



INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

**Filogeografia e demografia histórica de lagartixas-rabo-de-nabo  
*Thecadactylus rapicauda* e *T. solimoensis* (Squamata: Phyllodactylidae)**

Vanessa do Nascimento Braga

**Manaus – Amazonas**

**Abril/2021**

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***Thecadactylus rapicauda* e *T. solimoensis* (Squamata: Phyllodactylidae)**

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**Manaus – Amazonas**

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## SUMÁRIO

LISTA DE TABELAS .....	VIII
LISTA DE FIGURAS .....	IX
RESUMO .....	X
ABSTRACT .....	XI
INTRODUÇÃO GERAL.....	12
REFERÊNCIAS.....	16
<b>DIFFERENT HISTORIES OF AMAZONIAN DIVERSIFICATION: PHYLOGEOGRAPHY AND NICHE MODELING OF TWO CRYPTIC SPECIES OF TURNIP- TAILED GECKOS.....</b>	<b>22</b>
<b>ABSTRACT.....</b>	<b>22</b>
<b>INTRODUCTION.....</b>	<b>24</b>
<b>MATERIAL AND METHODS.....</b>	<b>26</b>
SAMPLING AND DATA PREPARATION .....	26
PHYLOGENETIC ANALYSES AND MOLECULAR DATING .....	27
POPULATION STRUCTURE AND HISTORICAL DEMOGRAPHY.....	28
SPECIES DISTRIBUTION MODELING .....	29
ENVIRONMENTAL VARIABLES AND OCCURRENCE DATA TREATMENT .....	29
MODEL BUILDING AND TESTING OF NICHE CONSERVATISM AND OVERLAP .....	30
<b>RESULTS .....</b>	<b>32</b>
PHYLOGENETIC RELATIONSHIPS, POPULATION STRUCTURE AND DEMOGRAPHY .....	32
SPECIES DISTRIBUTION MODELS AND NICHE OVERLAP AND DIVERGENCE .....	33
<b>DISCUSSION .....</b>	<b>34</b>
BIOGEOGRAPHY OF TURNIP-TAILED GECKOS IN THE AMAZON BASIN .....	35
DEMOGRAPHY, SPECIES DISTRIBUTIONS AND NICHE OVERLAP .....	37
<b>CONCLUSIONS .....</b>	<b>40</b>
<b>REFERENCES.....</b>	<b>41</b>
<b>GUISAN A, THULLER W, ZIMMERMANN NE. 2017. <i>HABITAT SUITABILITY AND DISTRIBUTION MODELS: WITH APPLICATIONS IN R</i>. CAMBRIDGE UNIVERSITY PRESS. KINDLE EDITION.....</b>	<b>45</b>
<b>TABLES.....</b>	<b>51</b>
<b>LEGENDS .....</b>	<b>54</b>
<b>SUPPORTING INFORMATION.....</b>	<b>62</b>

**Lista de Tabelas**

<b>Table 1.</b> Mitochondrial DNA genetic distance matrix (%) for the populations of <i>T. rapicauda</i> and <i>T. solimoensis</i> .....	51
<b>Table 2.</b> Genetic diversity indexes and neutrality tests for populations of <i>Thecadactylus</i> .....	52
<b>Table 3.</b> Contribution of bioclimatic variables used in species distribution models for <i>Thecadactylus</i> .....	53
<b>Table S1 1.</b> Geographic coordinates of the samples used, including identification number, species, location, and geographic coordinates. Samples obtained from GenBank are highlighted in bold.....	62
<b>Table S 2.</b> Primers used for amplification and sequencing in this study, with details of the PCR conditions used.....	68



## Lista de Figuras

<b>Figure 1. Distribution of samples obtained from <i>Thecadactylus</i> .....</b>	<b>56</b>
<b>Figure 2. Bayesian phylogenetic tree of <i>Thecadactylus</i> with divergence time estimates based on four loci. ....</b>	<b>57</b>
<b>Figure 3. Populations found in Baps based on mitochondrial DNA sequences. ....</b>	<b>58</b>
<b>Figure 4. Demography and stability areas respectively inferred through Extended Bayesian Skyline Plots and overlap of inferred current and paleodistributions....</b>	<b>59</b>
<b>Figure 5. Species Distribution Models for <i>Thecadactylus rapicauda</i>. ....</b>	<b>60</b>
<b>Figure 6. Analyses of similarity and niche equivalence for two <i>Thecadactylus</i> species. ....</b>	<b>61</b>
<b>Figure S1 1. Maximum likelihood genetic trees for markers (a) Cytb, b) PRLR, c) MXRA5, d) RAG2. The green circles indicate the probabilities 100% and the blue circles the probabilities &lt;90% and &lt;80%. ....</b>	<b>69</b>
<b>Figure S1 2. Estimated haplotype networks for <i>Thecadactylus</i> species.....</b>	<b>74</b>

## Resumo

Estudos filogeográficos associados a análises ecológicas se tornaram importantes ferramentas para entender como modificações históricas na paisagem atuaram na diversificação de espécies crípticas em regiões megadiversas, como as regiões neotropicais. Nós apresentamos uma abordagem integrativa entre filogeografia e modelos de distribuição de espécies (SDM) para investigar os potenciais agentes históricos e ecológicos que governam a estrutura filogeográfica e dinâmica demográfica de *Thecadactylus rapicauda* e *T. solimoensis*, lagartixas crípticas de hábito noturno, distribuídas ao longo da Amazônia. Nós usamos dados de sequências de genes mitocondrial e nuclear e dados geográficos dos táxons para delimitar as unidades evolutivas dentro dessas espécies de lagartixas. Empregamos datação molecular, análises demográficas coalescentes e modelagem de nicho para inferir os padrões de diferenciação de linhagem no espaço e tempo, e avaliamos o grau de sobreposição de nicho entre *T. rapicauda* e *T. solimoensis*. Apesar de serem espécies irmãs, essas lagartixas possuem histórias de diversificação bastante distintas. Em *T. rapicauda*, distribuído na porção leste da Amazônia, as flutuações climáticas Pleistocênicas e a dinâmica de estabelecimento dos rios, foram responsáveis por maior estruturação filogeográfica, e populações que não compartilham haplótipos. A instabilidade climática Pleistocênica também foi responsável por uma leve retração demográfica nas populações. Os paleomodelos estimados evidenciam momentos de transições de adequabilidade entre os períodos e que podem estar relacionados à retração demográfica. Por outro lado, em *T. solimoensis*, cuja distribuição está centrada na porção oeste, a maior estabilidade climática no Quaternário que ocorreu na região, influenciou na baixa estruturação filogeográfica, com compartilhamento de haplótipos entre as populações. Nos cenários de paleodistribuição estimados, fica evidente que a estabilidade da vegetação durante os períodos de moderadas transições climáticas na região, favoreceu a estabilidade demográfica recuperada em *T. solimoensis*. Por fim, o baixo conservatismo de nicho encontrado entre as duas espécies indica que, divergência ecológica junto a eventos de diversificação associados à evolução da paisagem e das condições climáticas são responsáveis pela diversidade críptica entre as espécies. As elevadas distâncias genéticas das populações em ambas as espécies e a hipótese de um possível clado Trans-Andino reforçam a necessidade de investigações mais detalhadas sobre os limites entre as espécies e estudos taxonômicos futuros.

## Abstract

Phylogeographic studies associated with ecological analyzes have become important tools to understand how historical changes in the landscape have acted in the diversification of cryptic species in megadiverse regions, such as the Neotropical regions. We present an integrative approach between phylogeography and species distribution models (SDM) to investigate the potential historical and ecological agents that govern the phylogeographic and demographic dynamics of *Thecadactylus rapicauda* and *T. solimoensis*, cryptic nocturnal geckos, distributed throughout the Amazon. We used data from mitochondrial and nuclear gene sequences and geographic data from taxa to delimit the evolutionary units within these species of geckos. We employ molecular dating, coalescent demographic analysis and niche modeling to infer patterns of lineage differentiation in space and time, and we assess the degree of niche overlap between *T. rapicauda* and *T. solimoensis*. Despite being sister species, these geckos have very different histories of diversification. In *T. rapicauda*, distributed in the eastern portion of the Amazon, Pleistocene climatic fluctuations and the dynamics of establishing rivers, were responsible for greater phylogeographic structuring, and populations that do not share haplotypes. Pleistocene climate instability was also responsible for a slight demographic decline in populations. The estimated paleomodels show moments of adequacy transitions between periods and which may be related to the demographic retraction. On the other hand, in *T. solimoensis*, whose distribution is centered in the western portion, the greater climatic stability in the Quaternary that occurred in the region, influenced the low phylogeographic structure, with the sharing of haplotypes among the populations. In the estimated paleodistribution scenarios, it is evident that the stability of vegetation during periods of moderate climatic transitions in the region, favored the demographic stability recovered in *T. solimoensis*. Finally, the low niche conservatism found between the two species indicates that ecological divergence along with diversification events associated with the evolution of the landscape and climatic conditions are responsible for the cryptic diversity between species. The high genetic distances of populations in both species and the hypothesis of a possible Trans-Andean clade reinforce the need for more detailed investigations on the limits between species and future taxonomic studies.

## INTRODUÇÃO GERAL

A biodiversidade global tem sido cada vez mais ameaçada pelas modificações antropogênicas predatórias (Bongaarts, 2019). Por isso, quantificar e catalogar as espécies existentes antes da extinção precoce tem sido uma importante atribuição dos biólogos. Entretanto, a vasta quantidade de espécies ainda hoje desconhecidas revela o quanto a biodiversidade pode ser enigmática (Moura & Jetz, 2021). Parte da dificuldade se deve ao fato de existirem táxons morfológicamente semelhantes, porém geneticamente distintos, que muitas vezes são erroneamente classificados como uma única espécie, quando na realidade representam duas ou mais espécies distintas, as espécies crípticas (Bickford *et al.*, 2007). Não obstante, estudos de sistemática filogenética e biogeografia têm revelado cada vez mais espécies crípticas devido ao avanço na obtenção e análise de conjuntos de dados integrativos (e.g., sequências de DNA e coordenadas geográficas) (Werneck *et al.*, 2012; Rosser *et al.*, 2019). Assim, entender os processos históricos e ecológicos que moldaram a evolução dessas espécies pode auxiliar na conservação da biodiversidade como um todo.

Em geral, a existência de espécies crípticas pode ser explicada por três mecanismos: divergência recente, estase morfológica e conservatismo de nicho ecológico (Fišer, Robinson, & Malard, 2018). A divergência recente sugere que táxons irmãos, ou que são parte de um complexo de espécies crípticas, não acumularam diferenças morfológicas devido ao curto tempo de divergência entre si (Egea *et al.*, 2016). Contudo, espécies crípticas também podem existir entre táxons com divergências relativamente antigas (Fišer *et al.*, 2018). Assim, a estase morfológica sugere que a morfologia pode ser conservada por longos períodos de tempo entre as espécies descendentes (Bickford *et al.*, 2007) em razão de fortes pressões seletivas estabilizadoras (Struck *et al.*, 2018). Por fim, o conservatismo filogenético de nicho sugere que a similaridade ecológica entre as populações ao longo do tempo, pode promover a diferenciação genética mediante isolamento geográfico (Wiens & Graham, 2005; Rato *et al.*, 2015).

Fatores ecológicos podem agir significativamente em eventos de especiação. Períodos de mudanças climáticas isolam geograficamente linhagens e conduzem diretamente a especiação, ou as linhagens podem manter o seu nicho ancestral. Em geral, descobertas de espécies crípticas através de estudos filogenéticos, além da sua

importância taxonômica, biogeográfica e para a biodiversidade, fomentam novos estudos para explicar como os componentes ecológicos implicam no surgimento de espécies crípticas em diferentes táxons (Sheu *et al.*, 2020). Com isso, estudos que avaliam a ocorrência de especiação ecológica através de métodos que quantificam a sobreposição de nicho de diferentes espécies como forma de inferir conservatismo de nicho têm ganhado força nos últimos anos, em especial por meio de métodos de modelagem de distribuição de espécies (SDM) (Cooper, Jetz, & Freckleton, 2010). A modelagem de nicho ecológico permite que traços ecológicos extraídos das áreas de ocorrência das espécies e linhagens evolutivas contribuam na detecção dos limites de nichos das espécies crípticas ao longo do espaço geográfico. Estudos têm mostrado que SDMs são abordagens poderosas para investigar o conservatismo e divergência de nicho (Culumber & Tobler, 2016; Scriven *et al.*, 2016). Devido ao poder de predição de nicho ecológico, a integração das SDM com estudos filogenéticos e filogeográficos são cada vez mais frequentes para entender os processos de especiação, aspecto de elevado interesse em regiões megadiversas como a região Neotropical e a Bacia Amazônica.

