catalog with synonymies and associated literature (Platnick 2020). About 49.136 species are recognized, placed in more than 4.207 genera distributed in 128 families (WSC 2021), but the number of species worldwide is believed to be much higher (Coddington & Levi 1991; Mora *et al.* 2011). Araneae are generally divided into two suborders (Mesothelae and

Opisthothelae). The suborder Mesothelae includes only a single family, the Liphistiidae; all the other families are placed in the Opisthothelae, that is generally divided into two smaller groups (infraorders), the Araneomorphae (the more typical spiders) and the Mygalomorphae (containing tarantulas and their relatives); the first comprises more than 94% of all spider species, and the second corresponds to about 6% (Coddington & Levi 1991; Coddington 2005; Santos *et al.* 2017).

Mygalomorph spiders includes tarantulas, trapdoor spiders, and some of the most venomous species of the world (Hedin *et al.* 2018). The infraorder comprises 30 families divided into 358 genera, with 3103 species (WSC 2021). The group is estimated to have diversified in the Carboniferous (300 Mya) (Ayoub *et al.* 2007; Starrett *et al.* 2013; Garrison *et al.* 2016; Opatova *et al.* 2020); several authors suggested that Mygalomorphae radiated in major lineages during the Mesozoic (Dunlop *et al.* 2008). Eskov & Zhonshtein (1990) called the Cretaceous as the "age of the mygalomorphs".

Mygalomorphae retain several features considered primitive, such as two pairs of book lungs, paraxial chelicerae, simple silk spigots, and biomechanically weak silk (Bond *et al.* 2012; Garrison *et al.* 2016). Their ancient origins and sedentary nature provide a rich biogeographical context for studying geological-scale continental vicariant events (Raven 1980; Hedin & Bond 2006; Opatova *et al.* 2013; Opatova & Arnedo 2014; Pérez-Miles & Perafán 2017). Since early taxonomic papers, Mygalomorphae has been considered a monophyletic group (Bond *et al.* 2012). However, the monophyly of some families and the inter-familiar relationships remain poorly supported, probably because of taxonomic difficulties for identification of species; knowledge on the biology of Mygalomorphae is relatively scarce in comparison with that of the Araneomorphae (Pérez-Miles & Perafán 2017).

Theraphosidae, comprises the largest Mygalomorphae family, with 147 genera and more than 985 species described (WSC 2020). It has a wide geographic distribution with records from the Americas, Asia, Africa, Southern Europe, Australia, and western North Pacific (Marianas). Theraphosidae are often large and hairy spiders, found in tropical and subtropical regions around the world, most living in natural cavities in the ground or in tree trunks, under rocks, fallen logs or loose tree bark (Guadanucci 2014).

Recent phylogenomic studies estimated the origin of the Theraphosidae between 34 and 71 Mya (Bond *et al*. 2014) and between 2 and 125 Mya (Garrison *et al*. 2016). More recently Opatova *et al*. (2020) estimated the split between Barychelidae and Theraphosidae around 106 Mya. Theraphosid fossil record is poor and recent (Dunlop *et al*. 2018). Probably the environmental conditions prior to the Neogene were not favorable for their fossilization of tarantulas, which could explain the absence of theraphosid fossils in earlier times (Perez-Miles 2020).

The arrangement of theraphosid subfamilies has been controverse, but the latest classifications (Lüddecke *et al*. 2018; Foley *et al*. 2019) consider five subfamilies in the New World: Aviculariinae, Ischnocolinae, Psalmopoeinae, Schismatothelinae, and Theraphosinae. Ischnocolinae Simon 1892 is recognized as the taxonomically most problematic group (Foley *et al.* 2019) as it has been used to place the species that could not assigned to other lineages (Guadanucci 2004). Ischnocolinae comprises relatively small spiders, often referred to as "dwarf tarantulas", ranging from 15 to 50 mm of body length. They usually live underneath rocks, crevices, tree barks and fallen trunks, where they spin silk sheets and tunnels. They are characterized by many spines on legs, without urticating setae, bearing simple palpal bulb, a narrow subtegulum, embolus usually long, thin and without keels (de Pikelin & Schiapelli 1973). They have a wide distribution, with species being found in the Americas, Asia, Africa, and Europe (Foley *et al.* 2019). The New World Ischnocolinae could only be recognized by exclusion, as they lack the synapomorphic characters of Theraphosinae and Aviculariinae; it comprises a non-monophyletic group (Guadanucci 2020).

In 2014, Guadanucci proposed the following changes: (1) Ischnocolinae as defined after Raven (1985) is paraphyletic and should be broken into less inclusive groups; the name "Ischnocolinae" should be restricted to a monophyletic group (Ischnocolinae *sensu stricto*) that includes the New World genera *Holothele*, *Acanthopelma*, *Reichlingia*, *Trichopelma* and the African and Middle East genus *Ischnocolus*; (2) part of the former Ischnocolinae comprises a monophyletic subfamily named Schismatothelinae, with the new world genera *Euthycaelus*, *Schismatothele*, *Guyruita*, *Sickius,* and *Neoholothele*; (3) other two New World genera *Catumiri* and *Dolichothele*, without clear defined relationships.

The genus *Dolichothele* Mello-Leitão 1923 is included in the subfamily "Ischnocolinae" *sensu lato*, with nine species distributed in Brazil and Bolivia (Fig. 1). The taxonomic history of the genus begun with Mello-Leitão (1923), who described the group based in *Dolichothele exilis* from the State of Paraíba, Brazil. Some *Dolichothele* species were described in the genus *Oligoxystre* by Guadanucci (2007, 2011). Lucas & Indicatti (2015) transferred *Oligoxystre* spp. to *Dolichothele*: *D. auratum* Vellard 1924, *D. bolivianum* Vol 2001, *D. diamantinensis* Bertani, Santos & Righi 2009, *D. dominguense* Guadanucci 2007, *D. mineirum* Guadanucci 2011, *D. rufoniger* Guadanucci 2007, and *D. tucuruiense* Guadanucci, 2007. Revollo *et al*. (2017)

recognized nine species, two new species closely related to *D. bolivianum*: *D. camargorum* and *D. mottai*. The genus includes mid-sized tarantulas, with setose legs, carapace, and abdomen; some species show colorful body patterns (e.g., *D. diamantinensis*, *D. dominguense*, *D. rufoniger*, *D. mottai*). Recent studies (Guadanucci 2007, 2011; Bertani *et al*. 2009; Lucas & Indicatti 2015; Revollo *et al*. 2017) have clarified many aspects of its diversity, taxonomy, morphology, and biogeography.

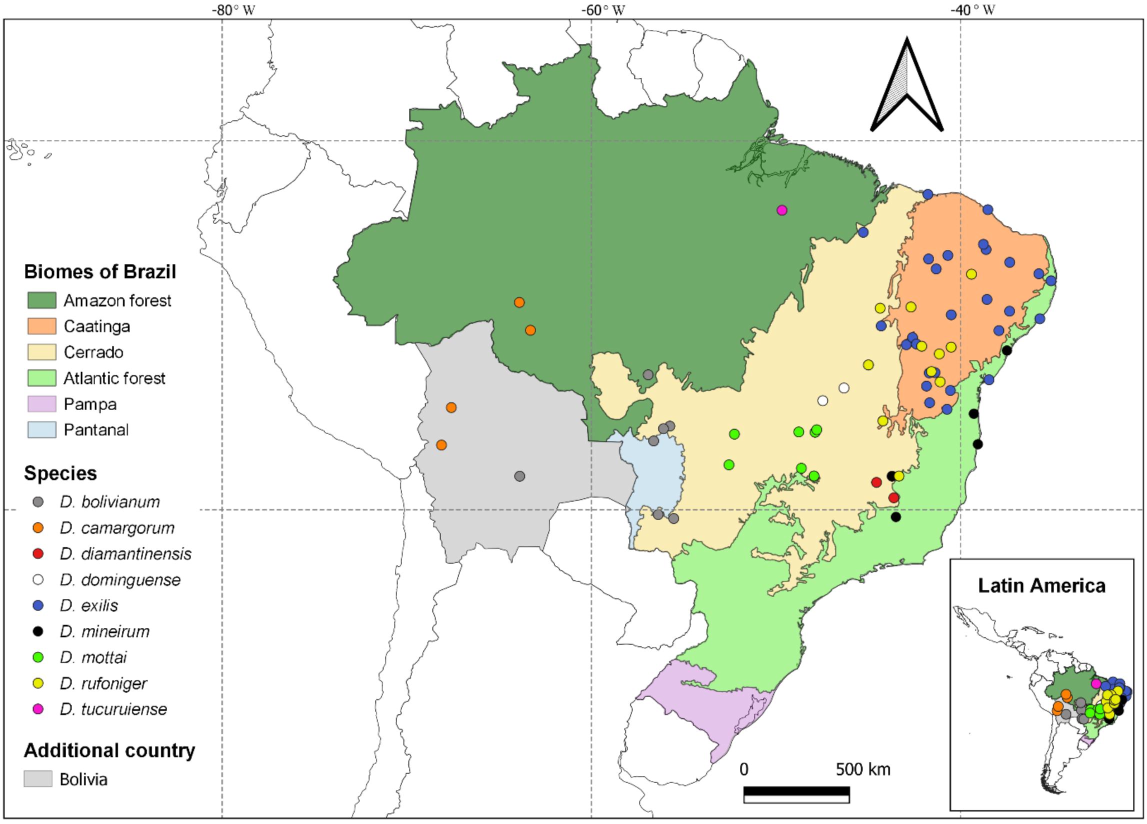

Figure 1. Map of distribution of *Dolichothele* species in South America.

Dolichothele species are mainly found in xeric habitats, like deserts and tropical dry forests (Guadanucci 2011; Motta 2014; Revollo 2016) but is also found in transition zones to tropical forests (Amazon and Atlantic Forest) (Fig. 1). *D. bolivianum* is found in Bosque Chiquitano, whereas *D. mottai*, *D.dominguense*, *D. diamantinensis* occurs on the Cerrado *stricto sensu* from Central-Western Brazil and *D. camargorum*, on the Amazon region, probably in Cerrado fragments (Revollo 2016). In general, the species show geographical endemism. The sympatric species, *D. exilis* and *D. rufoniger*, are found in the Caatinga and Cerrado and *D. mineirum* in humid areas of Brazilian Atlantic Rainforest (Fig. 1). The specimens has been collected at altitudes around 900 – 1.250 a.m.s.l, in rocky places and low soil depth.

There are three phylogenetic hypotheses for *Dolichothele* based on morphological data (Fig. 2): Guadanucci (2011) recovered the genus as monophyletic; the phylogenetic relationships of eight species were: (*D. mineirum* ((*D. tucuruiense* + *D. rufoniger*) (*D. diamantinensis* (*D. bolivianum* + *D. dominguense*)))) with *D. exilis* as the sister group of all remaining species of *Dolichothele*. Revollo (2016) proposed two phylogenetic hypotheses; the first did not recover *Dolichothele* as monophyletic since *D. exilis* was not included in the other species clade (Fig. 2a); the second hypotheses recovered the genus *Dolichothele* as monophyletic and established the relationships between ten species as follows: *D. exilis* (D. sp. Nov. 4 [(*D. dominguense*, (*D. camargorum*, *D. bolivianum*, *D. mottai*)) + ((*D. tucuruiense*, *D. rufoniger*) + (*D. mineirum*, D. sp. Nov. 3)]) (Fig. 2b).

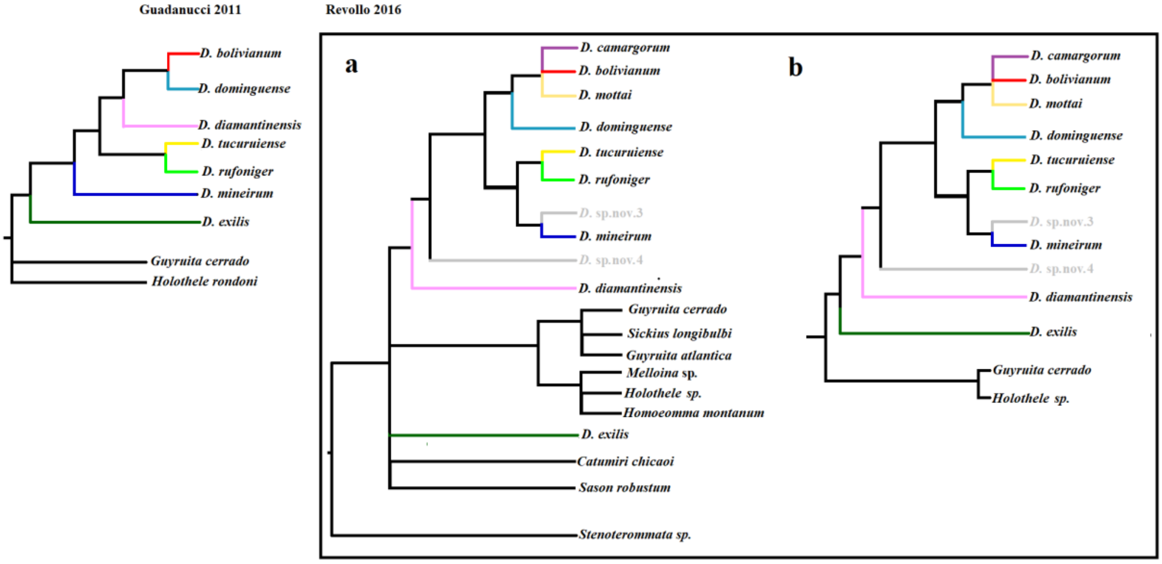

Figure 2. Phylogenetic hypothesis for Dolichothele species based in morfological data. (species in grey were not validate yet).

The few published phylogenies of theraphosids are based exclusively on morphological data and poses conflicts and/or incongruences, suggesting the need for additional phylogenetic research utilizing molecular data. In addition, phylogenies with estimates of divergence times are useful for testing hypothesis about geological events underlying diversification (e.g., Binford *et al.* 2008; Särkinen *et al.* 2012; Werneck *et al.* 2012b). *Dolichothele* genus is a good model for biogeographical studies because of its wide distribution among Neotropical biomes; several species occur in only one biome and can be informative in inferences about the history of the dry biomes and their neighbor’s rainforests. Our goal is to provide a first molecular hypothesis on the broadest scale of *Dolichothele* to test different scenarios associated with the formation of BDD and the influence of geomorphological surfaces (e.g., Serra do Espinhaço, Brazilian Shield) on the group’s diversification.

OBJECTIVES

The aim of this work is to do a biogeographic study of the genus *Dolichothele* to contribute to the understanding of the process associated with the current distribution of the Brazilian Dry Diagonal.

Specific objectives.