Em geral, táxons da região Neotropical possuem alta diversidade críptica (De Carvalho *et al.*, 2020; Marques-Souza *et al.*, 2020; Ribeiro-Júnior *et al.*, 2020), sendo que diversas hipóteses de diversificação envolvendo processos geomorfológicos e climáticos foram propostas para explicar eventos de especiação e origem de diversidade críptica. Por exemplo, estudos filogeográficos integrando SDMs identificaram a diversificação de espécies crípticas através da sua história evolutiva e o papel dos fatores ecológicos (Machado *et al.*, 2019), e processos de especiação e colonização de diferentes biomas combinando eventos históricos e evolução de nicho (Sheu *et al.*, 2020). As mudanças históricas na paisagem determinam os limites e padrões de distribuição das populações, além de influenciarem a variabilidade genética em nível intraespecífico ou entre espécies proximamente relacionadas (Avice, 1987). Flutuações climáticas (Gascon, Loughheed, & Bogart, 1998; Salariato, Acosta, & Cialdella, 2019; Ledo *et al.*, 2020), barreiras históricas, como os grandes rios (Pirani *et al.*, 2019; Dal Vechio *et al.*, 2020; Moraes *et al.*, 2020), e interações bióticas (Kumar & Kumar, 2018), são fatores abióticos que apoiam hipóteses de diversificação em estudos filogeográficos nos neotrópicos.

Especificamente na megadiversa Amazônia, há muitas hipóteses históricas envolvendo mudanças geoclimáticas dramáticas para explicar os padrões de biodiversidade (Wallace, 1854; Haffer, 1969; Hoorn *et al.*, 2010; Rull, 2011; Cheng *et*

*al.*, 2013). Há um consenso de que os processos climáticos e geomorfológicos foram os principais motores da diversificação na bacia amazônica, levando a uma dicotomia de condições ambientais e de diversidade de espécies entre as porções leste e oeste (Aleixo & de Fátima Rossetti, 2007; Leite & Rogers, 2013; Silva *et al.*, 2019), e a uma possível zona de sutura ecológica em espécies de ambientes alagáveis na bacia Amazônia Central (Thom *et al.*, 2020). A dinâmica histórica geológica da porção oeste da Amazônia foi marcada pela orogenia andina (Hoorn *et al.*, 2010), durante o Mioceno médio que influenciou o surgimento de zonas úmidas marinhas e o desenvolvimento do rio Amazonas transcontinental. Em proporções climáticas, o oeste permaneceu muito mais estável durante o Pleistoceno (Cheng *et al.*, 2013) e, com isso, a região possui uma biodiversidade maior (Rabosky *et al.*, 2019). Em condições geológicas, o leste da Amazônia, no geral, foi mais estável ao longo do Neógeno (Wesselingh *et al.*, 2010), contudo, em aspectos climáticos, teve uma história bastante dinâmica (Wang *et al.*, 2017).

O efeito das variações climáticas do Pleistoceno em promover a expansão e retração da floresta foi um dos principais mecanismos sugeridos para explicar os processos de especiação alopátrica, não apenas na região leste, mas em toda a Amazônia (Haffer, 1969). A hipótese de Haffer, conhecida como hipótese dos refúgios, sofreu críticas e releituras desde sua publicação. Cheng *et al.* (2013) propõem uma releitura da hipótese dos refúgios, sugerindo que a instabilidade climática, antes proposta para toda a Amazônia segundo Haffer, ocorreu apenas na porção leste da Amazônia, e a porção oeste permaneceu climaticamente estável durante o Pleistoceno. Entretanto, as incertezas em relação à hipótese propiciaram hipóteses contrárias a de Haffer, como a hipótese de distúrbio-vicariância que consiste em, nos períodos de flutuações de temperatura interglaciais, as espécies adaptadas ao frio, retraíram-se às áreas mais elevadas e iniciou-se uma especiação alopátrica (Bush, 1994). O processo de alopatria foi perturbado após o fim da era glacial, permitindo a dispersão das espécies através do contato secundário das elevações para as florestas das planícies.

Contudo, raramente essas hipóteses têm sido testadas por meio de estudos filogeográficos integrados à investigação de conservatismo de nicho, aplicado às espécies crípticas. Portanto, prever como as mudanças climáticas moldaram a distribuição geográfica, ecologia e genética das espécies, pode auxiliar a elucidar o surgimento da especiação críptica em regiões neotropicais e como essas espécies podem rastrear seu nicho ecológico em meio a flutuações climáticas.

Os lagartos *Thecadactylus rapicauda* (Houttuyn, 1972) e *T. solimoensis* (Bergmann & Russell, 2007) (Phyllodactylidae) pertencem a infraordem Gekkota, um grupo conhecido por sua alta diversidade ao redor do mundo e por incluir diversos complexos de espécies crípticas (Gamble *et al.*, 2011b, 2012; Meiri, 2020; Uetz *et al.*, 2020). Entretanto, os geconídeos da América do Sul pareciam inicialmente ter uma relativa menor concentração de diversidade de espécies, uma vez que a maior parte da diversidade descrita encontra-se em outras regiões tropicais (e.g., Austrália, Índia, países do sudeste da Ásia) (Uetz *et al.*, 2020). Porém, investigações recentes com base em dados moleculares indicam que a relativa menor diversidade de geconídeos Neotropicais, na verdade, é um padrão artificial, resultante dos altos níveis de diversidade críptica do grupo (Geurgas, Rodrigues, & Moritz, 2008; Gamble *et al.*, 2008, 2011a; Werneck *et al.*, 2012).

*Thecadactylus rapicauda* e *T. solimoensis* apresentam hábito de vida noturno e distribuição parapátrica abrangendo a bacia amazônica em um padrão leste-oeste. *Thecadactylus rapicauda* possui uma distribuição majoritariamente a leste da Bacia Amazônica sendo restrita a oeste pelo baixo rio Japurá e rios Purus e Madeira. Já *T. solimoensis* possui uma distribuição restrita ao oeste da Bacia Amazônica, com sua distribuição à leste delimitada pelo Rio Negro e bacia do Rio Madeira (Ribeiro-Júnior, 2015). Esse padrão biogeográfico de distribuição foi também detectado em diversas outras espécies de lagartos (Ávila-Pires, 1995; Ribeiro-Júnior, 2015). Inicialmente, todas populações eram atribuídas a uma única espécie, *T. rapicauda* (Houttuyn, 1972). Porém, as primeiras análises moleculares de Kronauer *et al.* (2005) sugeriram a presença de um clado com espécies crípticas na região sudoeste da Amazônia. Subsequentemente, com base em dados morfológicos e moleculares, o clado do sudoeste foi reconhecido como uma espécie válida, *Thecadactylus solimoensis* (Bergmann & Russell, 2007). No entanto, esses estudos foram baseados em apenas um único marcador mitocondrial, de forma que potenciais congruências e divergências entre as histórias dos genes e das espécies não foram consideradas (Edwards & Beerli, 2000; Knowles & Carstens, 2007). Além disso, os estudos não forneceram estimativas de tempos de divergência, tampouco de dinâmica demográfica, o que dificulta a compreensão dos fatores ecológicos e biogeográficos responsáveis pela divergência das duas espécies.

Se, a exemplo de outros táxons de geconídeos neotropicais, a datação molecular indicar uma divergência antiga entre as espécies (e.g., Mioceno), esperamos que a

diversidade morfológica críptica entre elas seja explicada pela ocorrência de conservatismo de nicho, com recuperação de alta sobreposição de nicho ecológico entre as mesmas se ainda for recente. Se a divergência for antiga, esperamos que ocorra mais tempo para a divergência dos nichos. Ainda, caso o gradiente de estabilidade climática leste-oeste tenha afetado as dinâmicas populacionais das duas espécies esperamos que *T. rapicauda* apresente assinaturas genéticas de maior instabilidade demográfica do que *T. solimoensis*, uma vez *T. solimoensis* possui distribuição restrita a uma região de maior estabilidade climática histórica (oeste).

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# Capítulo 1

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Rodrigues, M.T.; Torres-Carvajal, O; Azevedo, J.A.R.;  
Oliveira, A.F.S. & Leite, R.N.** Different histories of  
Amazonian diversification: phylogeography and niche  
modeling of two cryptic species of turnip-tailed geckos

## Different histories of Amazonian diversification: phylogeography and niche modeling of two cryptic species of turnip-tailed geckos

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### Abstract

Biogeographic studies associated with ecological analyses have become important tools to understand how historical changes in the landscape may have acted on the diversification of cryptic species in megadiverse regions, such as the Neotropics. Here we combine phylogeography and species distribution models (SDM) to investigate the potential drivers of the evolutionary history and demographic dynamics of two closely-related cryptic species *Thecadactylus rapicauda* and *T. solimoensis*, which are nocturnal geckos distributed mostly throughout Amazonia. We used DNA sequences from mitochondrial and nuclear genes to delimit evolutionary units within each species, and georeferenced occurrence data to infer ecological niches, paleodistributions, and historical stability during the Quaternary. We implemented molecular dating and demographic analyses integrated with SDMs to infer patterns of lineage differentiation and evaluate the degree of niche overlap between *T. rapicauda* and *T. solimoensis*. Although these geckos are cryptic sister species, they have very different histories of diversification. The establishment of river barriers and Pleistocene climatic fluctuations possibly resulted in a greater phylogeographic structuring of *T. rapicauda*, which is

mainly distributed in the eastern portion of Amazonia and its populations do not share haplotypes. Palaeomodels predicted transitions in habitat suitability during the Pleistocene, thus climate instability may have been related to a slight demographic retraction in *T. rapicauda*. On the other hand, *T. solimoensis*, restricted to western Amazonia, showed haplotype sharing among its populations, without apparent phylogeographic structure, and constant demography with large areas of historical stability, possibly due to climatic stability during the Quaternary. The low degree of niche conservatism between the two *Thecadactylus* species indicates that ecological divergence and events associated with landscape evolution and climatic conditions were responsible for the diversification of these cryptic gecko species.

**Keywords:** Phyllodactylidae, Neotropics, *Thecadactylus*, demography, niche conservatism, cryptic diversity

## Introduction

Different evolutionary mechanisms may explain the origin of the cryptic species (Wiens & Graham, 2005; Bickford *et al.*, 2007; Razgour *et al.*, 2011; Struck *et al.*, 2018; Zhao, Zhang, & Wei, 2019). Cryptic diversity in part arises from recent divergence between sister taxa that split recently in evolutionary time, reducing the potential to accumulate morphological differences (Egea *et al.*, 2016). Furthermore, at the beginning of speciation, selection presumably does not act as strongly on morphology as it does, for example, on physiological, reproductive or behavioral traits (Struck *et al.*, 2018). So, morphological disparity in young species can be low because not enough time has passed for differences between taxa to be evident. The restriction of morphological differentiation may be linked to the early stages of stasis by stabilizing selection (Struck *et al.*, 2018). Finally, there are also cases of cryptic species with relatively old divergence times (Fišer *et al.*, 2018).

Alternatively, morphological similarity may be retained for long periods of time in descendent lineages – morphological stasis (Bickford *et al.*, 2007), being related to the species ecology through phylogenetic niche conservatism (PNC) (Losos, 2008). PNC is the tendency of evolutionary lineages to maintain ancestral ecological characteristics over time, and consequently, their ecomorphology (Wiens, 2005; Pyron *et al.*, 2015), constraining the ecological niche divergence among closely-related species. For cryptic species, PNC may be a potential driver of morphology maintenance in scenarios where the environmental conditions selected the ancestral phenotype (Fišer *et al.*, 2018).

Landscape changes may affect the distribution of organisms and the limits among their populations or lineages, influencing genetic variability at the intraspecific level or between closely-related species (Avise, 1987). A number of geoclimatic factors contributed to shape the megadiversity of Amazonia (Hoorn & Wesselingh, 2010; Wang *et al.*, 2017), and several hypotheses have been proposed to explain the biogeographic patterns observed in this region (reviewed in Leite & Rogers, 2013; Rull & Carnaval, 2020). Despite the lack of consensus on the most important drivers of biotic diversification, there are marked differences in environmental conditions and biological diversity between the eastern and western portions of the Amazon Basin (Aleixo & de Fátima Rossetti, 2007; Leite & Rogers, 2013; Silva *et al.*, 2019). While the configuration of western Amazonia was subject to a dynamic geological history,



especially from the Miocene onwards with the intensification of the Andes orogeny, the landscape of eastern Amazonia remained relatively unchanged throughout the Neogene (Cheng *et al.*, 2013).

Lizards of the clade Gekkota are highly diverse around the world and known for harboring complexes of cryptic species (Gamble *et al.*, 2011b, 2012). In tropical South America, geckos have relatively fewer species in a few genera compared to other tropical regions (Abdala & Moro, 1996; Meiri, 2020; Uetz *et al.*, 2020). However, recent molecular studies indicate that this diversity conceals high levels of cryptic diversity (Geurgas *et al.*, 2008; Gamble *et al.*, 2008, 2011b; Geurgas & Rodrigues, 2010; Werneck *et al.*, 2012). Turnip-tailed geckos of the genus *Thecadactylus* (Phyllodactylidae) comprise three species with nocturnal habits and distinct geographic ranges in the Neotropical region. *Thecadactylus oskrobapreinorum* is endemic to the Lesser Antilles, whereas *T. rapicauda* and *T. solimoensis* have parapatric distributions in the Amazon Basin, forming an east-west axis bounded by the Negro and Madeira rivers. While *T. solimoensis* is restricted to southwestern Amazonia (Ribeiro-Junior, 2015), *T. rapicauda* is distributed from south of Mexico and Central America to northern South America, including both sides of the Andes and mostly eastern Amazonia (Bergmann & Russell, 2007). In fact, a widespread distribution encompassing the entire Amazon Basin has been attributed to *T. rapicauda*, which was initially described based on morphology only (Houttuyn, 1782). However, subsequent molecular analyses indicated the presence of a cryptic species in southwestern Amazonia, which was formally recognized as *T. solimoensis* (Kronauer *et al.*, 2005; Bergmann & Russell, 2007).

Previous molecular studies on turnip-tailed geckos did not provide estimates of divergence times or demographic history for the species and were based on a single mitochondrial marker, which hampers a better understanding of the diversification of *Thecadactylus* species in the Amazon Basin (Kronauer *et al.*, 2005; Bergmann & Russell, 2007). Although there are many morphological similarities between these species, we would expect signals of demographic fluctuation for *T. rapicauda* due to the greater climatic instability in eastern Amazonia, whereas *T. solimoensis*, which is restricted to the historically more stable western Amazonia, would have a genetic signature of constant demography. In addition, it is possible that both *Thecadactylus* species will present phylogenetic niche conservatism and ecological overlap if they

have an ancient history of diversification in the Amazon Basin, as is the case for other Neotropical phyllodactylids (Werneck *et al.*, 2012).

Species distribution modelling (SDM) is widely used to inform about geographic patterns of current and past distribution and to test for PNC between species (Elith *et al.*, 2006; Cooper *et al.*, 2010). SDM uses species occurrences (i.e., geographic coordinates) and environmental data to identify areas of predicted distribution for each species or lineage (Elith *et al.*, 2006). Thus, SDMs are an important tool for understanding how ecological processes may shape speciation and patterns of biodiversity distribution. Biogeographic studies that integrate genetic data sets with environmental data from SDM projections (Alvarado-Serrano & Knowles, 2014) are indispensable for a better understanding of the patterns of Neotropical cryptic diversity, the ecological factors affecting species distribution and niche evolution, and the role of historical events for biotic diversification (Machado *et al.*, 2019; Sheu *et al.*, 2020). Here, we integrate phylogeographic and SDM approaches using an expanded molecular and geographic sampling for the two species of *Thecadactylus* geckos that are distributed in Amazonia (*T. rapicauda* and *T. solimoensis*) to assess their evolutionary history and ecological divergence. Our study aims to: (1) investigate the occurrence of additional cryptic diversity and the spatial and temporal phylogeographic patterns for *Thecadactylus rapicauda* and *T. solimoensis* throughout Amazonia; (2) obtain a better interspecific and biogeographic resolution using multiple loci and molecular dating; (3) infer the demographic history of these two species and the potential impact of the Quaternary climatic fluctuations and (4) test whether they have conserved ecological niches using SDMs and analyses of niche overlap or divergence.

## **Material and methods**

### ***Sampling and data preparation***

We gathered 84 samples (liver or muscle tissue) of *Thecadactylus rapicauda* and 68 samples of *T. solimoensis* from 75 localities across their known distribution range (Fig. 1; Table S1). Specimens were deposited in the Amphibians and Reptiles Collection of the National Institute of Amazonian Research (INPA-H), Zoology Museum of the University of São Paulo (MZUSP), Royal Belgian Institute of Natural Sciences (RBINS), Louisiana Museum of Natural History (LSU), Zoological Collection

of the Federal University of Mato Grosso do Sul (ZUFMS), Museo de Zoología (QCAZ) Pontificia Universidad Católica del Ecuador and Emílio Goeldi Museum of Pará (MPEG). Genetic data collection was carried out at the Thematic Laboratory of Molecular Biology at the National Institute of Amazonian Research (LTBM/INPA).

The genomic DNA was extracted using the DNA Wizard Genomic Purification Kit (Promega). We amplified through polymerase chain reaction (PCR) a fragment of the mitochondrial gene Cytochrome b (*mtDNA* - *Cytb*) and three nuclear genes (nuDNA), Recombination Activating Gene 2 (*RAG2*), Prolactin Receptor (*PRLR*) and Matrix-remodeling Associated 5 (*MXRA5*), using GoTaq Master Mix (Promega Corporation). Information about primers and PCR conditions are listed in Table S2. We purified the PCR products with 1 g/ml Polyethylene Glycol (PEG). We performed sequencing reactions using BigDye terminator kit v.3.1 and precipitated them with EDTA/Ethanol. Sequencing reactions were carried out using an automated sequencer ABI3130xl at LTBM/INPA. The resulting chromatograms were visually inspected and sequences edited in Geneious v.7.0 (Kearse *et al.*, 2012). We performed multiple sequence alignments in the program Aliview v.1.17.1 (Larsson, 2014) using Muscle algorithm (Edgar, 2004).

### ***Phylogenetic analyses and molecular dating***

In order to infer a more robust phylogeny, we included only those individuals with sequences available for at least two genes. Phylogenetic relationships of each gene alignment and the concatenated data set were inferred based on maximum likelihood optimization (ML) using the program RAxML v.8.0 (Stamatakis, 2014). The concatenated data were partitioned into each independent gene with a distinct model of nucleotide evolution. We applied the GTR + Gamma model for all genes, and analyses were run using the rapid hill-climbing mode, 1,000 auto-initiation replicates and a complete search for the best-scoring ML tree. We used two gecko species of the family Phyllodactylidae (*Phyllopezus pollicaris* and *P. maranjonensis*) as outgroups. We built haplotype networks for each *Thecadactylus* species to better visualize genealogies and patterns of haplotype sharing among populations. We converted the maximum likelihood tree estimated from the concatenated nuclear and mitochondrial genes using the program Haploviewer v. 1.6 (Salzburger, Ewing, & Von Haeseler, 2011).

We estimated a time-calibrated Bayesian tree for the Amazonian *Thecadactylus* using the program BEAST 2 v.2.6 (Bouckaert *et al.*, 2014) based on the concatenated data set. We used a HKY site model and estimated gamma parameter with four categories and empirical frequencies for each gene using the program Partitionfinder 2 v.2.1 (Lanfear *et al.*, 2012). We implemented an uncorrelated lognormal relaxed clock to allow rate heterogeneity between lineages and used the Yule speciation model as tree prior. We calibrated the divergence time estimates with a rate of 0.0065 substitutions per million years for the mtDNA (Macey *et al.*, 1998), which is widely used for molecular dating in the order Squamata, including members of Phyllodactylidae (Werneck *et al.*, 2012). The substitution rates of nuclear genes were independently estimated based on the mtDNA substitution rates, using default values to set the uniform and gamma distributions of the priors `ucl.mean` and `ucl.stdev`. After preliminary runs, we ran the Markov Chain for 100 million generations with a sampling interval of 10,000. We verified the stationarity of posterior distributions and effective sample size (ESS) above 200 in the program Tracer v.1.7 (Rambaut *et al.*, 2018). We used the program TreeAnnotator v.2.6 (Drummond & Rambaut, 2007) to calculate the maximum clade credibility (MCC) phylogenetic tree, after discarding the first 10% of posterior trees as burn-in.

### ***Population structure and historical demography***

We inferred the number of population clusters and individual assignments of the Amazonian *Thecadactylus* samples, except for the trans-Andean clade samples, using a Bayesian probabilistic clustering method implemented in the program BAPS v.6 (Corander *et al.*, 2013) for the mitochondrial dataset. We applied the mixture model varying the number of clusters (K) from 1 to 15. Based on mixture results, we then performed admixture analyses with 100 interactions, 200 reference individuals per population and 20 interactions per individual. After defining these population clusters, we calculated genetic *p*-distances them to identify cryptic diversity within and between populations. We used mtDNA data in the MEGA v.7 program with runs of 1,000 automatic starts to obtain the standard deviation (Kumar, Stecher, & Tamura, 2016).

We assessed signals of demographic fluctuations with summary statistics calculated for the mitochondrial and nuclear genes, using the neutrality Tajima's *D* (Tajima, 1989) and Fu's *F<sub>s</sub>* (Fu, 1997), based on 10,000 simulations implemented in the

program DNASP v.6 (Rozas *et al.*, 2017). We also reconstructed the historical demography through Extended Bayesian Skyline Plots (EBSP) (Heled & Drummond, 2008) generated in the program BEAST 2 (Bouckaert *et al.*, 2014). To that purpose, the dataset for each species consisted of only those individuals with at least three loci. The Markov Chain was run for 100 million generations, with a thinning interval of 10,000 samples. The strict molecular clock was applied to each gene separately. We checked for the stationarity of posterior distributions and ESS values using the program Tracer v.1.7 (Rambaut *et al.*, 2018) and EBSP analyses were performed after removing a 10% burn-in.

### ***Species distribution modeling***

The potential distributions of *T. rapicauda* and *T. solimoensis* were predicted for the present and the past (~6Kya Middle Holocene, ~21Kya Last Glacial Maximum, and ~120Kya Last Interglacial) to infer the potential impact of the Quaternary climatic fluctuations on each gecko species, and to assess scenarios of niche overlap or divergence among the species. Details about the geographic occurrence data, bioclimatic variables and the analysis settings used to estimate SDMs are described below.

### ***Environmental variables and occurrence data treatment***

We obtained 19 bioclimatic variables from WorldClim (Hijmans *et al.*, 2005) with a resolution of 2.5 arcmin. From these initial variables, we eliminated those that had multicollinearity problems as inferred with an analysis of Variance Inflation Factor (VIF) (Naimi *et al.*, 2014) in R v.3.6 (R Studio Team, 2020), which evaluates collinearity and avoids redundancy between variables. We kept nine variables with VIF values lower than 10 relevant for species distribution in the Amazonian region: Mean Diurnal Range (BIO2); Isothermality (BIO3); Temperature Seasonality (BIO4); Max Temperature of Warmest Month (BIO5); Precipitation of Wettest Month (BIO13); Precipitation of Driest Month (BIO14), Precipitation Seasonality (BIO15); Precipitation of Warmest Quarter (BIO18); Precipitation of Coldest Quarter (BIO19). We also obtained the same set of variables estimated for the Middle Holocene (6 kya), Last Glacial Maximum (21 kya, LGM), and the Last Interglacial (120 kya, LIG) based on the CCSM4 (Community Climate System Model). (Gent *et al.*, 2011).

We obtained georeferenced samples from biological collections and we georeferenced the occurrence data for records of *Thecadactylus* species from a recent

catalogue of Amazonian lizards (Ribeiro-Junior, 2015). We performed a spatial filtering to exclude records in areas with denser sampling to minimize sampling bias due to the clustering of points. We kept unique occurrence records at least 50 km apart from one another using the spThin v.3.6 package (Aiello-Lammens *et al.*, 2015). After this filtering, we retained 162 points of *T. rapicauda* and 82 of *T. solimoensis*.