- To infer the phylogenetic relationships of species of the genus *Dolichothele* using molecular markers and estimate their divergence time.
- To analyze temporal and geographical patterns of diversification of these species and reconstruct the ancestral range of distribution of the genus *Dolichothele*.
- To test hypotheses about the diversification of the genus *Dolichothele* based on current hypotheses of diversification of the Brazilian Dry Diagonal.

MATERIALS AND METHODS

Taxon sampling

Specimens were obtained from museums or collected in the field (Table 1). Individuals were identified by Prof. Dr. José Paulo Guadanucci - Laboratory of Arachnology, Universidade Estadual Paulista (UNESP), Campus Rio Claro.

Table 1. Detail of the localities of occurrence of species of the genus *Dolichothele* and list of museums where there are specimens in biological collections.

Species	Occurrence area		Country	Deposit Museums
D. bolivianum	Minas Gerais, Bolivia	Cerrado	Bolivia e Brazil	**Bolivia** 1. Museo de Historia Natural Noël Kempff Mercado, Santa Cruz de la Sierra - HNNKM
D. camargorum	Rondônia	Amazon	Brazil	**Brazil** 1. University of São Paulo Zoology Museum – MZSP 2. Museum of Science and Technology from PUCRS. 3. National Institute for Research in the Amazon – INPA 4. Center for Taxonomic Collections of the Federal University of Minas Gerais – UFMG. 5. UNESP - Rio Claro Campus 6. National Museum from the Federal University of Rio de Janeiro. 7. Department of Zoology, University of Brasília 8. Institute Butantã – IBSP from University of São Paulo
D. diamantinensis	Serra do Espinhaço – Minas Gerais.	Cerrado		
D. dominguense	Goiás	Cerrado		
D. exilis	Bahia	Caatinga		
D. mottai	Distrito Federal e Goiás	Cerrado		
D. tucuruiense	Pará	Amazon		
D. rufoniger	Minas Gerais, Bahia e Piaui	Caatinga		
D. mineirum	Estado do Pará Estados de Minas Gerais, Bahia, Ceará e Sergipe	Atlantic Forest		

Molecular protocols and sequence alignment

DNA was extracted from the second and third left legs; for fresh samples, the Wizard Tissue kit (Promega: Valencia, CA, USA) was used according to the manufacturer’s instructions; for museum samples, 20ul of Proteinase K (20mg/ml/sample) was added to lysis buffer and kept overnight. DNA was eluted into 30 – 40 ul of buffer and stored at 4 °C.

Six DNA markers were chosen for this study: three mitochondrial regions (cytochrome *c* oxidase subunit 1, 12S rRNA and part of 16S rRNA) and three nuclear regions (ITS2, 28S rRNA and Histone H3 A). The primers are presented in Table 2.

PCR reactions were performed with Invitrogen kit in 25 µl solution containing 15-17 µl of dd water, 2.5 µl of 10X PCR Buffer, 2.5 µl of 25 mM MgCl2, 0,5 µl of 10mM dNTP Mix, 0.2 µl (5U) of Taq DNA Polymerase, 0.4 µl of each 10 pmol primer, and 3-5 µl of DNA.

The programs, amount and concentrations of the reagents used in the PCR reaction for each marker are detailed in the Appendix (Appendix: Table S1).

Table 2. Detail of primers used, in the PCR reactions, in this study

Marker	Primer name	Primer sequence (5′-3′)	References
COI	C1-J-1751‘‘SPID’’	GAGCTCCTGATATAGCTTTTCC	Hedin & Maddison 2001
	C1-N-2776	GGATAATCAGAATATCGTCGAGG	
	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
12S	12S-StL	GGTGGCATTTTATTTTATTAGAGG	Kocher *et al.* 1989
	12S-bi	AAGAGCGACGGGCGATGTGT	
ND1-16S	16SA/12261	CGCCTGTTTACCAAAAACAT	Hedin 1997
	SPID-ND1/13398	TCRTAAGAAATTATTTGAGC	Arnedo *et al.* 2004
ITS2	ITS4	TCCTCCGCTTATTGATATGC	White *et al.* 1990
	ITS3(5.8S)	GCATCGATGAAGAACGCAGC	
28S	28S-rd5b	CCACAGCGCCAGTTCTGCTTAC	Schwendinger & Giribet 2005
	28SA	GACCCGTCTTGAAACACGGAGGA	Whiting *et al.*1997
H3A	H3a F1	ATGGCTCGTACCAAGCAGACVGC	Colgan *et al.* 1998
	H3a R1	ATATCCTTRGGCATRATRGTGAC	

PCR products were purified using ExoSAP-IT (Affymetrix: Santa Clara, CA, USA). Sequencing was conducted at Center for Molecular Biology and Genetic Engineering – CBMEG – Unicamp, Life Sciences Core Facility (LaCTAD – Unicamp) and Macrogen, Inc services, Korea in 3500 XL Genetic Analyzer (Life Technologies: Foster City, CA, USA), using the manufacturer’s BigDye® Terminator v3.1 cycle sequencing kit. One to four specimens per species were sequenced.

Sequences were assembled using BIOEDIT 7.2.5 (Hall 1999). Sequence alignments were performed with MAFFT 7.187 (algorithm Q-INS-i) (Katoh & Standley 2013) using the default parameters and usually inspected.

Phylogenetic analyses and divergence time estimation

The Bayesian phylogenetic trees for each marker and for all sequences were inferred in BEAST 1.8.0 (Drummond *et al*. 2012). Alignments were evaluated with Partition Finder v 2 (Lanfear *et al.* 2017) to identify data partitions and the most appropriate evolutionary model for each dataset (Appendix: Table S2). The molecular clock hypothesis was using the Mega X program (Kumar *et al*. 2018) by comparing ML values with and without data restriction for all molecular markers. The hypothesis was rejected at the significance level of 5%. Therefore, the divergence time estimates do not assume a constant rate of evolution in all branches. The Yule process prior for speciation and uncorrelated lognormal relaxed clock models were used. Since there are no fossil information, substitution rates available in the literature were used (Kornilios *et al*. 2016) to calibrate the nodes in the trees and estimate divergence times for the mitochondrial phylogenetic tree (Table 3). Sequences of *Catumiri parvum* and from GenBank of *Aphonopelma seemanni* and *Avicularia avicularia* (Theraphosidae) and from species of family Nemesiidae were included as outgroups (Table 4). Analyses with 200 million generations, sampling trees every 20000 generations were performed. Mixing, convergence and effective sample size (>200) were assessed with TRACER 1.7 (Rambaut *et al.* 2018); were discarded the first 10% of the trees as burn-in and combined the trees from independent runs in LogCombiner 1.8 (Rambaut & Drummond 2013). The maximum clade credibility species and gene trees were annotated with TREEANNOTATOR 1.8 (Rambaut & Drummond 2015), and the trees were visualized in FIGTREE 1.4. (http://tree.bio.ed.ac.uk/software/figtree) Branches with posterior probability values ≥ 80 were considered well (or strongly) supported.

Table 3. Summary statistic used in Bayesians analyses in *Beast 1.8

Molecular marker	Mean rate	ucld.mean	ucld.stdev	Coefficient variance	Covariance
COI	0.0136 (0.0101 – 0.0174)	0.048 (0.0454 – 0.0505)	1.3505 (1.0432– 1.6749)	1.568 (0.9303– 2.3792)	-0.0213 (-0.2005– 0.2068)
NAD1 – 16S	0.0171 (0.0133 – 0.0209)	0.0208 (0.0178 – 0.0239)	0.8822 (0.5698– 1.208)	1.0111 (0.5929 – 1.4614)	0.0309 (-0.1608 – 0.2543)
12S	0.034 (0.0228 – 0.0463)	0.0293 (0.027 – 0.0316)	0.8513 (0.4393 – 1.2847)	0.9193 (0.4217 – 1.495)	0.0239 (-0.1868 – 0.2535)
28S	4.7783E-4 (2.5125E-4 – 7.2006E-4)	5.2749E-4 (2.2252E-4 – 8.7387E-4)	0.6229 (1.1103E-5 – 1.2819)	0.6441 (3.1162E-5 – 1.3635)	-0.0211 (-0.2964 – 0.2722)

Molecular marker	**Mean rate**	**ucld.mean**	**ucld.stdev**	**Coefficient variance**	**Covariance**
H3	5.1922E-3 (3.5654E-3 – 6.8981E-3)	3.4929E-3 (2.8055E-3 – 4.1972E-3)	0.9135 (0.5813 – 1.2508)	0.8318 (0.5692 – 1.0936)	-0.0506 (-0.3283 – 0.24)
ITS2	3.4393E-3 (1.9121E-3 – 5.1879E-3)	2.6968E-3 (1.7182E-3 - 3.6967E-3)	1.1053 (0.6268 – 1.6166)	1.231 (0.6883 – 1.8031)	6.7624E-3 (-0.2647 – 0.3159)

Table 4. Sequences from GenBank used as outgroup for the phylogenetic analyzes.

Family	**Species**	**Subfamily**	**Accession GenBank/Marker Molecular**
Theraphosidae	*Aphonopelma seemanni*	Theraphosinae	JN018124.1 / COI KX758196.1 / ND1 – 16S MG273539.1 / H3A JN018338.1 / 28S
	Avicularia avicularia	Aviculariinae	MK234709.1 / COI KX758147.1 / ND1 – 16S MG273499.1 / 12S MG273647.1 / 28S MG273564.1 / H3A
Nemesiidae	"Nemesiidae sp. South Africa MY546"		KY017808.1 / COI KY015984.1 / 16S KY015480.1 / 12S KY017214.1 / 28S KY018323.1 / H3A

Ancestral range reconstruction

To understand the historical spatiotemporal evolution of the *Dolichothele* genus, different ancestral range reconstructions were performed. The first reconstruction used a discrete phylogeographical model with Bayesian Stochastic Search Variable Selection (BSSVS) to simultaneously assesses the uncertainties associated with the phylogenetic inference and the ancestral ranges (Lemey *et al.* 2009), also implemented in BEAST 1.8. The Bayesian inference of the mtDNA dataset was used and the biomes of each species were assigned as terminals: Cerrado, Caatinga, Atlantic Forest and Pampas (outgroup species – *Catumiri parvum*). The nodes were dated with the TMRCA estimated in the previous analysis. A Yule tree prior was applied, and the ancestral areas was inferred using a symmetric substitution model with strict clock for the location trait. Two independent analyses with 200 million generations were run, with samples taken every 1000 generations. The parameters were checked in TRACER 1.6, the trees were combined in LOGCOMBINER 1.8, and the maximum clade credibility species and gene trees were annotated with TREEANNOTATOR 1.8, discarding the first 10% as burn-in. The final tree was visualized in FIGTREE 1.4.

The second reconstruction used the R package v 4.0, BioGeoBEARS (Matzke 2013), to estimate ancestral ranges and infer speciation modes. The species tree obtained with bayesian inference was used and the biomes of each species were assigned as terminals: Cerrado, Caatinga, Atlantic Forest and Pampas (outgroup species – Catumiri parvum). BioGeoBEARS implements three likelihood-based models: Dispersal-Extinction-Cladogenesis (DEC; Ree & Smith, 2008), the likelihood version of dispersal–vicariance (DIVA; Ronquist, 1997; herein DIVALIKE), and the likelihood version of BayArea model (Landis *et al.*, 2013; herein BAYAREALIKE). In each model, an additional j parameter for founder events was added, resulting in six models resulted (Matzke 2013), using 16, 9, 5 and 2.5 Mya to reflect the expanding vegetation of open biomes from Mid-Miocene and climates changes along Late Pleistocene (Werneck 2011). As most *Dolichothele* species are endemic, the parameter “max_range_size” was two or three. Log-likelihoods (LnL) with Akaike information criterion (AIC) and sample-size corrected AIC (AICc) scores were used to test each model’s suitability that were using the AIC weighted approach (Burnham & Anderson, 2002).

RESULTS

Taxon sampling

We studied 49 specimens of 7 species of genus *Dolichothele*, comprising all species except for *D. camargorum* and *D. tucuruiense*. Most specimens were from museums and some spiders were collected in the field (Table 4). Specimens were identified by Dr. José Paulo Guadanucci (UNESP, Campus Rio Claro).

At least one DNA region was sequenced for each specimen. The combined dataset was composed by 3263 bp (COI: 876 bp, ND1+16S+L1: 923 bp, 12S: 302 bp, 28S: 389 bp, ITS2: 400 bp, H3A: 373 bp).

Table 5. Specimens used in this study. CAD - Collection of arachnologic Diamantina, UNESP, Rio Claro; UFMG - University Federal of Minas Gerais; IBSP - Institute Butantã; UNB - University of Brasilia; MZSP - Museum of Zoology of the University of Sao Paulo. *Born in captivity.