#### *Model building and testing of niche conservatism and overlap*

We inferred species distributions models for the two species of *Thecadactylus* using the MaxEnt algorithm (Phillips, Anderson, & Schapire, 2006) in the ENMeval 0.3.1 package (Muscarella *et al.*, 2014) implemented in R v.3.6 (R Studio Team, 2020). Analyses were performed with the ENMevaluate function with 10,000 points of pseudo-absence (background), and with the checkerboard2 approach that partitions occurrence localities into four bins with a checkerboard pattern, minimizing effects of spatial autocorrelation (Muscarella *et al.*, 2014). We evaluated variable importance from their contributions to the model's performance through the jackknife test implemented in the ENMeval var.importance function (Phillips *et al.*, 2006). We built models with regularization multipliers ranging from 0.5 to 4 (increments of 0.5) and with three feature class combinations (L, LQ, LPQ) where L = linear, Q = quadratic, P = product), allowing for simple and flexible parameters optimization to the observed data (Muscarella *et al.*, 2014). As we were also projecting the models into past climate conditions, we did not include the more complex parameters H = hinge and T = threshold (Guisan *et al.*, 2017). After selecting the best models with delta-AIC smaller than two, we evaluated the performance of the models by the average value of the area under the curve (AUC) parameter. In the cases where more than one model presented delta-AIC smaller than 2, we averaged model predictions weighted by AUC.

After projecting the models to the remaining times (Holocene, LGM and LIG), we inferred the potential distribution of each species across time using a threshold that maximized sensitivity (se) and specificity (sp) (se + sp), which generally corresponds to maximizing other metrics such as True Skill Statistics and Kappa (Guisan *et al.*, 2017). From the resulting binary models, we produced a stability map by overlaying the resulting models.

To evaluate the degree of niche overlap between the *T. rapicauda* and *T. solimoensis*, we used the 19 WorldClim bioclimatic variables for the present climate

and followed the PCA-Env approach proposed by Broennimann *et al.*, (2012). The approach uses a Principal Component Analysis (PCA-Env) to estimate the environmental space of the species under comparison by transforming the set of environmental variables (many of these highly correlated) into two uncorrelated linear combinations of the original variables. The distribution of species in the environmental space is estimated by smoothing the occurrence records in each cell with a Kernel density function (Di Cola *et al.*, 2017). This method of estimating niche overlap in the environmental space is less subject to statistical biases of methods such as minimal convex polygon or niche modeling (Broennimann *et al.*, 2012).

From the smoothed densities of occurrence, we measured the niche overlap for the species-pair using the Schoener  $D$  metric (Schoener, 1968), which ranges from 0 (no overlap/complete divergence) and 1 (complete/high overlap). Finally, to test the niche conservatism or divergence hypotheses, we conducted niche equivalence and similarity tests (Warren *et al.*, 2008). The equivalence test determines whether the niche overlap remains constant as the occurrence of the two species is randomly relocated between the two distributions and is considered a test that assesses whether the two compared niches are identical (Warren *et al.*, 2008). From this randomization of occurrences, niche overlap  $D$  is calculated in 100 repetitions and a histogram of simulated values is generated. If the observed value of  $D$  falls within 95% of the density of the simulated values, the null hypothesis of equivalence is not discarded. The niche similarity test is calculated by randomly changing the position of the entire distribution of a species in the environmental space of the PCA (the central point of the distribution is randomly positioned at different points), while the other species remains at the original position, and then the niche overlap  $D$  is calculated in 100 repetitions. The same process is then repeated for the second species. If the observed overlap is greater than 95% of the simulations, the species occupy niches more similar to each other than the random overlap. These analyzes were performed using the ecospat v.3.0 package (Di Cola *et al.*, 2017) with 1000 repetitions in R v.3.6 (R Studio Team, 2020).

## Results

### *Phylogenetic relationships, population structure and demography*

We sequenced a total of 547 base pairs (bp) from 105 individuals for *Cytb*, 545 bp from 64 individuals for *MXRA5*, 473 bp from 87 individuals for *PRLR*, and 339 bp from 95 individuals for *RAG2*.

The *Cytb* gene trees were highly structured and individuals from several of the unique localities were inferred as monophyletic groups having strong bootstrap support (>90%) (Fig. S1). The concatenated gene trees showed cases of paraphyly and polytomies in ML tree with weaker structuring (Fig. 1A). ML and Bayesian topologies based on the concatenated data set (Fig. 1A) presented similar relationships and had high support for the separation between the two species of *Thecadactylus*. In general, *T. rapicauda* had a stronger phylogenetic structure, noticeable as subclades with different support values. One of which includes a highly supported trans-Andean subclade from western Ecuador and the others are distributed in eastern Amazonia (Fig. 1A). The trans-Andean subclade of *T. rapicauda* from western Ecuador is sister to the remaining *T. rapicauda* (Fig. 1A). However, there are still uncertainties whether this truly is a monophyletic group or may be associated with other subclades from the Amazon Basin, as sampling in our data set was limited to only one individual per locality. At the same time, *T. solimoensis* had no apparent phylogenetic sub-structure, with a clade in western Amazonia having short branches and shallower divergences (Fig. 1A-B).

The dated tree recovered high support values (>0.98) mainly for those nodes representing the oldest divergence events (Fig. 2). Time estimates indicated that the first split between *T. rapicauda* and *T. solimoensis* occurred in the Early Miocene, at approximately 24 million years ago (Ma; 15.03–32.60 95% highest posterior density [95% HPD] interval). The divergence between trans-Andean and the remaining *T. rapicauda* was estimated in ~21 Ma (18.75–24.60 95% HPD), but this clade received poor support (Fig. 2). The crown diversification of the Amazonian *T. rapicauda* started subsequently, at ~15 Ma (9.37–18.46 95% HPD), whereas the crown diversification of *T. solimoensis* started more recently in the Middle-to-Late Miocene transition, at ~8 Ma (6.89–11.78 95% HPD) (Fig. 2).

The BAPS analysis based on mtDNA data revealed a total of six population clusters, four clusters (R1 to R4) for the Amazonian *T. rapicauda* and two clusters (S5 and S6) for *T. solimoensis* (Fig. 3). Although all four populations of *T. rapicauda* are



distributed in the Brazilian Shield (encompassing the Belem, Xingu, Tapajós, Rondônia areas of endemism; Fig. 3), only three of those (R1, R3 and R4) apparently occur in the Guiana Shield (corresponding to the Guiana area of endemism). The populations R4 and R2 seem to be restricted to the lower reaches of the Amazon tributaries for the most part (but see locality in the upper Madeira for R4). However, while R4 is found on both margins of the Amazon river stem, R2 is present only in the southern bank. In contrast, R1 is distributed along the Branco and lower Negro rivers as well as in the Madeira-Tapajós interfluve, while R3 this paraphyletic population occurs in the Tapajós-Xingu interfluve and also extends into Guianan coastal areas and trans-Andean Ecuador. As for *T. solimoensis* populations, while S6 has a wide geographic range throughout western Amazonia, S5 has a much smaller distribution along the Purus and Madeira rivers (Fig. 3).

We found a total of six haplotypes in *T. rapicauda*, three haplotypes are present only in R1. Populations R2, R3 and R4 share the remaining haplotypes. In *T. solimoensis*, 12 unique haplotypes are distributed in the two populations S5 (two haplotypes) and S6 (10 haplotypes). The genetic *p*-distances varied between population clusters from 19% to 40%, in *T. solimoensis* with high variation in values Genetic *p*-distances varied between population groups from 19% to 40% in both *Thecadactylus* species (Table 1).

Demographic summary statistics indicated that the values obtained were not significant, suggesting that signs of demographic change are non-detectable among populations based on these metrics (Table 2). Nevertheless, trends of Extended Bayesian Skyline Plots indicated that the two gecko species had distinct demographic histories (Fig. 4). We detected a signal of demographic population retraction for *T. rapicauda* recently, at around 200 ka (Fig. 4A), whereas we found evidence of a demographic stability of *T. solimoensis* over the last five million years (Fig. 4B).

### ***Species distribution models and niche overlap and divergence***

Models selected for both species recovered high AUC values (> 0.89), indicating high model accuracy (Table 3). The variables that contributed most to the models of both species were Mean diurnal Range (BIO2), Isothermality (BIO3), Precipitation of Warmest Quarter (BIO18) and Precipitation of Coldest Quarter (BIO19) (Table 3).

For *T. rapicauda*, the SDM for the present illustrates broad environmental suitability, concentrated in the central and eastern regions of the Amazon Basin, and portions of high suitability in the north of the continent and Andean slopes (Fig. 5). The SDM projections of this species indicate a reduction in the areas of suitability during the warmer periods (Holocene and LIG), and a smaller reduction during the colder LGM. During the LIG (~ 120 kya) the suitability areas almost disappeared from eastern and central Amazonia. Between the LIG and the LGM (~ 21 kya), areas of high suitability in the central portion of the Amazon basin were increased. From the LGM onwards it is possible to notice an increase in the potential distribution of *T. rapicauda*, reaching the largest extension of high suitability areas during current period (Fig. 5). The high variation in the predicted distribution of *T. rapicauda* resulted in very few areas with high stability since the Pleistocene, with areas with four overlapping model predictions (i.e., refugia) occurring only in the Andean part of the distribution, and a few patches of areas with 3 overlapping models occurring in the Guiana Shield and eastern Amazonia (Fig. 4).

On the other hand, the predictions for *T. solimoensis* showed less variation across the Quaternary climatic fluctuations, but with a tendency of increased suitability during the LIG and LGM. During these periods, areas of high suitability were extended to beyond the western Amazon basin (Fig. 5). As result, large areas of high stability with four overlapping model predictions are coincident with most of the current distribution of the species in the western Amazonia (Fig. 4).

The estimated niche overlap between the *Thecadactylus* species-pair was low, as inferred by the niche overlap index  $D$  ( $D = 0.056$ ). Niche equivalence was rejected, suggesting that the niches are more distinct than expected by chance ( $P > 0.95$ ). However, niche similarity was not rejected ( $P < 0.95$ ), indicating that the environmental conditions within the distributions of these species are not more different than by chance. The results of the PCA-Env characterization of the species environmental background conditions and the variable contributions to the niche overlap pairwise comparisons are detailed in Fig. 6.

## Discussion

We investigated the potential historical and ecological processes governing the phylogeographic structure, demographic dynamics and ecological niche conservatism of

two sister-species of nocturnal geckos that are widely distributed in Amazonia. Our analyses indicated that they have different evolutionary histories. After the two species of *Thecadactylus* diverged ~24 million years ago, *T. rapicauda* has had a more structured phylogeographic history compared to *T. solimoensis*. Regarding their demographic history, *T. rapicauda* experienced population retraction more recently at about 200 kya, while *T. solimoensis* exhibited population stability in the last 5 million years. In addition, SDMs showed that the predicted distribution of *T. rapicauda* experienced large variations in the extension of suitable areas in central and eastern Amazonia since the LIG, whereas for *T. solimoensis*, there was almost no variation in the areas corresponding to its current distribution across the Quaternary climatic fluctuations. Finally, there is a low niche overlap between these two species, potentially indicating a lack of climate niche conservatism between them. Thus, the integration of molecular and ecological datasets helped us to understand the processes that acted on the different diversification trajectories of two species of turnip-tailed geckos with cryptic morphology over wide geographical ranges in a west-east gradient across Amazonia.

### ***Biogeography of turnip-tailed geckos in the Amazon Basin***

In their phylogenetic study of *Thecadactylus*, Kronauer *et al.* (2005) detected two distinct clades within what was previously believed to be a single widespread species, *T. rapicauda*, highlighting its cryptic diversity. Subsequently, Bergmann and Russell (2007) recognized the clade in southwestern Amazonia as a distinct species, *T. solimoensis*. Although space-temporal patterns in *Thecadactylus* had not been explicitly investigated, the parapatric distribution of *T. rapicauda* and *T. solimoensis* in Amazonia suggested that processes related to the landscape evolution and establishment of the Solimões-Amazonas drainage played an important role in their diversification. Our phylogenetic reconstructions based on Bayesian and maximum likelihood methods using multilocus data corroborate the phylogenetic division between *T. rapicauda* and *T. solimoensis* in the Amazon Basin.

In addition, we recovered a well-supported clade from Ecuador that is sister to the large Amazonian clade of *T. rapicauda*. Despite the lower coverage of our dataset for that region, this trans-Andean lineage indicates that some populations of *T. rapicauda* are highly divergent from the larger Amazonian clade and may have been

under distinct speciation processes. The orogeny of the Andes was a gradual process from south to north, which was intensified at the beginning of the Miocene (~23 Ma) in the northern cordilleras, an age that coincides with the diversification of several mountain taxa (Hoorn *et al.*, 2010). Moreover, the peak of this Andean uplift (10 to 7 Ma) has been implicated in the speciation of cis-Andean taxa as well (Pirani *et al.*, 2019).