Species	Museum	Voucher	Locality	Collection date	Biome
			Dolichothele		
D. bolivianum	IBSP	134073	Mato Grosso, MS	13/11/2008	Cerrado
	CAD	VD1	Balneário Água viva, MS	03/08/2019	Cerrado
	CAD	VD2	Balneário Água viva, MS	03/08/2019	Cerrado
	CAD	VD3	Balneário Água viva, MS	03/08/2019	Cerrado
	CAD	MSP3Db	Balneário Água viva, MS	03/08/2019	Cerrado
D. dominguense	CAD	600	Chapada dos Veadeiros, GO	01/01/2014	Cerrado
	UFMG	20090	Itacarambi, Distrito de Fabião II - MG	23/03/2016	Cerrado
	UFMG	20091	Itacarambi, Distrito de Fabião II - MG	23/03/2016	Cerrado
	UFMG	20092	Itacarambi, Distrito de Fabião II - MG	23/03/2016	Cerrado
	UFMG	20093	Itacarambi, Distrito de Fabião II - MG	23/03/2016	Cerrado
	MZSP	26536	S. Domingos - GO	01/04/2003	Cerrado
D. exilis	CAD	591	Jaguaretama, CE	18/10/2013	Caatinga
	CAD	570	Jaguaretama, CE	15-18/10/2013	Caatinga
	CAD	97	São Desidério, BA	01/10/2014	Cerrado
	CAD	98	Jaguaretama, CE	15-18/10/2013	Caatinga

Species	Museum	Voucher	Locality	Collection date	Biome
D diamantinensis	UFMG	20087	Crato - CE	04/09/2016	Caatinga
	CAD	VW3	São Desidério - BA	* 2017	Cerrado
	CAD	VW4	São Desidério - BA	* 2017	Cerrado
	CAD	BIO4	Jaguaretama, CE	* 2017	Caatinga
	CAD	578	MG	06/04/2010	Cerrado
	CAD	579	MG	06/04/2010	Cerrado
	CAD	608	São Gonçalo do Rio Preto, MG	14-20/12/2010	Cerrado
	CAD	VGT100	Diamantina, MG	04/04/2018	Cerrado
	CAD	VGT72.2	Gouveia, MG	* 2017	Cerrado
	CAD	BIO1	Diamantina, MG	* 2015	Cerrado
	CAD	BIO2	Diamantina, MG	* 2017	Cerrado
	CAD	BIO3	Diamantina, MG	* 2017	Cerrado
	CAD	489	Diamantina MG	Not Date	Cerrado
D. mineirum	IBSP	9141	Crasto (Santa Luzia do Itanhy) - SE	Not Date	Atlantic Forest
	CAD	VW1	Diamantina, MG	* 2017	Cerrado
	CAD	VW2	Diamantina, MG	* 2017	Cerrado
	CAD	BA01	Cordeiros, BA	* 2017	Atlantic Forest
D. rufoniger	CAD	473	São Gonçalo do Rio Preto, MG/Parque Estadual do Rio Preto	02/07/2005	Cerrado
	CAD	476		13-19/01/2010	Cerrado
	CAD	569		20-25/10/2010	Cerrado
	CAD	575		12-18/01/2010	Cerrado
	CAD	595		14/12/2010	Cerrado
	CAD	577		19/01/2010	Cerrado
	CAD	619		05/09/2011	Cerrado
	CAD	474		01/12/2008	Cerrado
	CAD	VGT99		04/04/2018	Cerrado
	CAD	LAB164	Serra Talhada, PE	03/10/2019	Caatinga
	UFMG	20086	Crato - Ceará	04/09/2016	Caatinga
D. mottai	UNB	9090	Distrito Federal, Brasilia	06/09/2018	Cerrado
	UNB	9103	Distrito Federal, Brasilia	10/15/18	Cerrado
	UNB	BGSP03	Barra do Garcas, MT	11/10/2019	Cerrado
	UNB	BGSP04	Barra do Garcas, MT	11/10/2019	Cerrado
	UNB	BGSP10	Barra do Garcas, MT	11/10/2019	Cerrado
	UNB	GVSP08	Goias Velho, GO	09/10/2019	Cerrado
			Outgroup		
Catumiri parvum	CAD	VGT78.1	Rio Grande do Sul	Not Date	Pampas

Phylogenetic analyses and divergence time estimation

Figure 3 presents the phylogenetic trees generated for each DNA marker, showing *Dolichothele* as a monophyletic genus and *Catumiri* as sister genus. The trees generated by ITS2 and H3A did not recover *D. bolivianum* and *D. mottai* as monophyletic lineages. According to all trees, *D. rufoniger* and *D. mineirum* do not comprise monophyletic lieneages.

The multilocus phylogenetic hypothesis (Fig. 4) indicates that *Dolichothele exilis* was the first species to diverge, followed by the divergence of two clades, one composed by *D. dominguense*, *D. rufoniger* and *D. mineirum* and the other composed by *D. diamantinensis* (the first to diverge), *D. bolivianum* and *D. mottai*.

The TMRCA of the *Dolichothele* genus were ca. 9.7 Mya (95% HPD = 7.4 – 12.5 Mya) (Fig. 5), with support of the nodes high (BPP: 0.87 – 0.99). The oldest divergences occurred between 7.4 – 12.5 Mya. The most recent divergences took place during the Quaternary (~2.5 Mya); and the divergence between *Catumiri* and *Dolichothele* was estimated in 15.40 Mya.

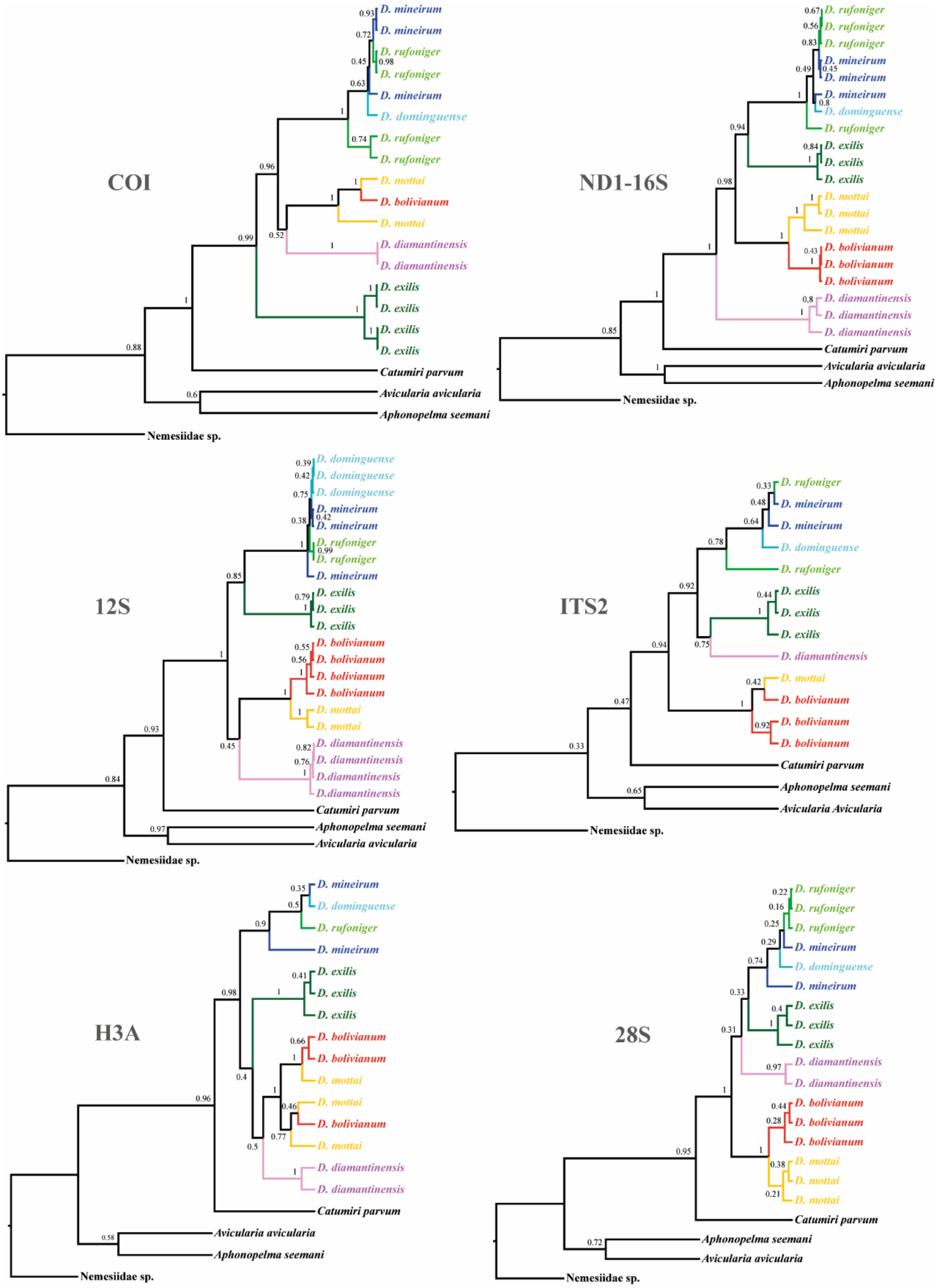

Figure 3. Bayesian phylogenetic inferences for each DNA marker (COI, ND1-16S, 12S, 28S, H3 and ITS2). Nodes with strong support (P 0.95) are represented.

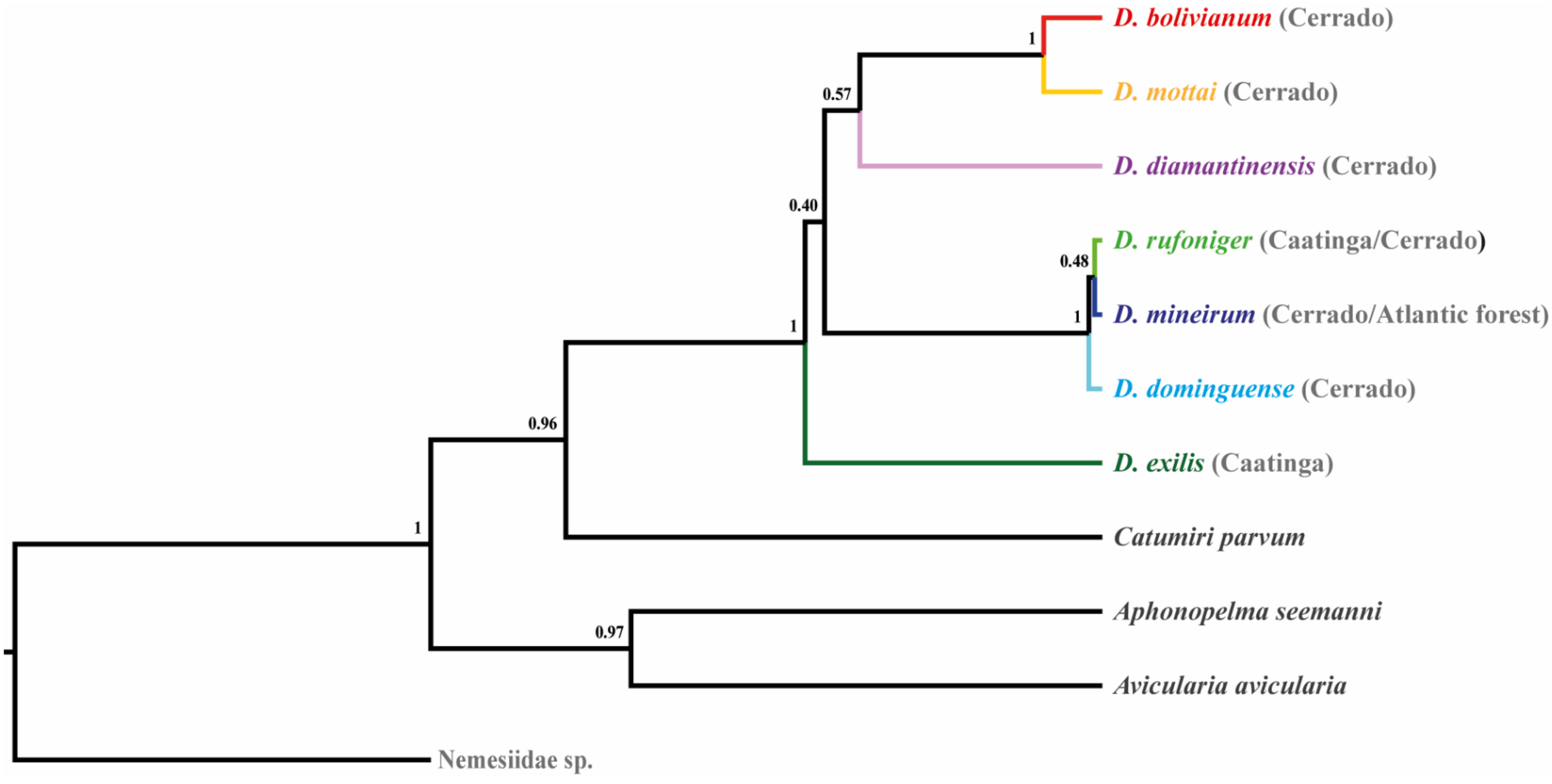

Figure 4. Multilocus phylogenetic hypotheses for the genus Dolichothele (*Beast 1.8). Terminal taxa are colored by species, in parentheses the biome where it occurs. On branches the probably posterior support.

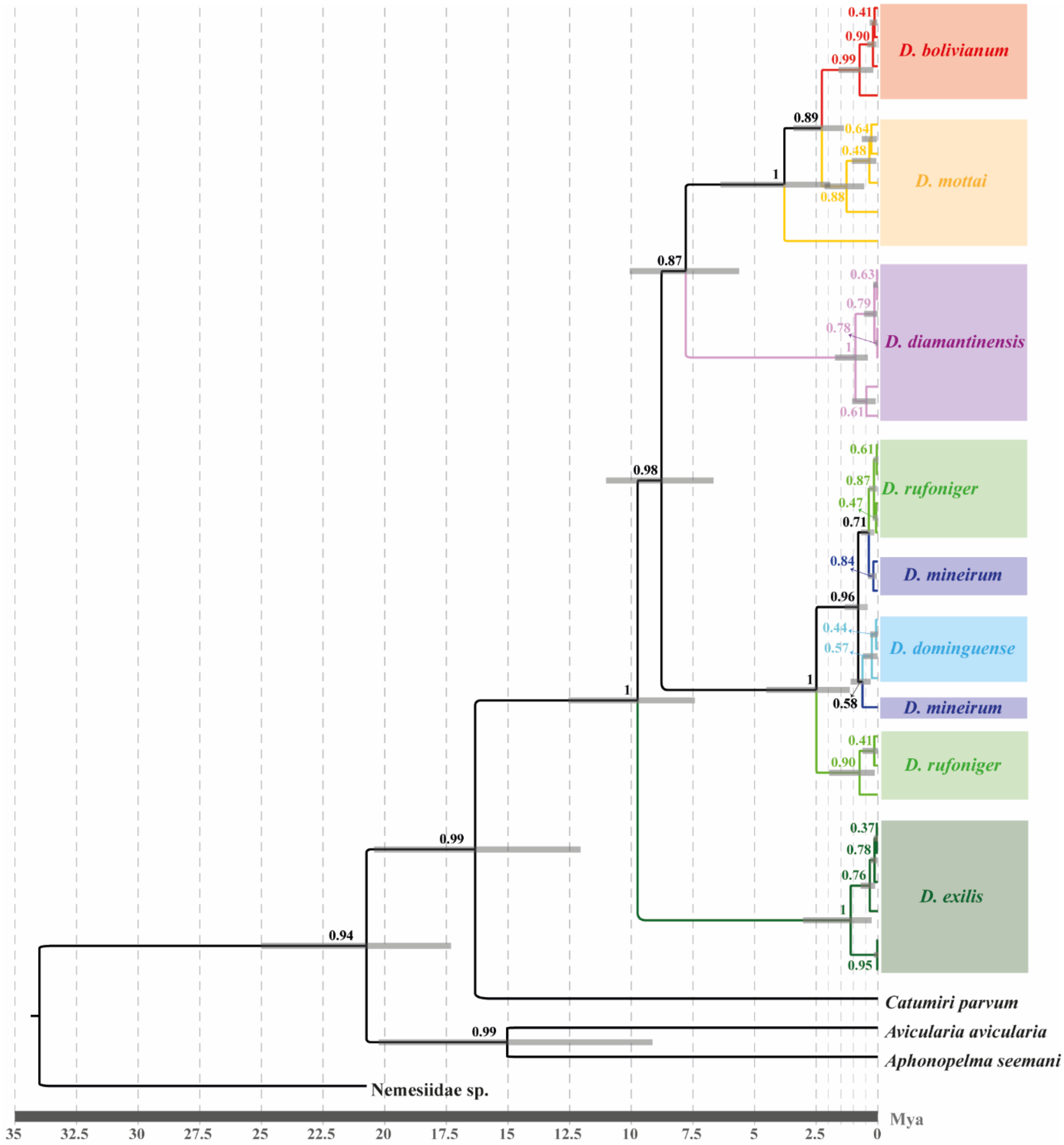

Figure 5. mtDNA tree of the Dolichothele genus (*Beast 1.8). On branches the posterior probably. Gray bars: 95% HDP (Highest Posterior Density) confidence intervals of node ages

Ancestral range reconstruction

The BSSV algorithm analyses indicated *Dolichothele* originated and diversified within the Cerrado biome (59%), with independent events of occupation of the Caatinga biome and Atlantic Forest (Fig. 6).

The BioGeoBEARS analyses also suggests that *Dolichothele* diversified from the Cerrado (48%) or from Caatinga + Cerrado (48%) (Fig. 7). The analysis, using the most suitable model for our data (DEC+J, max_range_size =3), revealed that Cerrado biome was most likely colonized first; after that, *Dolichothele* diversified within Cerrado and subsequently dispersed from there to other biomes. The details about the probabilities on nodes numbers are in Appendix (Table S3, Fig. S1 and S2).

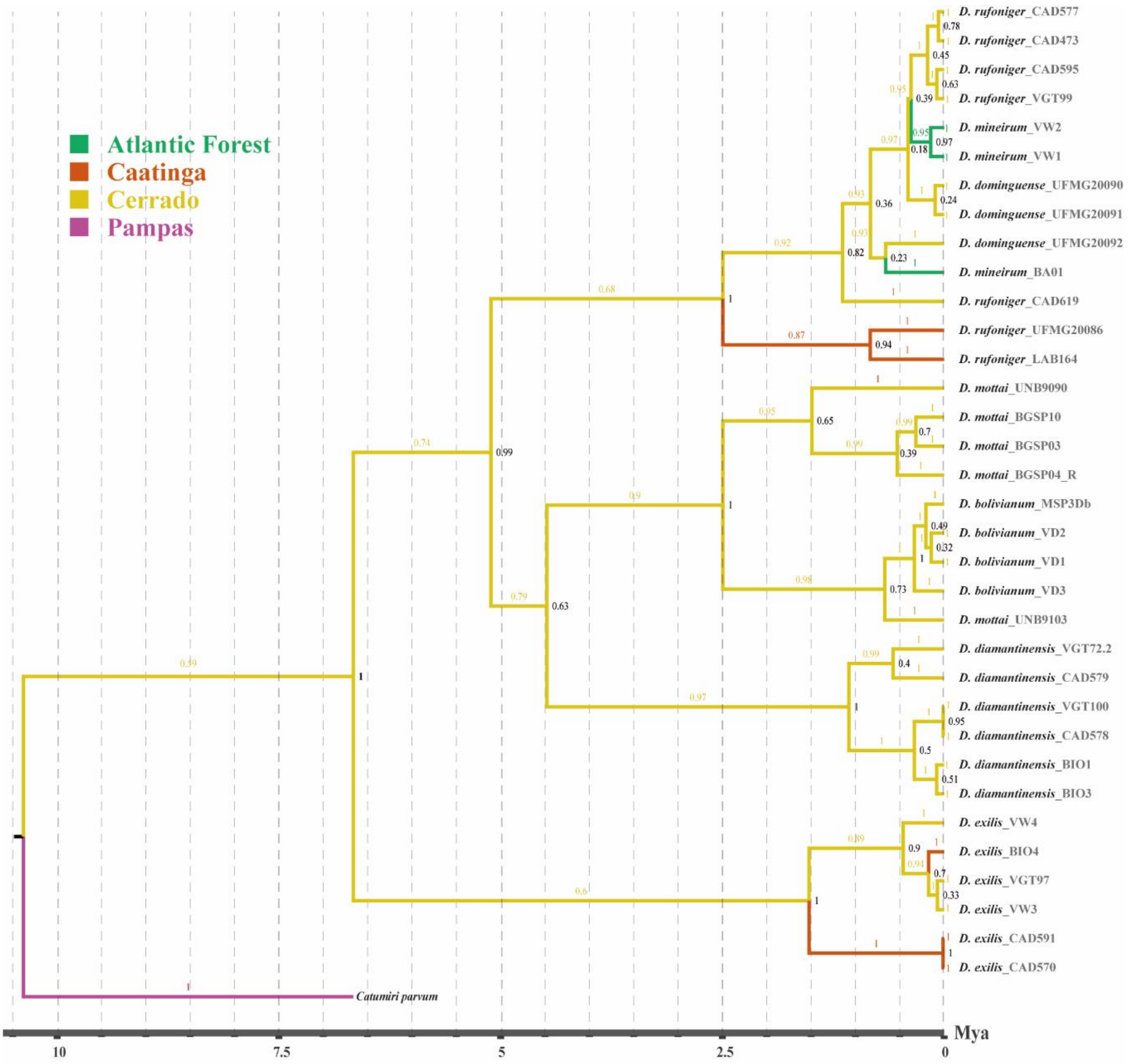

Figure 6. Ancestral range reconstruction for the Dolichothele species (BSSV algorithm on Beast 1.8). Branches are colored with the most probable location. Numbers on branches: probability of the location (%). On nodes: posterior probably support. Sample species ID and number correspond to Table 2

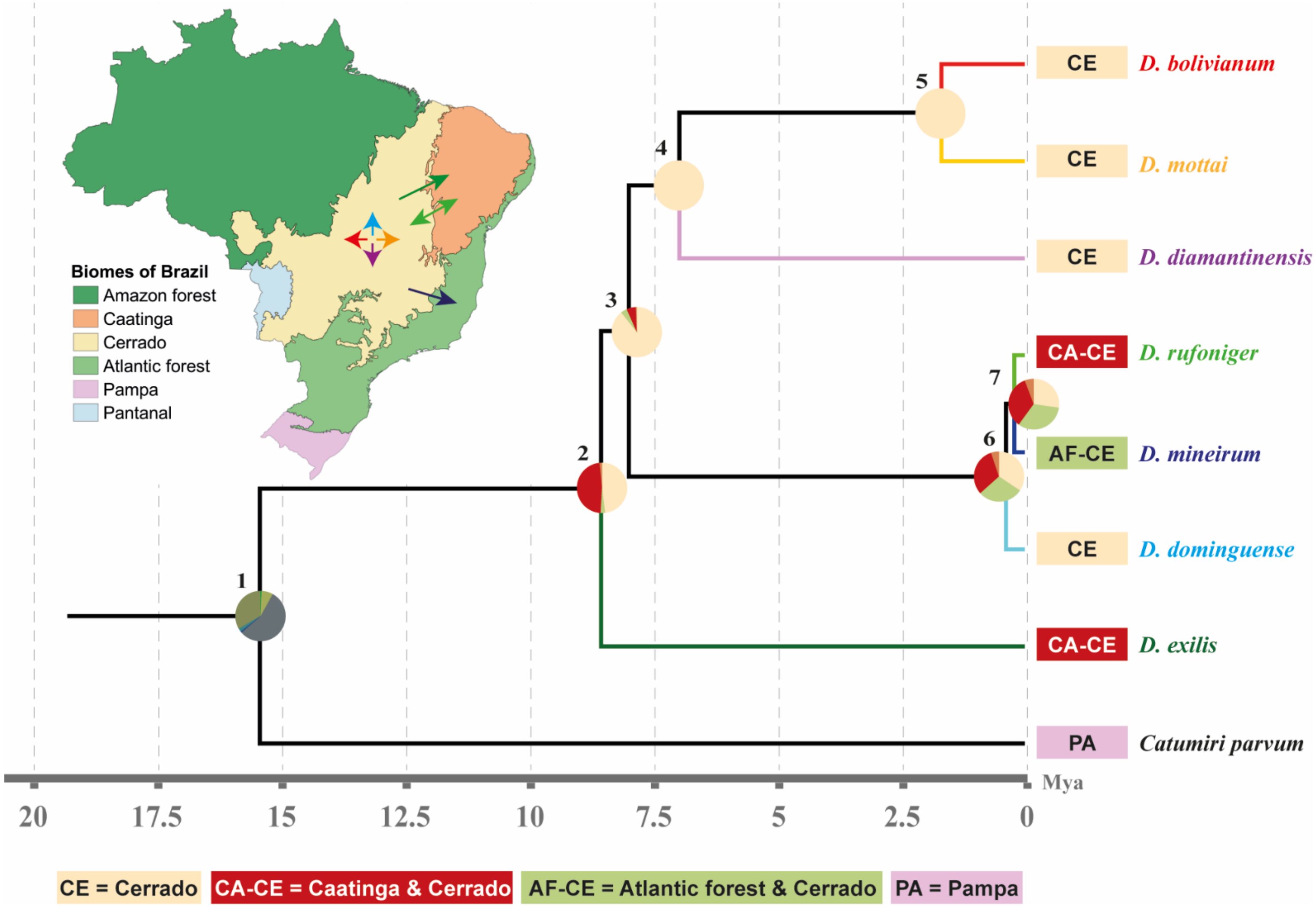

Figure 7. Ancestral area reconstruction of Dolichothele (BioGeoBEARS). The best-fit model:DEC+J using max_range_size =2. Most likely biogeographic areas are shown in the circles; colors in the squares indicate current species distribution. Combinations of two areas are not shown on the map. On the circles the number of the node detailed in Appendix Fig. S2.

DISCUSSION

Phylogenetic relationships of the Dolichothele genus

Previous phylogenetic hypothesis for genus *Dolichothele* were built on morphological characters and this is the first time that molecular data are used to study this genus.

Guadanucci (2014) suggested that *Catumiri* and *Dolichothele* are sister clades, what has been corroborated in the present analysis (Figs. 3 and 4). These genera have sympatric distribution in Brazil, are among the smallest spiders of Theraphosidae family and have similar behaviors (Guadanucci 2004). According to our analysis, the split between them occurred around 15 Mya (Fig. 5).

This study recovered *Dolichothele* as a monophyletic clade for all molecular markers, but the tree topology varied among the trees (Fig. 3), indicating that the relationships among species should be interpreted with caution. Indeed, systematics of *Dolichothele* species had been revised, with conflicting results (Guadanucci 2007, 2011; Lucas & Indicatti 2015; Revollo *et al.* 2017).

Guadanucci (2007, 2011) considered eight species for the genus *Dolichothele*, including two with wide distribution: *D. bolivianum* (central Brazil to eastern Bolivia) and *D. mineirum* (Brazilian Atlantic Forest). Based on morphology, Revollo (2016) split *D. bolivianum* and *D. mineirum* in six species; for this author, the genus *Dolichothele* have eleven species including two new species close to *D. bolivianum*: *D. mottai* and *D. camargorum* from Goias (GO) and Rondonia (AM), respectively (Revollo *et al.* 2017). These three distinct species are not supported by the cladistic analysis of Revollo (2016) that displayed *D. bolivianum*, *D. mottai* and *D. camargorum* as polyphyletic. The molecular phylogenies show *D. bolivianum* and *D. mottai* as very close lineages, that separated during Pleistocene (Figs. 3, 4 and 5) in a well-supported node (Fig.4) although they are not monophyletic in the trees generated with ITS2 and H3A sequences. Whether this indicates they belong to a single species (as suggested by Guadanucci, 2007, 2011) or to two species presenting incomplete lineage sorting due to their recent separation remains to be addressed in future studies.

In the species tree (Fig. 4), the first node within *Dolichothele* is well supported, corresponding to the diversification of *D. exilis*. The node corresponding to the separation of *D. bolivianum* and *D. mottai* has a high probability as well as the node corresponding to the separation of *D. dominguense* and *D. rufoniger* + *D. mineirum*.

The comparison of the molecular phylogenetic tree with the two available ones, built with morphological characters, show distinct topologies (Fig. 8), but in all of them *D. exilis* in the first species to diverge. However, in the gene trees (Fig.3) just COI presents *D.exilis* as the first branch to diverge. The incongruences between the molecular tree and the morphological ones might be due to homoplasy of morphological features. The low support of several nodes in the molecular tree indicates that the systematic study of this genus should use approaches like integrative taxonomy and genomic data.

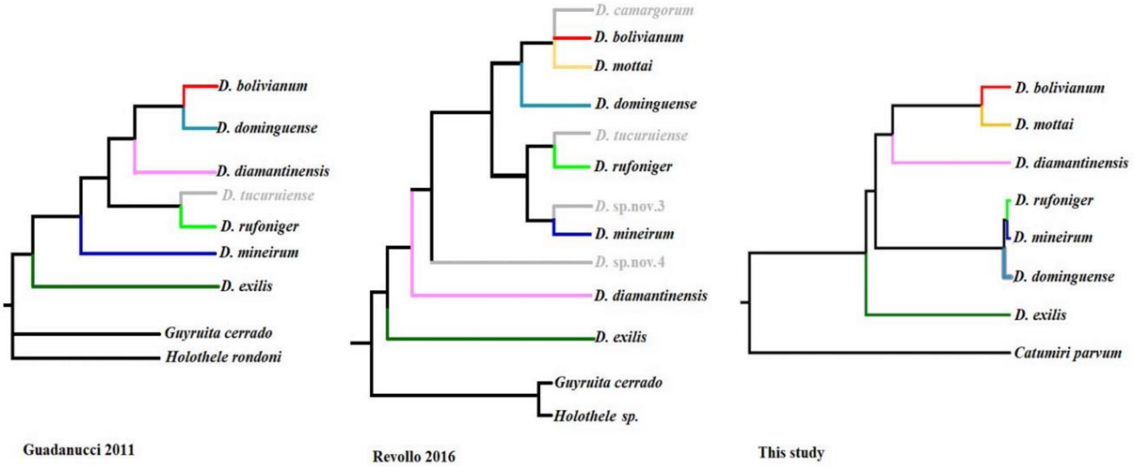

Figure 8. Phylogenetic hypothesis for *Dolichothele* species. (Species in grey were not included in this study).

Diversification of Dolichothele in the Brazilian landscape

Although tarantulas occur around the globe, mostly in tropical and sub-tropical regions, most known diversity is endemic to the New World. Brazil is the most diverse country with 3855 species of spiders (WSC 2021). Among the Brazilian biomes, the highest species richness of spiders is found in the Atlantic Forest, followed by the Amazon, in spite of large sampling gaps and high concentration of records around major cities and rivers. There are plenty of records from Pampas, mostly concentrated in the transition zone with the Atlantic Forest. The Cerrado, Caatinga and Pantanal have been poorly sampled and, consequently, are among the lesser-known biomes regarding spiders (Santos *et al*. 2017).