Our analyses evidenced different levels of cryptic diversity for these two sister-species of *Thecadactylus* and distinct evolutionary trajectories in the Amazon Basin. The phylogeographic structuring of cryptic lineages is a pattern commonly found in Neotropical geckos (Geurgas & Rodrigues, 2010; Werneck *et al.*, 2012; Domingos *et al.*, 2014), and typical of low vagility organisms such as small amphibians and lizards (Werneck *et al.*, 2015; Domingos *et al.*, 2017; Miranda *et al.*, 2019). Populations of *T. rapicauda* from eastern Amazonia had limited haplotype sharing and overall higher intraspecific distances, which may in fact represent species complexes that deserve further in-depth taxonomic studies. In *T. rapicauda*, we recovered a population from the Guiana Shield and three populations from the Brazilian Shield. The population R1 occurs in northern South America and lowland areas of central Amazonia. The SDMs showed moderate to low stability of climate conditions for *T. rapicauda*. The few areas with adequate suitability for this species in at least three time periods is coincident with part of the Guiana Shield and small patches in eastern Amazonia, which could represent a climate refugia from which the species expanded to its current range.

The populations R2, R3 and R4 of *T. rapicauda* located in lowland areas of the Brazilian Shield differentiated amid the Pleistocene period, thus climate instability and the greater landscape heterogeneity in eastern Amazonia may have promoted their isolation in climatic refugia, consequently causing population structuring and detectable demographic signatures (Cheng *et al.*, 2013; Wang *et al.*, 2017; Baker *et al.*, 2020). These populations seem to be spatially constrained by large interfluves. The Tapajós River region experienced a number of neotectonic events and recent climatic fluctuations that likely affected the adjacent terrestrial ecosystems (Moraes *et al.*, 2020), possibly driving allopatric differentiation in population R2, and representing a secondary contact zone for haplotype sharing of populations R3 and R4. These two populations share a single haplotype along the Xingu River and on the left bank of the Amazon River, but these rivers apparently did not act as barriers. Still, in population R4

there are haplotypes restricted to the left bank of the Amazon and Rio Negro, where these rivers may have acted as a barrier.

In contrast, *T. solimoensis* did not show any evident indication of phylogeographic structure, with haplotype sharing among populations. Studies point out that high diversification may be associated with environmental stability during periods of past climatic fluctuations (Olivares *et al.*, 2017; Arruda *et al.*, 2018; Nascimento *et al.*, 2019; Rabosky *et al.*, 2019; Baker *et al.*, 2020). Although populations S5 and S6 are located in western Amazonia, which is the most climatically stable region along the Quaternary (Cheng *et al.*, 2013), the relative stability of this region may hinder intraspecific differentiation of cryptic lineages in *T. solimoensis* given that the selective pressures probably did not diverge substantially among them.

The dates indicate that the divergence between *T. rapicauda* and *T. solimoensis* happened during the Miocene, around 24 million years ago, showing an ancient diversification between the two species of *Thecadactylus*. Ancient diversification of geckos in the New World has already been reported in different taxonomic groups and Neotropical biomes (Gamble *et al.*, 2012; Werneck *et al.*, 2012; Domingos *et al.*, 2014; Ramírez-Reyes *et al.*, 2020). The diversification period of *Thecadactylus* coincides with geomorphological and climatic changes that occurred in Amazonia, caused by the intensification of Andean orogeny (Hoorn *et al.*, 2010). During the Middle Miocene, the Andean uplift was responsible for the emergence of wetlands and subsequent development of the transcontinental Amazon River, which favored population isolation and speciation. The diversification of *T. rapicauda* started around 15 million years ago, and *T. solimoensis* diverged approximately 9 million years ago. Hoorn *et al.* (2010) report that the Andean elevation reached its highest peak at the end of the Oligocene until the beginning of the Miocene (~13Ma). In parallel, precipitation intensified in the western region, which led to the formation of the Lake Pebas system. This marshy formation confined forest islands with a floristic composition similar to the modern composition (Gomes *et al.*, 2020). As a result, there was the diversification of the existing terrestrial biota, such as mammals, insects, fish, amphibians and reptiles (Réjaud *et al.*, 2020).

### **Demography, species distributions and niche overlap**

The recent Population decline inferred for *T. rapicauda* coincides with the climatic instability that occurred in eastern Amazonia during the Pleistocene, at

~200,000 years ago (Cheng *et al.*, 2013). During the climatic fluctuations of this period, the vegetation underwent periodic cycles of retraction and expansion (Arruda *et al.*, 2018). This scenario may have caused population reductions and extinctions in eastern areas. The paleomodels for *T. rapicauda* indicated two suitability transitions which may be related to the population retraction estimated in demographic analysis at ~200 ka: 1) the decrease in extension of suitable area during the warmer periods (LIG and Holocene) and; 2) increase in suitability in the subsequent LGM and current periods. In contrast, *T. solimoensis* demography remained constant in western Amazonia for the past 5 million years, a region that was climatically more stable during the Pleistocene. The SDMs models for *T. solimoensis* showed this stability, with low variation of suitability over time and a tendency to increase the extension of suitable areas during the LIG (towards south) and LGM (towards eastern Amazonia).

Species Distribution Models are important tools for understanding the potential distributions and the ecological divergence of organisms in different periods of time, helping to generate hypotheses and interpret phylogeographic patterns (Alvarado-Serrano & Knowles, 2014). The SDMs projections into different periods of time indicated that areas of historical suitability varied differently for the two species of *Thecadactylus*. The paleodistribution models showed that during the LIG, a period with hot and humid temperatures, the central and eastern portions of the Amazon Basin were less suitable for *T. rapicauda*, and the persistence of the species in this region could be related to the presence of microrefugia (e.g., forest patches), which would not be detectable at the scale of our study. Cheng *et al.*, (2013) report that eastern Amazonia has been through drastic changes in the regional climate from LGM to the beginning of the middle Holocene, from severely dry to substantially humid conditions. Thus, in drier glacial periods, the forest may have retracted, allowing the fragmentation and recolonization of animal and plant populations. However, speleothems (Wang *et al.*, 2017) and palynological (Reis *et al.*, 2017; Arruda *et al.*, 2018) studies state that even in periods of climatic instability the vegetation in central Amazonia remained with similar characteristics to the present. In contrast, our results indicate a retraction of suitable habitats for *T. rapicauda* during the middle Holocene, even in conditions of greater humidity, in comparison to the LGM. Therefore, spatiotemporal and ancestral areas reconstruction could be used to test the hypothesis of an eastwards diffusion history of *T. rapicauda*, as proposed for other lowland Amazonian taxa (Rejaud, *et al.*, 2020; Silva *et al.*, 2019) The paleodistribution projections estimated for *T. solimoensis* showed a

distinct pattern compared to *T. rapicauda*. In general, during the LIG and LGM, models showed more extensive suitable habitats even outside the western portion of the Amazon Basin. Evidence from paleovegetation modeling and pollen validation studies (Costa *et al.*, 2018) indicates that forests in western Amazonia were not fragmented during the LGM, providing stability of humid tropical forests. Unlike the Holocene, during the LGM, precipitation in the region increased, which may have contributed to the persistence and stability of tropical vegetation. Although western Amazonia experienced a reduction in humidity in warmer periods, the vegetation underwent few changes during the Quaternary (Cheng *et al.*, 2013; Maksic *et al.*, 2019; Gomes *et al.*, 2020). The moderate fall in precipitation and a drier climate during the middle Holocene in western Amazonia favored the expansion of dry vegetation in ecotone regions (Cheng *et al.*, 2013; Cohen *et al.*, 2014; Lombardo *et al.*, 2019; Nascimento *et al.*, 2019). However, the persistence of the tropical forest in western Amazonia during the middle Holocene enabled those organisms associated with forest environments, such as *T. solimoensis* according to our models.

In addition to the effects of vegetation fluctuations during the glacial and interglacial cycles, ecophysiological aspects of these nocturnal geckos species may have contributed to their establishment and diversification. Thermoconforming nocturnal species have a physiology adapted to relatively milder night temperatures. Therefore, they may be less susceptible to water stress, as their nocturnal habits should help mitigate the effects of climate change (Sinervo *et al.*, 2010). Temperature fluctuations can impact patterns of animal activity, which tend to shift temporal niches towards nocturnal habits to avoid thermal stress due to climate changes (Levy *et al.*, 2019). Thus, of the fluctuations during the Quaternary may have not been as negative for *Thecadactylus* as it could be for a diurnal taxon.

Our ecological analyses indicate a low degree of niche overlap between *T. rapicauda* and *T. solimoensis*, suggesting that since their separation there has been a substantial divergence between ecoclimatic preferences, indicating a low degree of niche conservatism. Diversification may be related to climatic niche divergence (Castro-Insua *et al.*, 2018; Sheu *et al.*, 2020), which influences inter and intraspecific evolution and, consequently, the segregation of ecological niche. Thus, climatic conditions were important factors that influenced the occupation of different niche ranges of these sister-species of *Thecadactylus*. By rejecting the niche equivalence hypothesis, we suggest that the niches of these turnip-tailed geckos diverged since their first split in the

Miocene, in support that the evolutionary history of gecko species appears to be the result of ecoclimatic factors and landscape changes (Rodríguez-Rodríguez *et al.*, 2020). In addition, considering the niche divergence and the lack of marked geographical barriers between species, there is evidence for ecological parapatric speciation of *Thecadactylus*. This is demonstrated with the low niche similarity of these species, which would be expected given the habitat availability (Blair *et al.*, 2013) and their similar lifestyles.

## Conclusions

Past climatic and geomorphological changes in Amazonia such as climatic instabilities and changes in river courses had different impacts on the evolutionary history of sister-species of *Thecadactylus* geckos. Geomorphological events that occurred in the Amazon Basin facilitated their evolutionary divergence ~24 million years ago, and Pleistocene climatic fluctuations influenced the phylogeographic structure of *T. rapicauda*, with the emergence of populations and haplotypes restricted by some major rivers. Climatic instability in the Quaternary is related to a slight demographic retraction of *T. rapicauda*. Even under unstable climatic conditions in the central and eastern portion of the Amazon Basin, populations of *T. rapicauda* persisted and potentially expanded its distribution when climatic conditions became more suitable in the present. Conversely, *T. solimoensis*, distributed in western Amazonia, had shallower phylogeographic structure, with evident haplotype sharing, demographic stability, and probably occupied areas of ecological suitability overall stable during the Quaternary climatic fluctuations. The two gecko species also showed divergence in their climatic preferences, indicating low niche conservatism. As such, PNC likely did not act as the main mechanism responsible for their cryptic diversity, and events associated with landscape evolution and paleoclimatic conditions may have played more important roles in the diversification of these geckos.

The high intra and interspecific genetic distances in both species of *Thecadactylus* and the trans-Andean clade noticed in this study reinforce the need for further investigations on species limits and taxonomy of Neotropical geckos. These should include new samples throughout the genus distribution and using genomic data sets integrated with ecological and evolutionary inferences. Finally, the approach used here contributes to future work on the diversification of Neotropical vertebrates, employing an integrative evolutionary ecological perspective.



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**TABLES**

**Table 1.** Mitochondrial DNA genetic distance matrix (%) for the populations of *T. rapicauda* (R1 to R4) and *T. solimoensis* (S5 and S6). Intra-population distance (bold diagonal), inter-population distance.

	<b>R2</b>	<b>S6</b>	<b>S5</b>	<b>R3</b>	<b>R1</b>	<b>R4</b>
<b>R2</b>	<b>0.22</b>					
<b>S6</b>	19.0	<b>0.05</b>				
<b>S5</b>	20.0	19.0	<b>0.25</b>			
<b>R3</b>	40.0	40.0	36.0	<b>0.23</b>		
<b>R1</b>	26.0	38.0	29.0	27.0	<b>0.07</b>	
<b>R4</b>	23.0	40.0	26.0	29.0	20.0	<b>0.06</b>

**Table 2.** Genetic diversity indexes and neutrality tests for populations of *Thecadactylus* using the mitochondrial Cytochrome b gene. n - number of individuals; Hn - number of haplotypes; Hd - haplotypic diversity;  $\pi$  - nucleotide diversity; s. d. - standard deviation; D - Tajima's test; Fs - Fu's test. Non-significant values.

Species	population	n	Hn	Hd ( $\pm$ s.d)	$\pi$ ( $\pm$ s.d)	D*	Fs*
<i>T. rapicauda</i>	R1	21	13	0.919 ( $\pm$ 0.042)	0.051 ( $\pm$ 0.0044)	-0.10	2.71
	R2	22	17	0.978 ( $\pm$ 0.019)	0.062 ( $\pm$ 0.0062)	0.84	0.61
	R3	9	8	0.972 ( $\pm$ 0.064)	0.150 ( $\pm$ 0.0207)	0.29	0.16
	R4	5	5	1.000 ( $\pm$ 0.126)	0.154 ( $\pm$ 0.0421)	1.60	1.68
<i>T. solimoensis</i>	S5	36	24	0.937 ( $\pm$ 0.033)	0.044 ( $\pm$ 0.0058)	-0.82	-0.32
	S6	9	7	0.944 ( $\pm$ 0.070)	0.142 ( $\pm$ 0.0169)	0.82	0.68

**Table 3.** Contribution of bioclimatic variables used in species distribution models for *Thecadactylus*. Contribution values are percentages for each period and AUC values are in bold.