Large-scale changes in the physical environment may influence diversification patterns by changing the distribution and connectivity of landmasses or global and/or regional climates (Dimitrov & Hormiga 2021). While geological processes have certainly played a role in shaping the biogeographic patterns of spiders at various scales (e.g., Arnedo, Oromí & Ribera 2001; Hormiga, Arnedo & Gillespie 2003; Dimitrov, Arnedo & Ribera 2008; Bidegaray-Batista & Arnedo 2011; Xu *et al*. 2016), they have been suggested as a main cause of diversification in only a few cases (e.g., Bidegaray-Batista & Arnedo 2011; Xu *et al*. 2016). The results presented here indicate that *Dolichothele* genus originated in the Late Miocene (7.4 – 12.5 Mya) and that most events of cladogenesis occurred more recently, around 2.5 Mya, a radiation that may be linked to climatic fluctuations in the Quaternary. Studies with other arthropods also indicated a late Miocene origin of taxa associated with the savannas, with open domains acting as geographical barriers for taxa of the rain forests (Morrone 2006, 2014; Ferrari *et al.* 2015). Similarly, for Angiosperms, the diversification of the dry diagonal lineages occurred in the Neogene, whereas diversification within lineages occurred mainly during the Pleistocene (Collevatti *et al.* 2020). A similar pattern has been described for other spiders: in *Sicarius cariri* (Magalhaes *et al.* 2014) and *Nephila sexpuntacta* (Bartoleti *et al.* 2017) diversification of intraspecific lineages occurred during the Pliocene or in the Pleistocene period. Simulations for both *Nephila sexpunctata* and *Sicarius cariri* populations detected bottlenecks in the Pleistocene with subsequent demographic expansions, a pattern compatible with the scenario inferred by Sobral-Souza *et al.* (2015).

Many DD taxa originated in the Miocene (Pennington *et al*. 2004; Werneck *et al*. 2012b; Beati *et al*. 2013; Côrtes *et al*. 2015; Lanna *et al*. 2018; Fonseca *et al*. 2018; Lima *et al*. 2021), suggesting that the history of dry forests taxa may be older than previously thought. Our results corroborate that Neotropical and seasonally dry environments have stood as cohesive biogeographic units for a long evolutionary time. Nevertheless, while it seems likely that Neotropical dry biomes have been connected in the past, the causes of diversification events remain unclear (Pennington *et al.* 2005; Werneck 2011).

Climate changes are considered to affect the diversification rates of organisms (e.g., Clarke & Gaston 2006; Thom *et al*. 2020), but there are controversies on the impact of the Quaternary glacial cycles on the evolution of open areas for Neotropical organisms (Rull 2008; Werneck 2011). Rocha *et al*. (2020) hypothesized that if habitat fragmentation associated to climate cycles impacted of open area organisms, it would be expected an association between intraspecific lineages and regions with long term habitat stability. While climatic stability may be a predictor of squamate species richness (Werneck et al., 2012d), other studies indicate no correlation between climatic stability and levels of genetic diversity or main phylogeographical patterns (Bartoleti *et al*. 2017; Magalhaes *et al*. 2014; Pinheiro *et al*. 2013; Santos *et al*. 2014).

Pleistocene climatic refugia has been invoked to explain the genetic structure of some Neotropical Forest species, like mammals (Carnaval *et al*. 2009), frogs (Koscinski *et al*. 2008) and birds (Batalha-Filho *et al*. 2012; Silva *et al*. 2019; Thom *et al*. 2020), but few studies discuss the effect of climate on spider evolution (Dimitrov & Hormiga 2021). This work's results indicate that the Pleistocene climatic cycles (~2 Mya) influenced the more recent cladogenetic events in *Dolichothele*. Because of the low dispersal capacity of these mygalomorphs, the climate changes might have affected the spiders in a way different from the expected by the Pleistocene refugia model (Haffer 1997; Garzón-Orduña, Benetti-Longhini & Brower 2014).

Ancestral range reconstruction

Dolichothele comprises three species that occur mostly or exclusively in tropical forests and six species that occur mostly or exclusively in the Dry Diagonal and open formations (Fig. 1.) Our results indicate that the ancestral area of the *Dolichothele* was the Cerrado (Figs. 6 and 7), leading to the hypotheses of independent events of dispersion to the neighbor tropical forests and Caatinga. Although geomorphological events have been considered decisive in the vicariant speciation of several Neotropical groups (Giugliano, Collevatti & Colli, 2007; Antonelli *et al*. 2010; Werneck *et al*. 2009), our results are consistent with a scenario of dispersal playing a role on the divergence of *Dolichothele*.

In the clade *D. dominguense* + *D. rufoniger* + *D. mineirum*, *D. mineirum* represents a dispersion from Cerrado to the Atlantic Forest (Fig. 7). Biogeographical and geomorphological evidence indicate that during the glacial periods, forests might have contracted (Haffer 1969; Prance 1982), forcing transitions in plant and animal populations and fragmentation and recolonization processes at the large scale (Dutech *et al.* 2003; Flanagan *et al*. 2004; Scotti-Saintagne *et al*. 2013). It has been suggested that South American savannas reached their maximum extension during the LGM, connecting savannah patches currently located at the North (Guiana Shield), South (in central Brazil - Cerrado), East (Atlantic Forest) and West (Chiquitano dry forests) of South American (Webb 1991). These episodes of savanna expansions might have facilitated dispersal events of *Dolichothele* to the other biomes. *D. exilis* and *D. rufoniger* that co-occur in the Cerrado-Caatinga ecotone are relatively phylogenetically distant (Fig. 4 and 5). Guadanucci (2011) suggested that the Caatinga species experienced allopatric speciation and secondary sympatry. Ando Teixeira (2017) and Souza (2018) studied the mating behavior of *D. exilis* and *D. rufoniger*, finding that they share many courtships' behavioral steps that are performed in distinct sequences and intensities according to the species, indicating reproductive isolation.

Cerrado is the largest savanna region in South America and the second largest South American biome, exceeded only by Amazonia. It includes much of Central Brazil and parts of Northeastern Paraguay and eastern Bolivia (Ab'Saber 1977), with extensive borders with the two largest forest biomes (Amazonia and Atlantic Forest) and with the two largest dry regions

(Caatinga and Chaco). Hypotheses about the evolution of Cerrado lineages includes migration from adjacent biomes (Werneck 2011), deep-rooted isolated lineages with *in situ* diversification, long term isolation caused by dispersal limitations (Pennington *et al*. 2004, 2006; de Queiroz & Lavin 2011; Särkinen *et al*. 2012; de Queiroz *et al*. 2017; Hurbath *et al*. 2021) and long-distance dispersal along DD (Gentry 1982; Lavin *et al*. 2004; Mayle 2006; Pennington *et al*. 2009; Werneck 2015). Recent studies have indicated different mechanisms underlying the diversification of different groups in the Cerrado. Data from some groups support the effect of old events, such as the final uplift of the Central Brazilian shield (Domingos *et al*. 2014; Werneck *et al*. 2012a), others, the late Tertiary marine transgressions (Garda & Cannatella 2007; Werneck *et al*. 2009; Giugliano *et al*. 2013), while others support the role of recent Quaternary cycles (Ramos *et al*. 2007) in promoting diversification. A broad biogeographic meta-analysis combining distribution data and phylogenies for several taxonomic groups detected a high proportion of biotic interchange among Neotropical regions, mostly associated with dispersal shifts from the forest into the open biomes (Antonelli *et al*. 2018a). Maciel *et al*. (2010) suggested that barriers and dispersion routes were created by the elevation of the Central Brazilian Shield and, together with climate changes explain the diversification and current distribution of species of *Rhinella*. A pattern like *Dolichothele*'s, with both old and recent events of diversification was also found in frogs (Prado *et al*. 2012) and lizards (Santos *et al*. 2014; Guarnizo *et al*. 2016).

Future molecular analyses using specimens from all habitats will certainly help to provide more detailed biogeographical hypothesis.

CONCLUSIONS

The molecular phylogeny of genus *Dolichothele* is for first time presented here. The analysis showed *Dolichothele* spiders as a monophyletic group. Despite being the most comprehensive phylogenetic study for the *Dolichothele* genus, some questions remain open. We point to directions for future studies using UCEs or other method for resolved uncertain taxonomies.

Divergence time estimates for *Dolichothele* suggest that the deepest branches split in the Late Miocene and recent diversification events happened during the Quaternary.

Our findings allow us to hypothesize that the evolutionary history of *Dolichothele* comprises expansions within the Cerrado biome and independent colonization events to the neighboring biomes. We highlight the South American open diagonal as a significant diversification center.

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APPENDIX

Appendix 1: PCR protocols.

Table S1. Detail of the amounts of reagents, times, and PCR amplification protocols for each of the markers used in this study.

Marker	PCR program	Quantities by reactive
NAD1-16S	1 cycle of 94°C / 2 min 35 cycles of 94°C / 45 sec 50°C / 40 sec 65°C / 1 min 1 cycle of 65°C / 10 min 1 cycle of 4°C / ∞	Buffer10X [10X]: 2,5ul $MgCl_2$ [25mM]: 2,5ul dNTPmix [10mM]: 0,5ul Primer F [10uM]: 0,5ul Primer R [10uM]: 0,5ul TaqPol [5U/ul]: 0,3ul DNA [40ng/ul]: 3ul H_2O MQ: 15,2ul
COI	1 cycle of 95ºC / 4min 35 cycles of 95ºC / 45 sec 60ºC / 45 sec 72ºC / 2 min 1 cycle of 72ºC / 10 min 1 cycle of 4ºC/ ∞	
ITS2	1 cycle of 95ºC / 4min 35 cycles of 95ºC / 45 sec 60ºC / 45 sec 70ºC / 2 min 1 cycle of 70ºC / 10 min 1 cycle of 4ºC/ ∞	
12S	1 cycle of 94ºC / 3min 35 cycles of 94ºC / 45 sec 46ºC / 30 sec 72ºC / 1 min 1 cycle of 72ºC / 3 min 1 cycle of 4ºC/ ∞	
28S	1 cycle of 96ºC / 7min 35 cycles of 96ºC / 45 sec 50ºC / 45 sec 72ºC / 1 min 1 cycle of 72ºC / 10 min 1 cycle of 4ºC/ ∞	
H3A	1 cycle of 94ºC / 3min 30 cycles of 94ºC / 45 sec 46ºC / 1 min 72ºC / 1 min 1 cycle of 72ºC / 10 min 1 cycle of 4ºC/ ∞	

Appendix 2: Partition scheme.

Table S2. Detail of the best partitioning scheme for our concatenate data.

Scheme Name: step_1
Scheme lnL: -12288.25764465332
Scheme AICc: 24748.6664521
Number of params: 84
Number of sites: 3525
Number of subsets: 9

Subset	Best Model	# sites	Partition names
1	TRN+I+G	392	COIcodon1
2	TVM+I+G	391	COIcodon2
3	TRN+G	391	COIcodon3
4	GTR+I+G	1126	12S, ND1-16S
5	F81+G	402	ITS2
6	TRN+I	530	28S
7	GTR+I	98	H3Acodon1
8	JC	98	H3Acodon2
9	K81	97	H3Acodon3

```
#nexus
begin sets;
        charset Subset1 = 1-1174\3;
        charset Subset2 = 2-1174\3;
        charset Subset3 = 3-1174\3;
        charset Subset4 = 2013-2300 1175-2012;
        charset Subset5 = 2301-2702;
        charset Subset6 = 2703-3232;
        charset Subset7 = 3233-3525\3;
        charset Subset8 = 3234-3525\3;
        charset Subset9 = 3235-3525\3;
        charpartition PartitionFinder = TRN+I+G:Subset1, TVM+I+G:Subset2, TRN+G:Subset3,
GTR+I+G:Subset4, F81+G:Subset5, TRN+I:Subset6, GTR+I:Subset7, JC:Subset8, K81:Subset9;
end;
```

<u>MrBayes block</u> for partition definitions

```
begin mrbayes;
        charset Subset1 = 1-1174\3;
        charset Subset2 = 2-1174\3;
        charset Subset3 = 3-1174\3;
        charset Subset4 = 2013-2300 1175-2012;
        charset Subset5 = 2301-2702;
        charset Subset6 = 2703-3232;
        charset Subset7 = 3233-3525\3;
        charset Subset8 = 3234-3525\3;
        charset Subset9 = 3235-3525\3;
        partition PartitionFinder = 9:Subset1, Subset2, Subset3, Subset4, Subset5, Subset6, Subset7,
Subset8, Subset9;
        set partition=PartitionFinder;

        lset applyto=(1) nst=6 rates=invgamma;
        lset applyto=(2) nst=6 rates=invgamma;
        lset applyto=(3) nst=6 rates=gamma;
```

```
        lset applyto=(4) nst=6 rates=invgamma;
        lset applyto=(5) nst=1 rates=gamma;
        lset applyto=(6) nst=6 rates=propinv;
        lset applyto=(7) nst=6 rates=propinv;
        lset applyto=(8) nst=1;
prset applyto=(8) statefreqpr=fixed(equal);
        lset applyto=(9) nst=6;

        prset applyto=(all) ratepr=variable;
        unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all) tratio=(all);
end;
```

Appendix 3: Summary statistics from BiogeoBears.

Table S3. Summary statistics AICc_rellike_formatted from BioGeoBears**.**

	LnL	**numparams**	**D**	**e**	**J**	**AICc**	**AICc_wt**
DEC	-21.39	2	0.055	0.021	0	49.18	0.069
DEC+J	-16.02	3	0.015	1.0e-12	1.0e-05	44.03	0.90
DIVALIKE	-22.7	2	0.063	0.021	0	51.8	0.019
DIVALIKE+J	-22.12	3	0.054	0.020	0.052	56.24	0.0020
BAYAREALIKE	-23.69	2	0.061	0.050	0	53.78	0.0069
BAYAREALIKE+J	-22.28	3	0.051	0.021	0.066	56.57	0.0017

Appendix 3 continuation…

Table S3.1. Summary statistics Test Table Models obtained from BioGeoBears

TEST_TABLE

	alt	null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	pval	test	tail	AIC1	AIC2	AICwt1	AICwt2	AICweight_ratio_model1	AICweight_ratio_model2
1	DEC+J	DEC	-16.02	-21.39	3	2	1	10.74	0.0010	chi-squared	one-tailed	38.03	46.78	0.99	0.012	79.21	0.013
2	DIVALIKE+J	DIVALIKE	-22.12	-22.7	3	2	1	1.16	0.28	chi-squared	one-tailed	50.24	49.4	0.40	0.60	0.66	1.52
3	BAYAREALIKE+J	BAYAREALIKE	-22.28	-23.69	3	2	1	2.81	0.093	chi-squared	one-tailed	50.57	51.38	0.60	0.40	1.5	0.67

Appendix 3 continuation…

Table S3.2. Summary statistics Relative_probs_of_each_state_at_branch_bottom_below_node_UPPASS obtained from BioGeoBears

Nodes/Area	Null Range	AF	CA	CE	PA	AF-CA	AF-CE	AF-PA	CA-CE	CA-PA	CE-PA	AF-CA-CE	AF-CA-PA	AF-CE-PA	CA-CE-PA
1	0,00	0,01	0,01	0,91	0,01	0,00	0,01	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00
2	0,00	0,01	0,01	0,91	0,01	0,00	0,01	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00
3	0,00	0,01	0,01	0,95	0,01	0,00	0,01	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00
4	0,00	0,33	0,03	0,60	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
5	0,00	0,03	0,33	0,59	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
6	0,00	0,20	0,21	0,50	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
7	0,00	0,07	0,07	0,38	0,13	0,01	0,04	0,04	0,04	0,04	0,09	0,01	0,00	0,04	0,04
8	0,00	0,09	0,15	0,35	0,08	0,03	0,05	0,03	0,05	0,03	0,05	0,03	0,00	0,02	0,03
9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	0,00	0,07	0,07	0,07	0,40	0,03	0,03	0,07	0,03	0,07	0,07	0,00	0,03	0,03	0,03
11	0,00	0,06	0,24	0,24	0,18	0,02	0,02	0,03	0,03	0,05	0,05	0,01	0,02	0,02	0,03
12	0,00	0,08	0,22	0,56	0,05	0,01	0,01	0,01	0,02	0,01	0,02	0,00	0,00	0,00	0,01
13	0,00	0,01	0,01	0,95	0,01	0,00	0,01	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00
14	0,00	0,01	0,02	0,84	0,03	0,00	0,01	0,01	0,02	0,01	0,03	0,00	0,00	0,01	0,01
15	0,00	0,02	0,02	0,86	0,02	0,00	0,02	0,00	0,02	0,00	0,02	0,00	0,00	0,00	0,00

Appendix 3 continuation…

Table S3.3. Summary statistics Relative_probs_of_each_state_at_branch_bottom_below_node_DOWNPASS obtained from BioGeoBears

Nodes/Area	**Null Range**	**AF**	**CA**	**CE**	**PA**	**AF-CA**	**AF-CE**	**AF-PA**	**CA-CE**	**CA-PA**	**CE-PA**	**AF-CA-CE**	**AF-CA-PA**	**AF-CE-PA**	**CA-CE-PA**
1	0,000	0,000	0,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
2	0,000	0,000	0,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
3	0,000	0,000	0,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
4	0,000	0,000	0,003	0,003	0,000	0,000	0,000	0,000	0,994	0,000	0,000	0,000	0,000	0,000	0,000
5	0,000	0,003	0,000	0,003	0,000	0,000	0,994	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
6	0,000	0,000	0,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
7	0,000	0,000	0,109	0,109	0,000	0,000	0,000	0,000	0,781	0,000	0,000	0,000	0,000	0,000	0,000
8	0,000	0,000	0,000	0,000	1,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	0,0000	0,0035	0,0542	0,3955	0,0000	0,0064	0,0315	0,0000	0,4737	0,0000	0,0000	0,0352	0,0000	0,0000	0,0000
11	0,0000	0,0019	0,0019	0,4566	0,0000	0,0014	0,2261	0,0000	0,2261	0,0000	0,0000	0,0859	0,0000	0,0000	0,0000
12	0,0000	0,0000	0,0000	1,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
13	0,0000	0,0000	0,0000	1,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
14	0,0000	0,0354	0,0354	0,1084	0,0000	0,0378	0,2901	0,0000	0,2901	0,0000	0,0000	0,2027	0,0000	0,0000	0,0000
15	0,0000	0,0008	0,0008	0,0081	0,0000	0,0027	0,3290	0,0000	0,3290	0,0000	0,0000	0,3296	0,0000	0,0000	0,0000

Appendix 3 continuation…

Table S3.4. Summary statistics Relative_probs_of_each_state_at_branch_top_AT_node_UPPASS obtained from BioGeoBears

	Range Null	**AF**	**CA**	**CE**	**PA**	**AF-CA**	**AF-CE**	**AF-PA**	**CA-CE**	**CA-PA**	**CE-PA**	**AF-CA-CE**	**AF-CA-PA**	**AF-CE-PA**	**CA-CE-PA**
1	0,00	0,01	0,01	0,85	0,01	0,00	0,03	0,00	0,03	0,00	0,03	0,00	0,00	0,00	0,00
2	0,00	0,01	0,01	0,85	0,01	0,00	0,03	0,00	0,03	0,00	0,03	0,00	0,00	0,00	0,00
3	0,00	0,00	0,01	0,69	0,01	0,00	0,07	0,00	0,08	0,00	0,08	0,02	0,00	0,02	0,02
4	0,00	0,33	0,03	0,59	0,04	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
5	0,00	0,03	0,33	0,58	0,04	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00
6	0,00	0,20	0,21	0,49	0,07	0,00	0,01	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00
7	0,00	0,04	0,04	0,26	0,09	0,02	0,06	0,04	0,06	0,04	0,10	0,04	0,03	0,09	0,09
8	0,00	0,04	0,07	0,17	0,04	0,04	0,07	0,03	0,07	0,04	0,06	0,11	0,05	0,10	0,11
9	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,07
10	0,00	0,05	0,05	0,05	0,29	0,03	0,03	0,08	0,03	0,08	0,08	0,02	0,07	0,07	0,07
11	0,00	0,06	0,24	0,24	0,18	0,02	0,02	0,03	0,03	0,05	0,05	0,01	0,02	0,02	0,03
12	0,00	0,08	0,21	0,53	0,05	0,01	0,02	0,01	0,03	0,02	0,03	0,00	0,00	0,01	0,01
13	0,00	0,00	0,01	0,75	0,01	0,00	0,06	0,00	0,06	0,00	0,06	0,01	0,00	0,01	0,01
14	0,00	0,01	0,01	0,59	0,02	0,00	0,07	0,01	0,08	0,01	0,09	0,03	0,00	0,03	0,04
15	0,00	0,02	0,02	0,85	0,02	0,00	0,02	0,00	0,02	0,00	0,02	0,00	0,00	0,00	0,01

Appendix 3 continuation…

Table S3.5. Summary statistics Relative_probs_of_each_state_at_branch_top_AT_node_DOWNPASS obtained from BioGeoBears

	Range Null	**AF**	**CA**	**CE**	**PA**	**AF-CA**	**AF-CE**	**AF-PA**	**CA-CE**	**CA-PA**	**CE-PA**	**AF-CA-CE**	**AF-CA-PA**	**AF-CE-PA**	**CA-CE-PA**
1	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
2	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
3	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
4	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00
5	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
6	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00
8	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
9	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,56	0,00	0,00	0,02	0,33
10	0,00	0,00	0,00	0,37	0,00	0,00	0,03	0,00	0,57	0,00	0,00	0,03	0,00	0,00	0,00
11	0,00	0,00	0,00	0,46	0,00	0,00	0,23	0,00	0,23	0,00	0,00	0,08	0,00	0,00	0,00
12	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
13	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
14	0,00	0,00	0,00	0,06	0,00	0,00	0,38	0,00	0,38	0,00	0,00	0,19	0,00	0,00	0,00
15	0,00	0,00	0,00	0,01	0,00	0,00	0,33	0,00	0,33	0,00	0,00	0,33	0,00	0,00	0,00

Appendix 3 continuation…

Table S3.6. Summary statistics ML_marginal_prob_each_state_at_branch_top_AT_node obtained from BioGeoBears

Nodes/Area	null range	**AF**	**CA**	**CE**	**PA**	**AF-CA**	**AF-CE**	**AF-PA**	**CA-CE**	**CA-PA**	**CE-PA**	**AF-CA-CE**	**AF-CA-PA**	**AF-CE-PA**	**CA-CE-PA**
1	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
2	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
3	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
4	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0
5	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
6	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0
8	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0
9	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,56	0,00	0,00	0,02	0,33
10	0	0,00	0,00	0,48	0,00	0,00	0,03	0,00	0,48	0,00	0,00	0,02	0,00	0,00	0,00
11	0	0,00	0,00	0,90	0,00	0,00	0,04	0,00	0,06	0,00	0,00	0,01	0,00	0,00	0,00
12	0	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
13	0	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
14	0	0,00	0,00	0,34	0,00	0,00	0,29	0,00	0,31	0,00	0,00	0,05	0,00	0,00	0,00
15	0	0,00	0,00	0,27	0,00	0,00	0,33	0,00	0,34	0,00	0,00	0,06	0,00	0,00	0,00

Appendix 3 continuation…

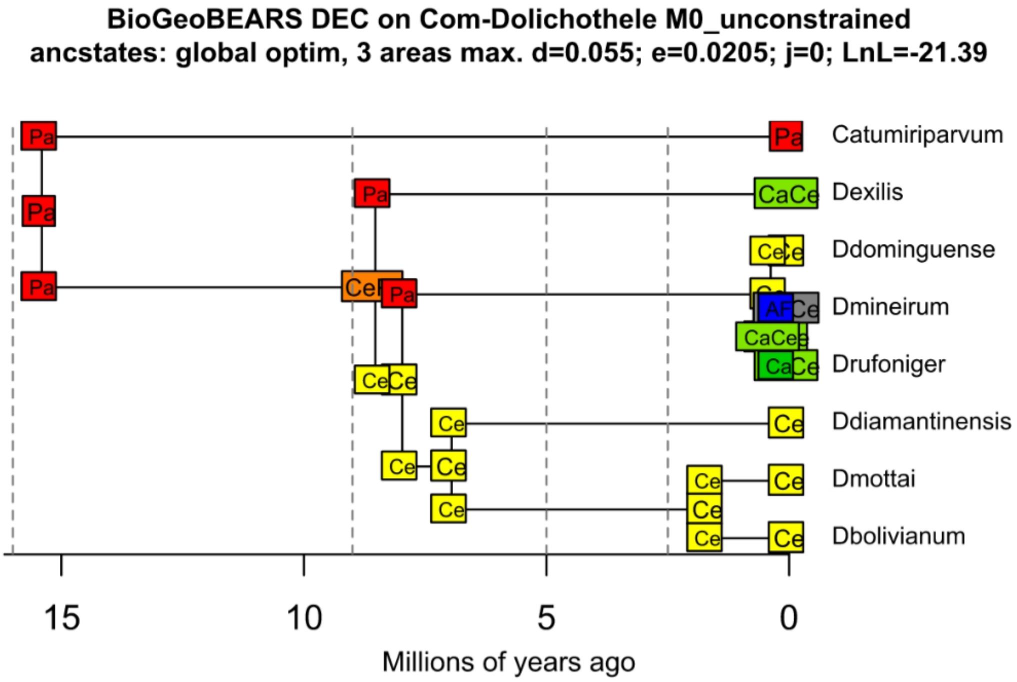
Table S3.7. Summary statistics ML_marginal_prob_each_state_at_branch_bottom_below_node obtained from BioGeoBears

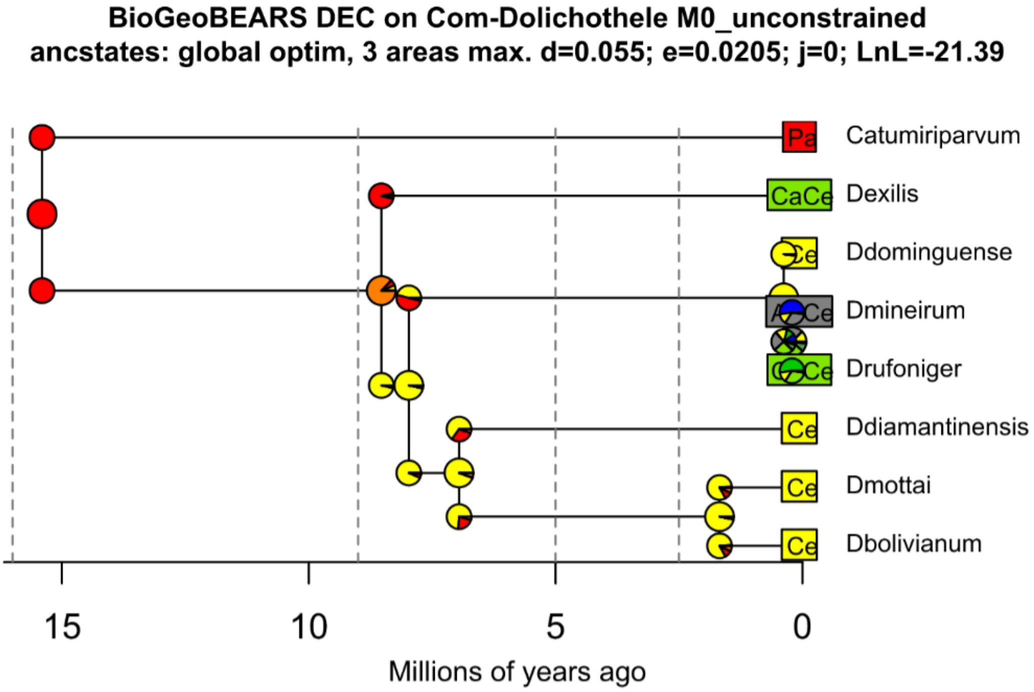
	null range	**AF**	**CA**	**CE**	**PA**	**AF-CA**	**AF-CE**	**AF-PA**	**CA-CE**	**CA-PA**	**CE-PA**	**AF-CA-CE**	**AF-CA-PA**	**AF-CE-PA**	**CA-CE-PA**
1	0,00	0,00	0,00	1,00	0,00	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
2	0,00	0,00	0,00	1,00	0,00	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
3	0,00	0,00	0,00	1,00	0,00	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
4	0,00	0,00	0,03	0,60	0,00	0,00	0,000	0,000	0,371	0,000	0,000	0,000	0,000	0,000	0,000
5	0,00	0,03	0,00	0,62	0,00	0,00	0,353	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
6	0,00	0,00	0,00	1,00	0,00	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
7	0,00	0,00	0,09	0,53	0,00	0,00	0,000	0,000	0,373	0,000	0,000	0,000	0,000	0,000	0,000
8	0,00	0,00	0,00	0,00	1,00	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	0,0000	4,9258	0,0764	0,5579	0,0000	0,0045	0,0222	0,0000	0,3341	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
11	0,0000	0,0010	0,0038	0,9029	0,0000	0,0002	0,0342	0,0000	0,0515	0,0000	0,0000	0,0064	0,0000	0,0000	0,0000
12	0,0000	0,0000	0,0000	1,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
13	0,0000	0,0000	0,0000	1,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
14	0,0000	0,0042	0,0064	0,8953	0,0000	0,0011	0,0345	0,0000	0,0524	0,0000	0,0000	0,0061	0,0000	0,0000	0,0000
15	0,0000	0,0007	0,0008	0,3449	0,0000	0,0005	0,2907	0,0000	0,3081	0,0000	0,0000	0,0542	0,0000	0,0000	0,0000

Appendix 4: Models evaluated in BioGeoBEARS

Figure S1. Results of the ancestral reconstruction according to each model [(A) DEC (B) DEC+J (C) DIVALIKE (D) DIVALIKE + J (E) BAYAREALIKE (F) BAYAREALIKE + J] evaluated in the Biogeobears program.