Specie	Variables	% contribution of the variable
<i>Thecadactylus rapicauda</i>	Mean diurnal Range (BIO2)	23.7
	Precipitation of Coldest Quarter (BIO19)	25.6
	Isothermality (BIO3)	34
	Precipitation Seasonality (BIO15)	6.7
	Precipitation of Driest Month (BIO14)	3.4
	Max Temperature of Warmest Month (BIO5)	0.8
	Precipitation of Wettest Month (BIO13)	1.6
	Precipitation of Warmest Quarter (BIO18)	4.2
	Temperature Seasonality (BIO4)	-
<b>Average AUC</b>		<b>0.911</b>
<b>Feature class - regularization</b>		<b>LQ - 0.5</b>
<i>Thecadactylus solimoensis</i>	Precipitation of Coldest Quarter (BIO19)	17.4
	Isothermality (BIO3)	47.3
	Precipitation of Warmest Quarter (BIO18)	21.4
	Temperature Seasonality (BIO4)	-
	Precipitation of Driest Month (BIO14)	5.3
	Precipitation Seasonality (BIO15)	4.2
	Precipitation of Wettest Month (BIO13)	1.3
	Max Temperature of Warmest Month (BIO5)	2.3
	Mean diurnal Range (BIO2)	0.9
<b>Average AUC</b>		<b>0.899</b>
		<b>LQ - 0.5</b>
<b>Feature class - regularization</b>		<b>LQP - 1.5</b>
		<b>LQ - 1</b>

## LEGENDS

**Figure 1. Distribution of samples obtained from *Thecadactylus*.** (A) Phylogenetic tree using concatenated genes estimated under maximum likelihood inference (ML). Blue and green circles indicate Bayesian posterior probabilities and ML bootstrap values (bold), respectively. (B) Geographic distribution of *Thecadactylus* samples. Orange and purple symbols represent *T. rapicauda* and *T. solimoensis* samples, respectively; samples of *T. rapicauda* from western Ecuador are depicted by orange stars.

**Figure 2. Bayesian phylogenetic tree of *Thecadactylus* with divergence time estimates based on four loci.** Blue bars represent 95% HPD credibility intervals. Blue and green circles indicate Bayesian posterior probabilities and ML bootstrap values (bold), respectively.

**Figure 3. Populations found in Baps based on mitochondrial DNA sequences.** (A) Maximum likelihood phylogenetic tree showing populations. (B) Population distribution map over the Amazon.

**Figure 4. Demography and stability areas respectively inferred through Extended Bayesian Skyline Plots and overlap of inferred current and paleodistributions (see Fig. 5) for *Thecadactylus rapicauda* (A) and *Thecadactylus solimoensis* (B).** The x-axis indicates time in millions of years and the y-axis indicates population size over time.

**Figure 5. Species Distribution Models for *Thecadactylus rapicauda* (A) and *T. solimoensis* (B).** Projections were inferred to the present, mid-Holocene (~6 k), Last Glacial Maximum (LGM, ~21k), and Last Interglacial (LIG, ~120k).

**Figure 6. Analyses of similarity and niche equivalence for two *Thecadactylus* species.** (A) and (B) estimate of the axes of PCA1 and PCA2 of the PCA-env represents the multidimensional space of occurrence of the species. The solid and dashed lines represent 100% and 50% of availability of the environment, respectively. (C) histogram of equivalence test; (D) similarity test histogram  $1 < 2$  (pairwise comparisons); (E) histogram of similarity  $2 < 1$  (pairwise comparisons); (F) PCA with the contributions of bioclimatic variables used in the analysis.

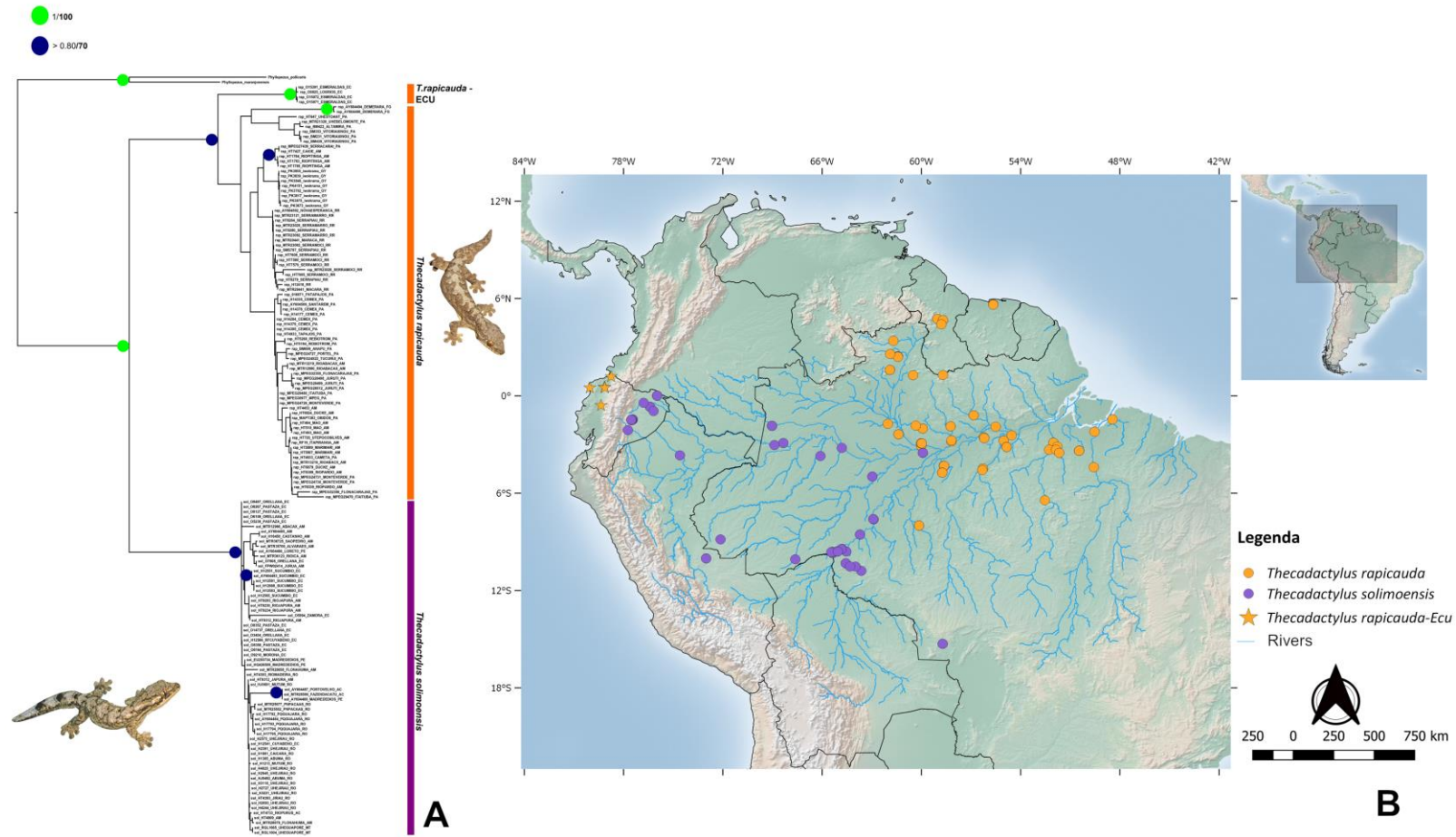


Figure 1.



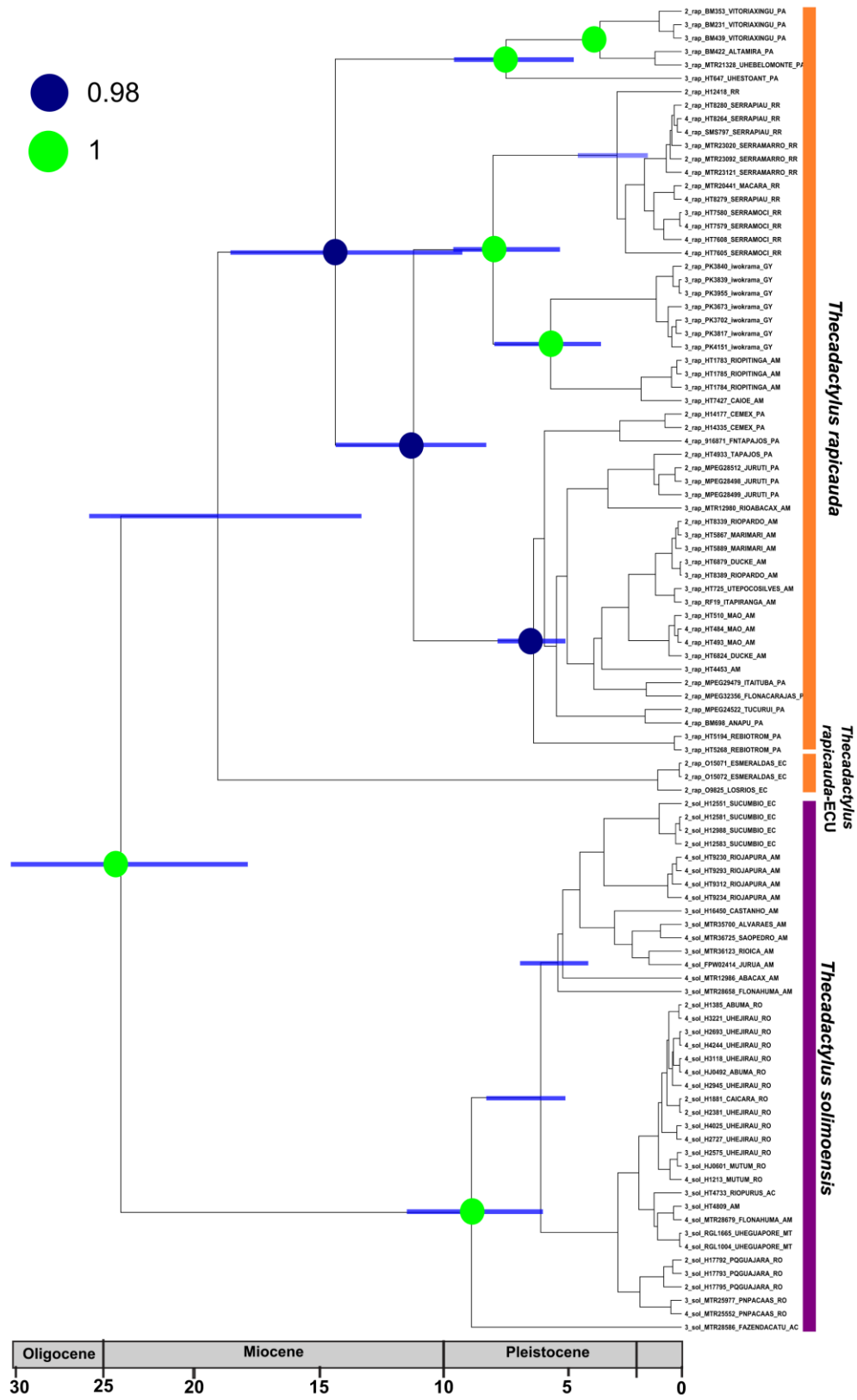


Figure2.