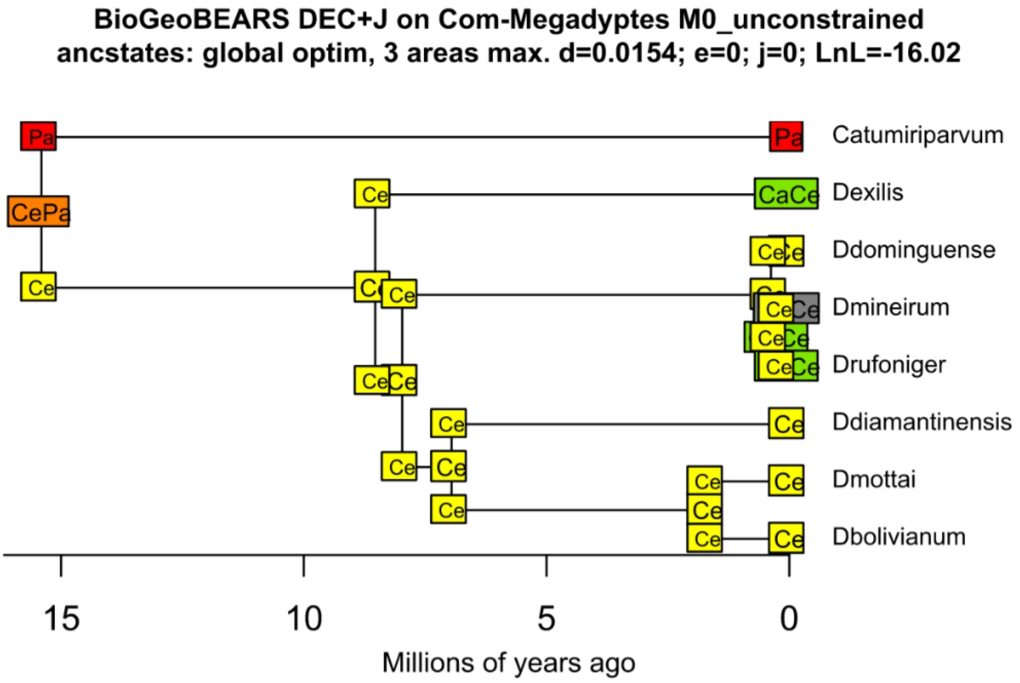
A) DEC Model.

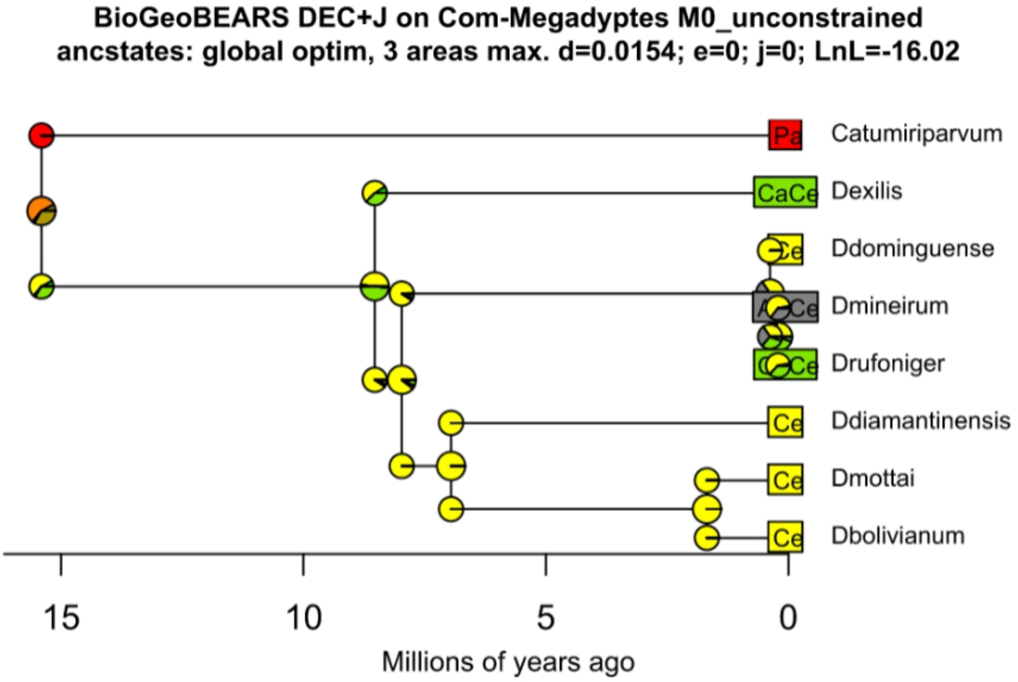




Appendix 4 continuation…

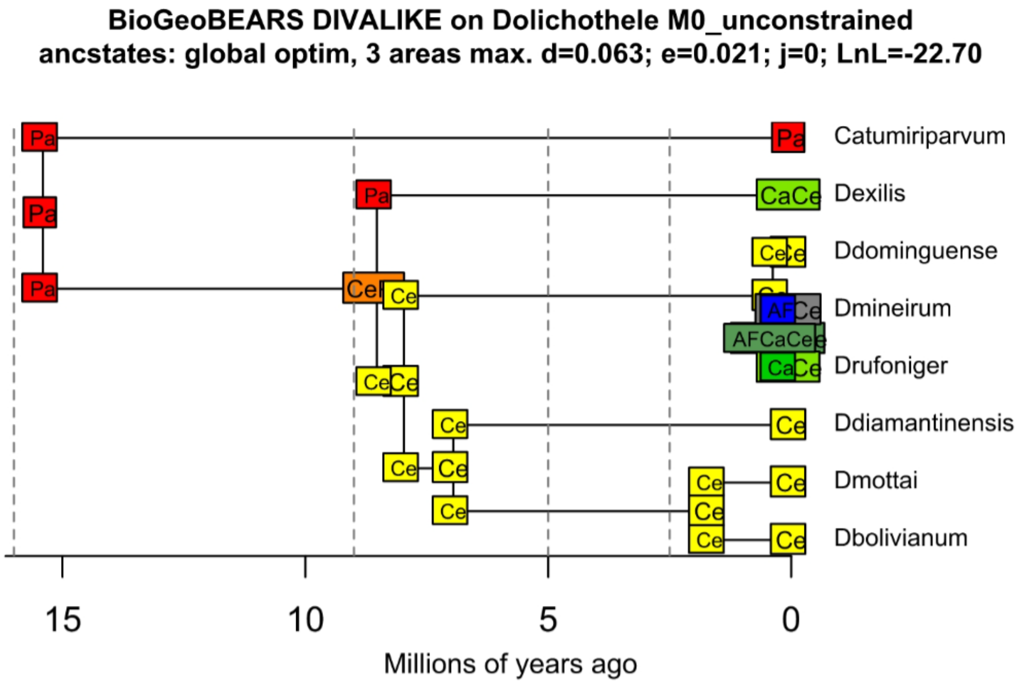
B) DEC + J Model.

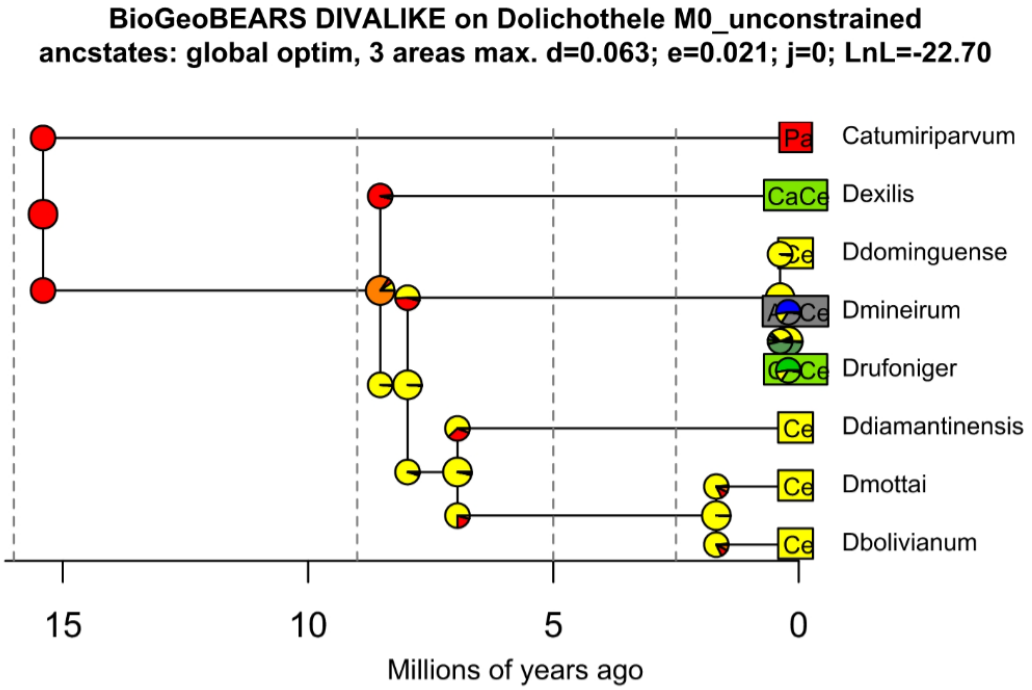




Appendix 4 continuation…

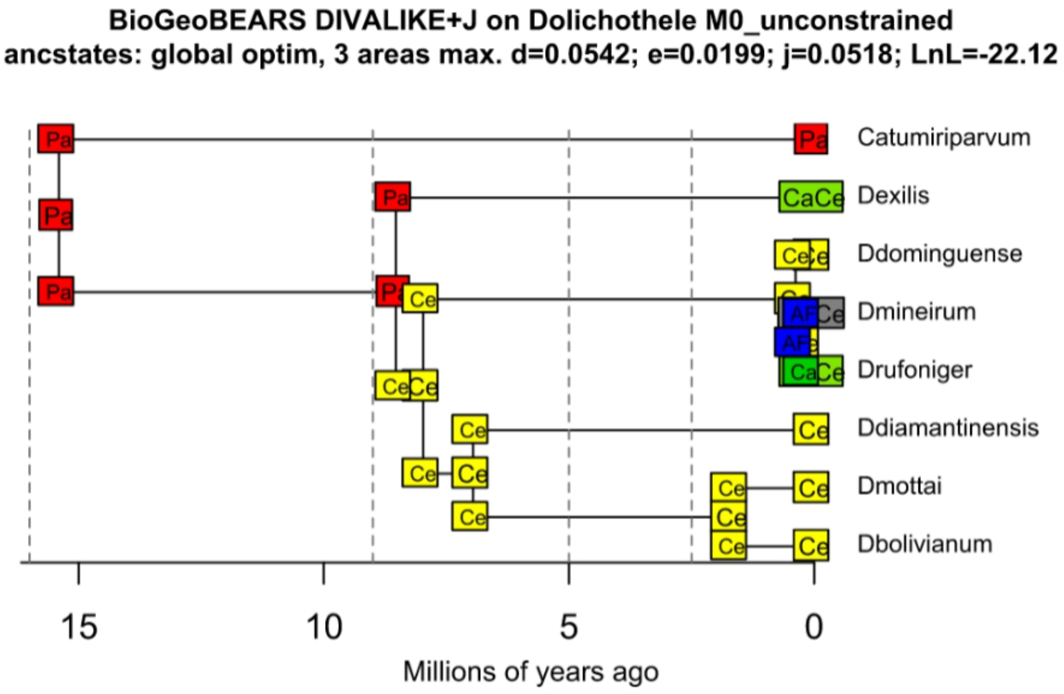
C) DIVALIKE Model

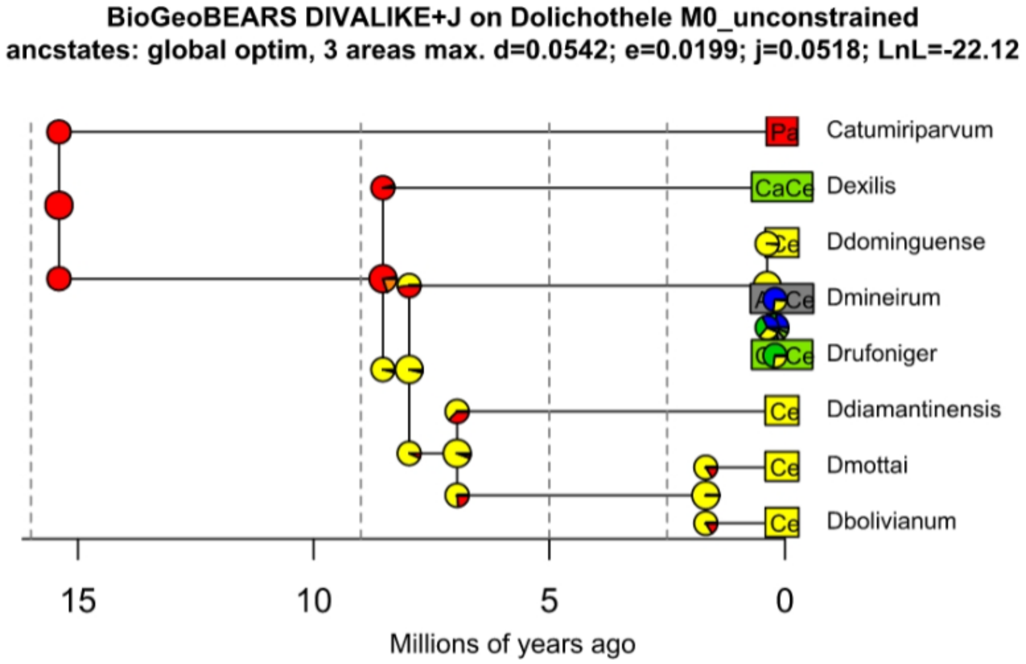




Appendix 4 continuation…

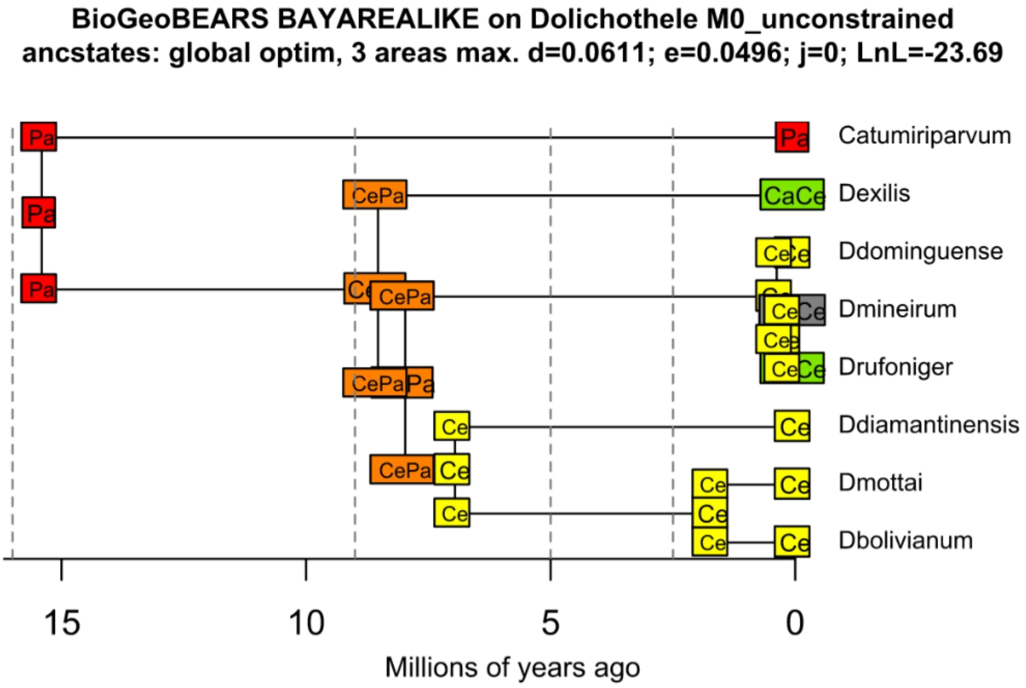
D) DIVALIKE + J Model

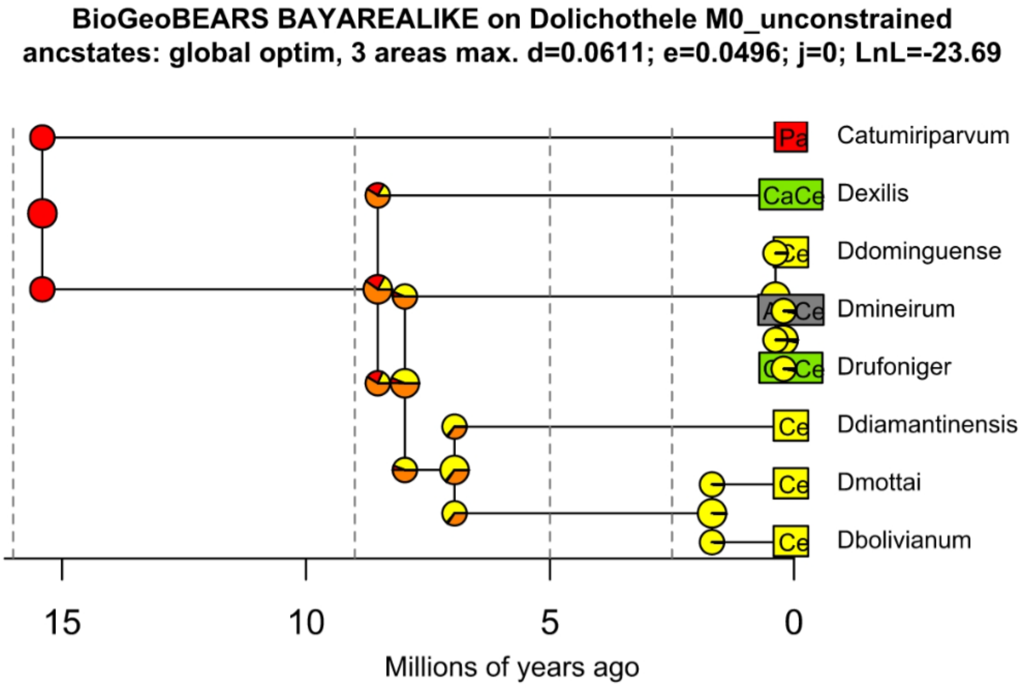




Appendix 4 continuation…

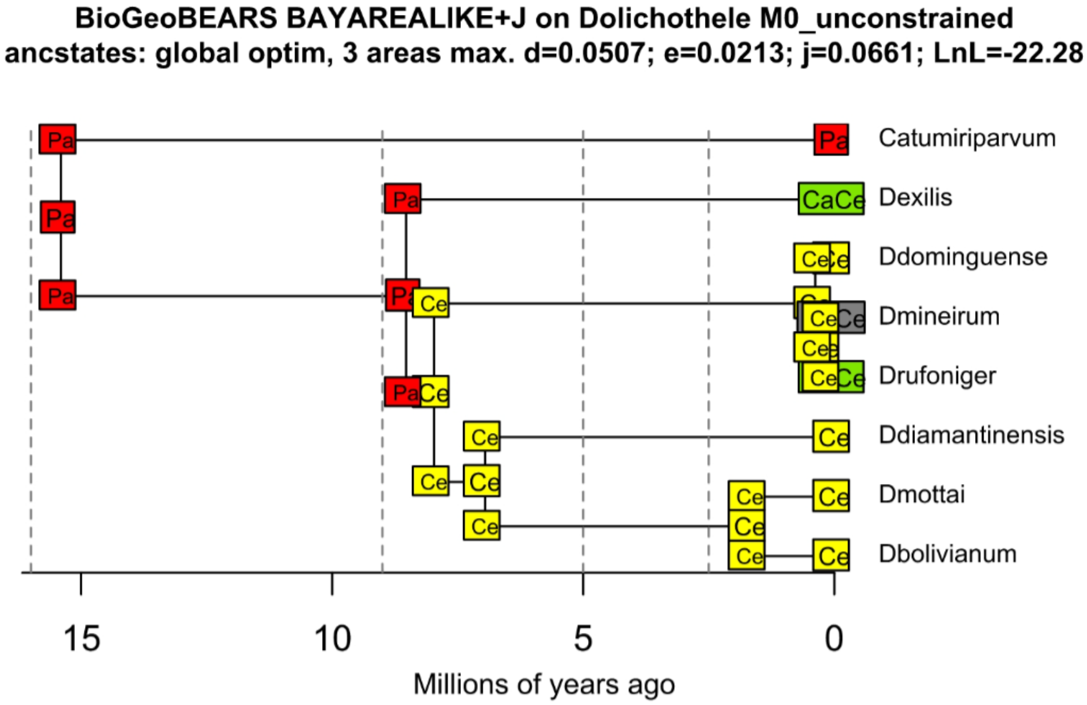
E) BAYAREALIKE Model

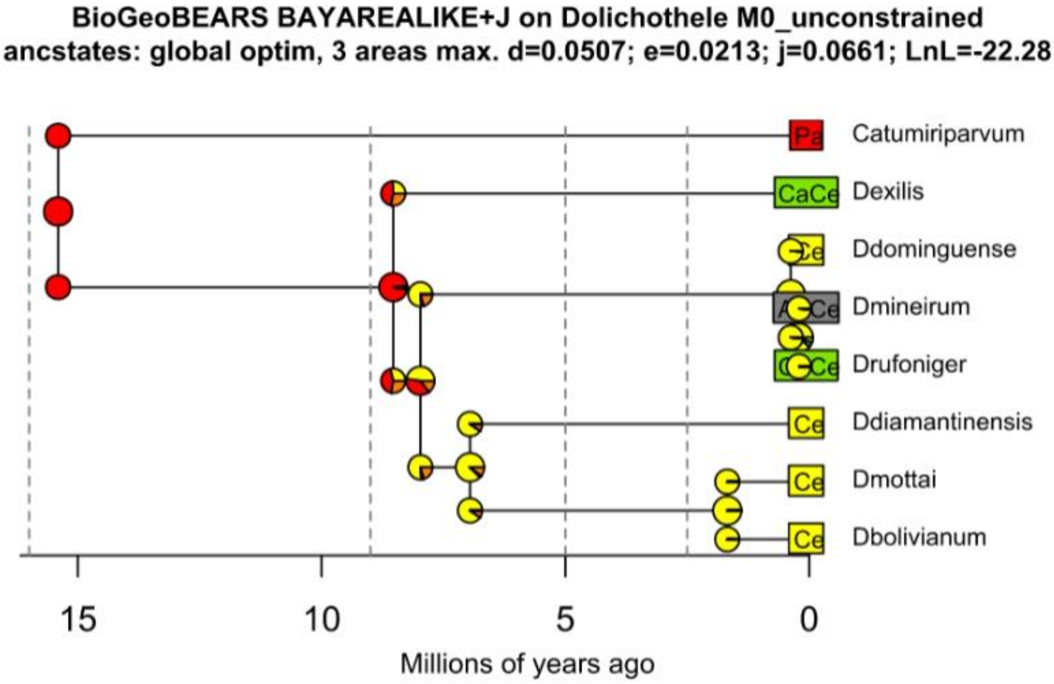

BioGeoBEARS BAYAREALIKE on Dolichothele M0_unconstrained
ancstates: global optim, 3 areas max. d=0.0611; e=0.0496; j=0; LnL=-23.69


BioGeoBEARS BAYAREALIKE on Dolichothele M0_unconstrained
ancstates: global optim, 3 areas max. d=0.0611; e=0.0496; j=0; LnL=-23.69

Appendix 4 continuation…

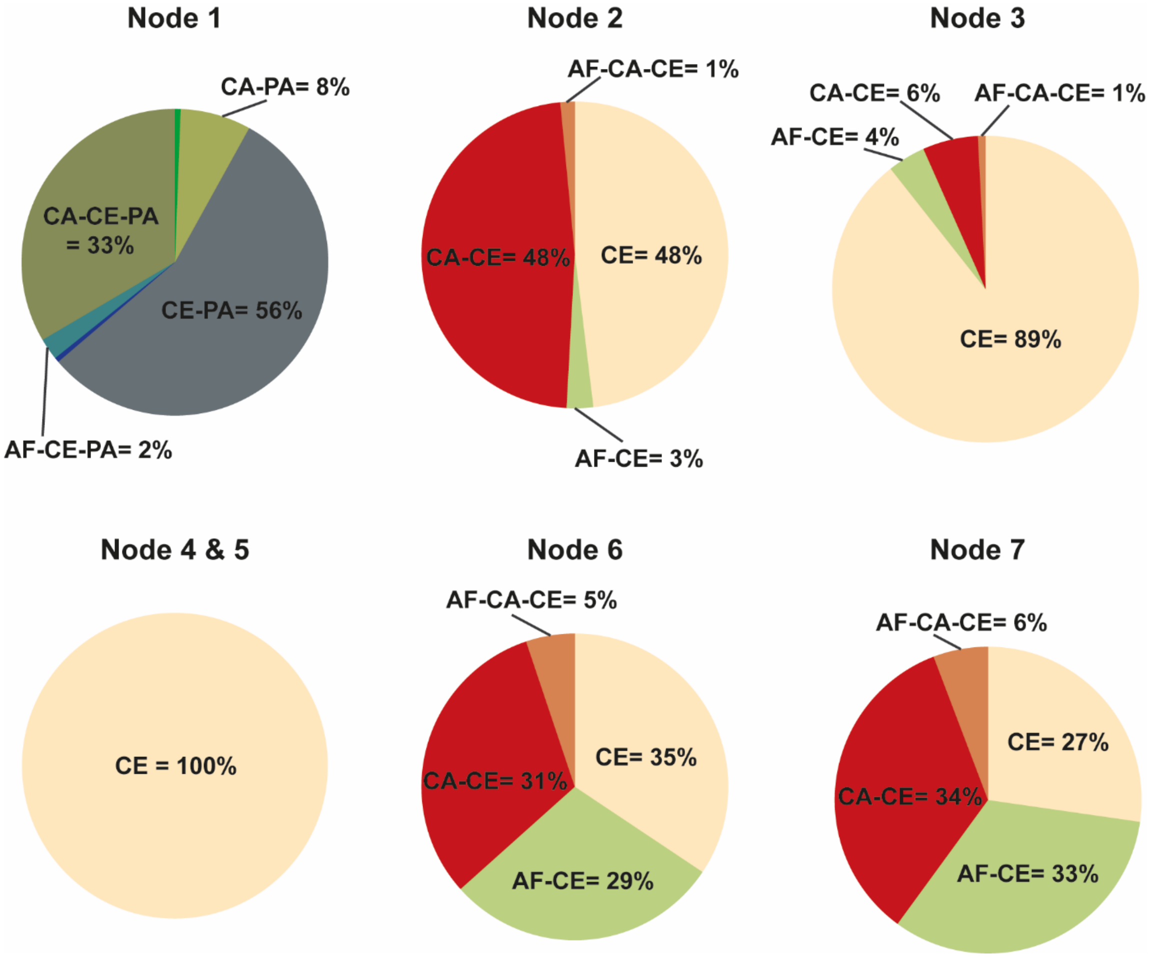
F) BAYAREALIKE + J Model





Appendix 5: Node’s probabilities Model DEC+J

Figure S2. Details of the probabilities obtained with the Model DEC+J for each node in the ancestral range reconstruction using BioGeoBears program.



UNICAMP
COORDENADORIA DE PÓS-GRADUAÇÃO
INSTITUTO DE BIOLOGIA
Universidade Estadual de Campinas
Caixa Postal 6109. 13083-970, Campinas, SP, Brasil
Fone (19) 3521-6378. email: cpgib@unicamp.br
IB

DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Biogeografia histórica del gênero Dolichothele (Araneae:Theraphosidae)***", desenvolvida no Programa de Pós-Graduação em Genética e Biologia Molecular do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: ______________________________
Nome do(a) aluno(a): Wendy Yohana Arroyo Pérez

Assinatura: ______________________________
Nome do(a) orientador(a): Vera Nisaka Solferini

Data: 20 de setembro de 2021

Declaração

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Campinas, 20 de setembro de 2021

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RG n.° G284156-I

Assinatura : ________________________________
Nome do(a) orientador(a): **Vera Nisaka Solferini**
RG n.° 4.763.396