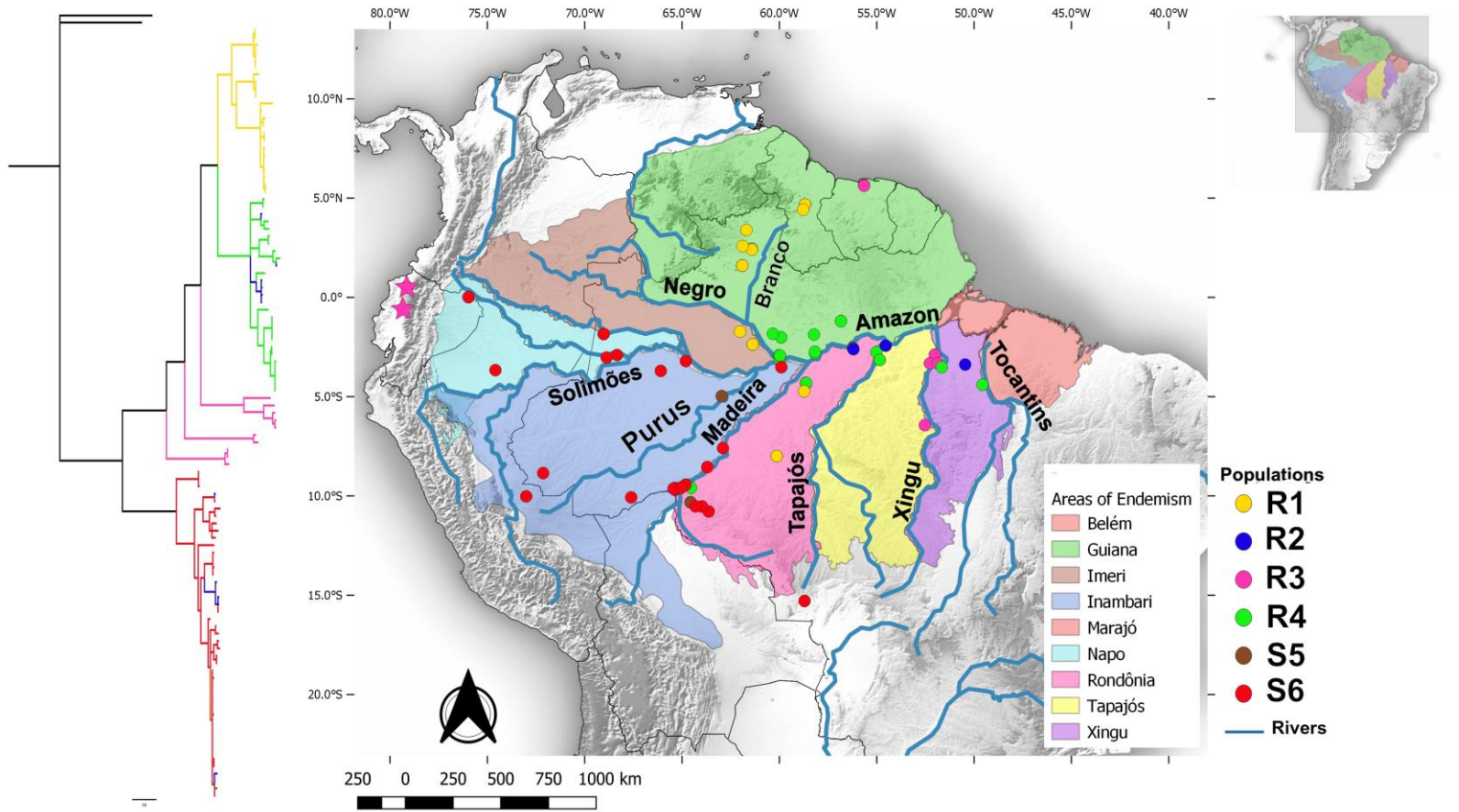


Figure 3.

## Demography and stability areas

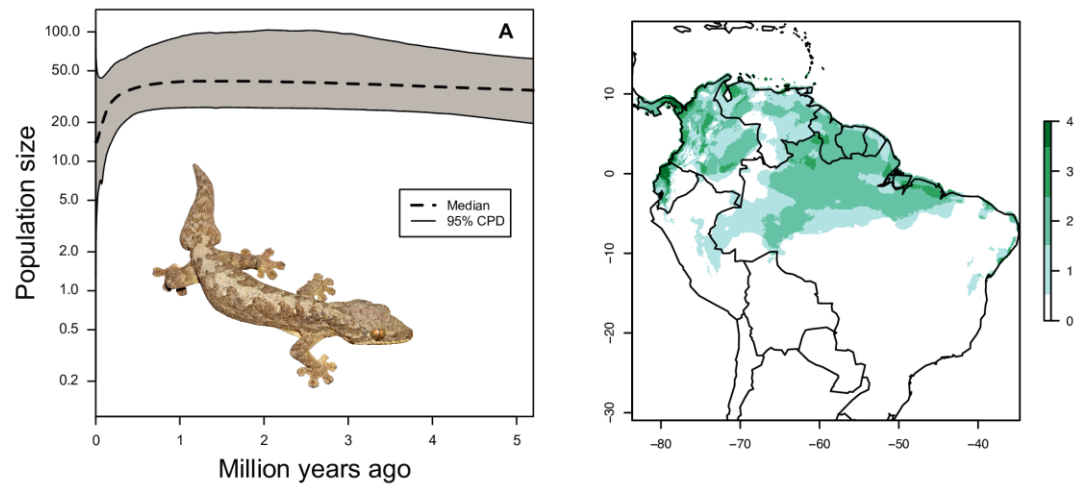
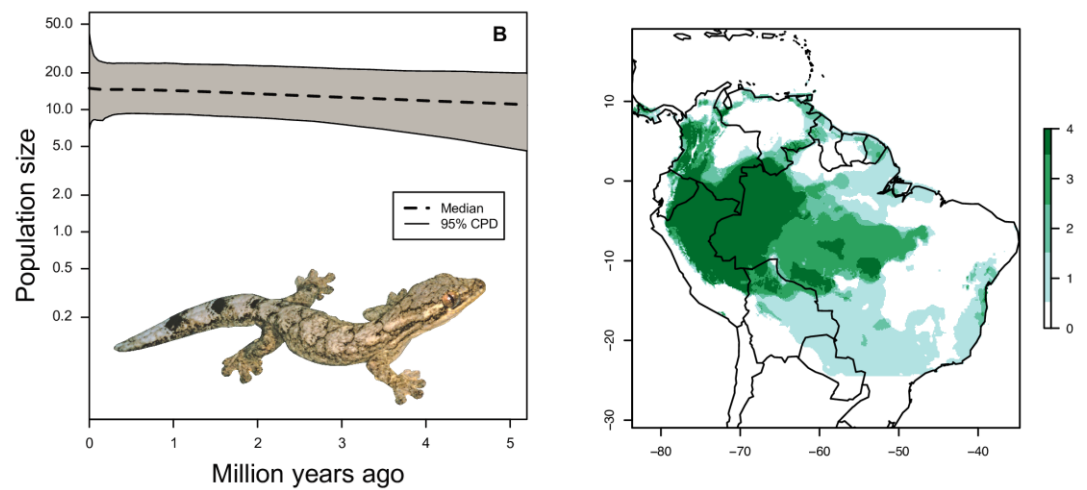
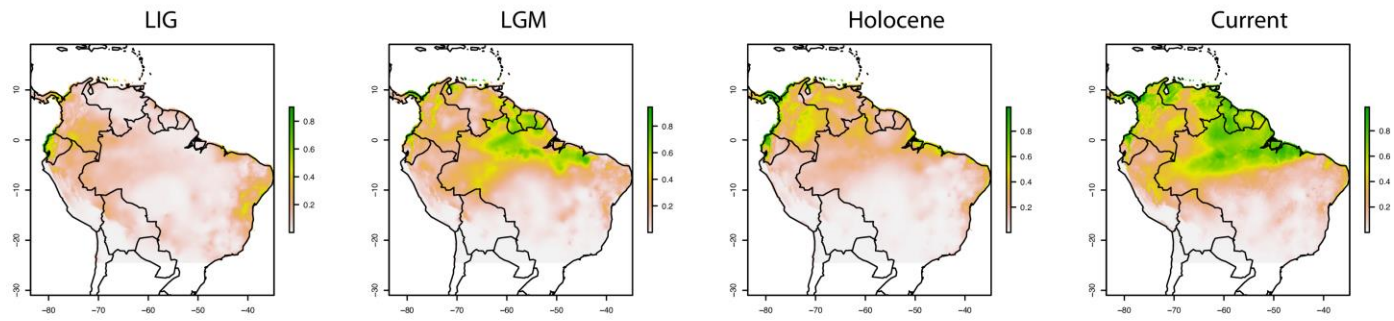
(A) *Thecadactylus rapicauda*(B) *Thecadactylus solimoensis*

Figure 4.

(A) *Thecadactylus rapicauda*



(B) *Thecadactylus solimoensis*

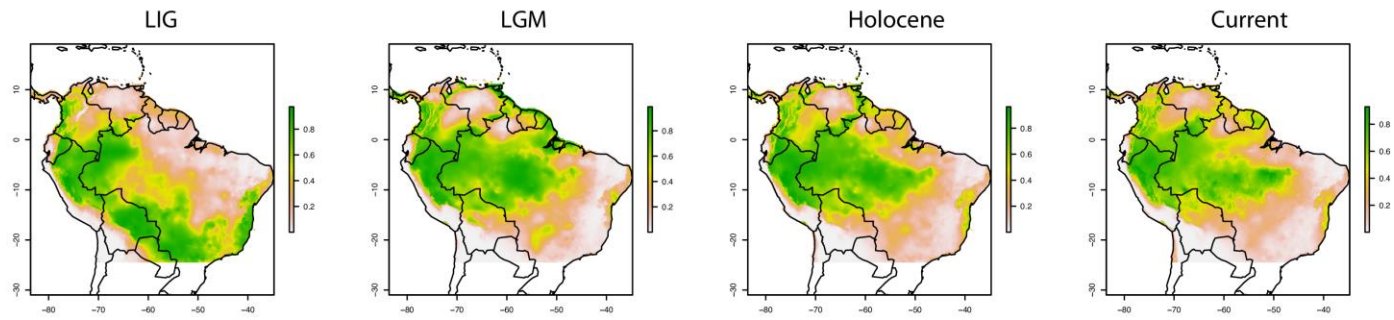


Figure 5.

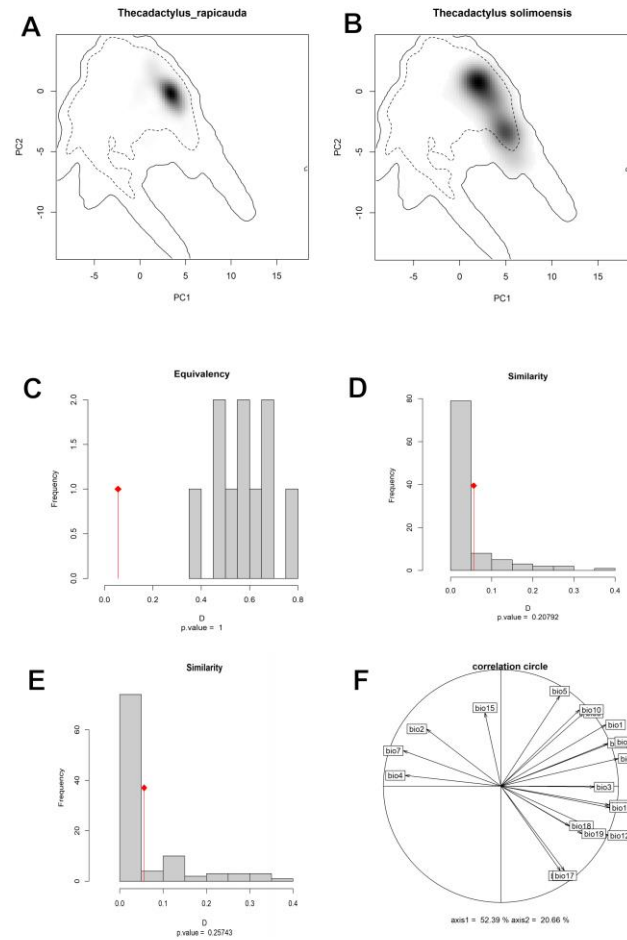


Figure 6.

## Supporting Information

**Table S1 1. Geographic coordinates of the samples used, including identification number, species, location, and geographic coordinates. Samples obtained from GenBank are highlighted in bold.**

<b>ID Number</b>	<b>Specie</b>	<b>Locality</b>	<b>Long</b>	<b>Lat</b>
HT484	<i>Thecadactylus rapicauda</i>	Manaus/AM	-60.013207	-2.923073
HT493	<i>Thecadactylus rapicauda</i>	Manaus/AM	-60.003289	-3.083583
HT510	<i>Thecadactylus rapicauda</i>	Manaus/AM	-60.013207	-2.923073
HT647	<i>Thecadactylus rapicauda</i>	UHE Sto Antônio do Jari/AM	-52.531388	-6.438110
HT725	<i>Thecadactylus rapicauda</i>	UTE Poço de Silves/AM	-58.177398	-2.813400
HT1783	<i>Thecadactylus rapicauda</i>	Mineração Taboca, Rio Pitinga/AM	-60.137228	-7.997300
HT1784	<i>Thecadactylus rapicauda</i>	Mineração Taboca, Rio Pitinga/AM	-60.137228	-7.997300
HT1785	<i>Thecadactylus rapicauda</i>	Mineração Taboca, Rio Pitinga/AM	-60.137228	-7.997300
HT4393	<i>Thecadactylus solimoensis</i>	Rio Madeira/RO	-58.225278	-1.875000
HT4453	<i>Thecadactylus rapicauda</i>	Urucará/AM	-58.225278	-1.875000
HT4933	<i>Thecadactylus rapicauda</i>	Rio Tapajós/PA	-49.523353	-2.213317
HT5194	<i>Thecadactylus rapicauda</i>	ReBio Trombetas/PA	-56.836579	-1.196286
HT5268	<i>Thecadactylus rapicauda</i>	ReBio Trombetas/PA	-56.836579	-1.196286
HT5867	<i>Thecadactylus rapicauda</i>	Presidente Figueiredo/AM	-59.911939	-2.016555
HT5889	<i>Thecadactylus rapicauda</i>	Presidente Figueiredo/AM	-59.911939	-2.016555
HT6824	<i>Thecadactylus rapicauda</i>	Reserva Ducke/AM	-59.975139	-2.929856
HT6879	<i>Thecadactylus rapicauda</i>	Reserva Ducke/AM	-59.975556	-2.930056
HT7427	<i>Thecadactylus rapicauda</i>	Comunidade Caioé/AM	-62.023333	-1.721694
HT7579	<i>Thecadactylus rapicauda</i>	Parque Nacional da Serra da Mocidade/RR	-61.900000	1.600000

HT7580	<i>Thecadactylus rapicauda</i>	Parque Nacional da Serra da Mocidade/RR	-61.900000	1.600000
HT7605	<i>Thecadactylus rapicauda</i>	Parque Nacional da Serra da Mocidade/RR	-61.900000	1.600000
HT7608	<i>Thecadactylus rapicauda</i>	Parque Nacional da Serra da Mocidade/RR	-61.900000	1.600000
HT8264	<i>Thecadactylus rapicauda</i>	Serra do Apiau/RR	-61.415472	2.436889
HT8279	<i>Thecadactylus rapicauda</i>	Serra do Apiau/RR	-61.415528	2.4349167
HT8280	<i>Thecadactylus rapicauda</i>	Serra do Apiau/RR	-61.415528	2.4349167
HT8339	<i>Thecadactylus rapicauda</i>	Rio Pardo/AM	-61.415528	2.434917
HT8389	<i>Thecadactylus rapicauda</i>	Rio Pardo/AM	-60.334083	-1.831861
HT9230	<i>Thecadactylus solimoensis</i>	Rio Japurá/AM	-69.029528	-1.846139
HT9234	<i>Thecadactylus solimoensis</i>	Rio Japurá/AM	-69.029528	-1.846139
HT9293	<i>Thecadactylus solimoensis</i>	Rio Japurá/AM	-69.029528	-1.846139
HT9312	<i>Thecadactylus solimoensis</i>	Rio Japurá/AM	-69.029528	-1.846139
HT4733	<i>Thecadactylus solimoensis</i>	Rio Purus/AC	-62.960583	-4.975677
HT4809	<i>Thecadactylus solimoensis</i>	Amazonas	-62.960583	-4.975677
RGL1004	<i>Thecadactylus solimoensis</i>	UHE Guaporé/MT	-58.708731	-15.290572
RGL1665	<i>Thecadactylus solimoensis</i>	UHE Guaporé/MT	-58.708731	-15.290572
MTR 12980	<i>Thecadactylus rapicauda</i>	São Sebastião, Rio Abacaxis/AM	-58.636389	-4.308889
MTR 12986	<i>Thecadactylus solimoensis</i>	Rio Abacaxis/AM	-58.625278	-4.344167
MTR 13219	<i>Thecadactylus rapicauda</i>	Rio Abacaxis/AM	-58.625278	-4.315556
916871	<i>Thecadactylus rapicauda</i>	Floresta Nacional do Tapajós/PA	-55.019625	-2.758042
HJ0492	<i>Thecadactylus solimoensis</i>	Abumã/RO	-65.356619	-9.694911
HJ0601	<i>Thecadactylus solimoensis</i>	Mutum/RO	-63.703700	-8.550742
H1213	<i>Thecadactylus solimoensis</i>	Mutum/RO	-63.703700	-8.550742
H1385	<i>Thecadactylus solimoensis</i>	Abumã/RO	-65.356619	-9.694911
MTR20441	<i>Thecadactylus rapicauda</i>	E.E. Maracá/RR	-61.689964	3.388664
H1881	<i>Thecadactylus solimoensis</i>	Caiçara/RO	-64.792269	-9.439171
BM231	<i>Thecadactylus rapicauda</i>	Vitória do Xingu/PA	-52.012564	-2.885578

BM353	<i>Thecadactylus rapicauda</i>	Vitória do Xingu/PA	-52.012564	-2.885578
MTR21328	<i>Thecadactylus rapicauda</i>	UHE Belo Monte/PA	-51.776400	-3.129156
MTR23020	<i>Thecadactylus rapicaudus</i>	Serra da Marroquina/RR	-61.373420	-2.367967
MTR23092	<i>Thecadactylus rapicauda</i>	Serra da Marroquina/RR	-61.373420	-2.367967
MTR23121	<i>Thecadactylus rapicauda</i>	Serra da Marroquina/RR	-61.373472	-2.367967
MTR25552	<i>Thecadactylus solimoensis</i>	Parque Nacional de Pacaás/RO	-63.627300	-10.786333
MTR25977	<i>Thecadactylus solimoensis</i>	Parque Nacional de Pacaás/RO	-63.973360	-10.527499
MTR28586	<i>Thecadactylus solimoensis</i>	Fazenda Experimental Catuaba/AC	-67.614780	-10.075711
MTR28658	<i>Thecadactylus solimoensis</i>	FLONA Humaita/AM	-62.885140	-7.616944
MTR28679	<i>Thecadactylus solimoensis</i>	FLONA Humaita/AM	-62.899440	-7.595133
H2381	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-64.824690	-9.445933
H2575	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-64.536740	-9.597544
H2693	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-65.065080	-9.594922
H2727	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-64.824690	-9.445933
H2945	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-64.824690	-9.445933
H3118	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-65.069320	-9.584644
H3221	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-65.377920	-9.608244
RF19	<i>Thecadactylus rapicauda</i>	Itapiranga/AM	-58.176300	-2.743411
BM422	<i>Thecadactylus rapicauda</i>	Altamira/PA	-52.271100	-3.322999
BM439	<i>Thecadactylus rapicauda</i>	Vitória do Xingu/PA	-51.858700	-3.324444
BM698	<i>Thecadactylus rapicauda</i>	Anapu/PA	-51.659200	-3.517888
H4025	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-65.439920	-9.634122
H4244	<i>Thecadactylus solimoensis</i>	UHE Jirau/RO	-65.049180	-9.591322
SMS797	<i>Thecadactylus rapicauda</i>	Serra do Apiaú/RR	-61.415190	2.408588
MTR35700	<i>Thecadactylus solimoensis</i>	Alvarães/AM	-64.805300	-3.210266
MTR36123	<i>Thecadactylus solimoensis</i>	Rio Içá/AM	-68.339830	-2.904911
MTR36725	<i>Thecadactylus solimoensis</i>	São Pedro/AM	-68.877070	-3.032088



PK3955	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.731390	-4.731399
PK4151	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.685560	4.671677
PK3840	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.731890	-4.731899
PK3875	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-59.026110	4.746944
PK3839	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.731890	-4.731899
PK3702	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.685560	4.671677
PK3673	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.685560	4.671677
PK3817	<i>Thecadactylus rapicauda</i>	Iwokrama/Guiana	-58.783890	4.412788
FPWerneck02414	<i>Thecadactylus solimoensis</i>	Juruá/AM	-66.096744	-3.706622
MAP-T383	<i>Thecadactylus rapicauda</i>	Óbidos/PA	-55.506467	-1.8991472
H12541	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12418	<i>Thecadactylus rapicauda</i>	Roraima	-60.492439	1.274333
H12551	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12565	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12566	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12581	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12583	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H12988	<i>Thecadactylus solimoensis</i>	Sucumbios,Reserva Faunistica Cuyabeno/ECU	-75.968826	0.01329973
H14177	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.842472	-3.150667
H14204	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.840417	-3.1495278
H14305	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.842472	-3.150667
H14335	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.538167	-2.433333
H14378	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.842472	-3.150667
H14379	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.842472	-3.150667
H16450	<i>Thecadactylus solimoensis</i>	Castanho/AM	-59.903333	-3.515555
H17792	<i>Thecadactylus solimoensis</i>	Parque Estadual Guajará-Mirim/RO	-64.563306	-10.321444
H17793	<i>Thecadactylus solimoensis</i>	Parque Estadual Guajará-Mirim/RO	-64.563306	-10.321444

H17794	<i>Thecadactylus solimoensis</i>	Parque Estadual Guajará-Mirim/RO	-64.563306	-10.321444
H17795	<i>Thecadactylus solimoensis</i>	Parque Estadual Guajará-Mirim/RO	-64.563306	-10.321444
MPEG24522	<i>Thecadactylus rapicauda</i>	Tucuruí/PA	-49.568889	-4.398333
MPEG24727	<i>Thecadactylus rapicauda</i>	Fazenda Riacho Monte Verde,Portel/PA	-50.457500	-3.383890
MPEG24728	<i>Thecadactylus rapicauda</i>	Fazenda Riacho Monte Verde, Portel/PA	-50.457500	-3.383890
MPEG24730	<i>Thecadactylus rapicauda</i>	Fazenda Riacho Monte Verde, Portel/PA	-50.457500	-3.383890
MPEG24731	<i>Thecadactylus rapicauda</i>	Fazenda Riacho Monte Verde, Portel/PA	-50.457500	-3.383889
MPEG27439	<i>Thecadactylus rapicauda</i>	Serra do Acarai, Oriximiná/PA	-58.688600	1.28486940
MPEG28498	<i>Thecadactylus rapicauda</i>	Juruti, Galiléia/PA	-56.225556	-2.547500
MPEG28499	<i>Thecadactylus rapicauda</i>	Juruti, mutum/PA	-56.196111	-2.609440
MPEG28512	<i>Thecadactylus rapicauda</i>	Juruti, mutum/PA	-56.196111	-2.609440
MPEG29479	<i>Thecadactylus rapicauda</i>	Itaituba/PA	-56.271667	-4.484167
MPEG29480	<i>Thecadactylus rapicauda</i>	Itaituba/PA	-56.312222	-4.575560
MPEG30977	<i>Thecadactylus rapicauda</i>	Campus de Pesquisa do MPEG/PA	-48.444928	-1.450920
MPEG32356	<i>Thecadactylus rapicauda</i>	Parauapebas, Flona Carajás/PA	9326469.0	576210.00
MPEG32355	<i>Thecadactylus rapicauda</i>	Parauapebas, Flona Carajás/PA	9326621.0	577130.00
QCAZR3404	<i>Thecadactylus solimoensis</i>	Orellana, Parque Nacional Yasuní/ECU	-76.397097	-0.674324
QCAZR5230	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.451710	-1.470977
QCAZR5894	<i>Thecadactylus solimoensis</i>	Zamora Chinchipe/ECU	-78.948958	-4.072222
QCAZR6109	<i>Thecadactylus solimoensis</i>	Orellana, Parque Nacional Yasuní/ECU	-76.398520	-0.674111
QCAZR7808	<i>Thecadactylus solimoensis</i>	Orellana, Parque Nacional Yasuní/ECU	-76.397097	-0.674324
QCAZR8127	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.444080	-1.450433
QCAZR8164	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.486470	-1.472254
QCAZR8267	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.511660	-1.507777
QCAZR8352	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.531000	-1.473999
QCAZR8358	<i>Thecadactylus solimoensis</i>	Pastaza/ECU	-77.535290	-1.474455
QCAZR9210	<i>Thecadactylus solimoensis</i>	Morona Santiago/ECU	-77.739870	-2.114446

QCAZR9497	<i>Thecadactylus solimoensis</i>	Orellana, Comunidad El Descanso/ECU	-76.786480	-0.431111
QCAZR9825	<i>Thecadactylus rapicauda</i>	Los rios, Centro Científico Río Palenque/ECU	-79.362280	-0.592099
QCAZR14737	<i>Thecadactylus solimoensis</i>	Orellana, Parque Nacional Yasuní/ECU	-76.188190	-0.935649
QCAZR15071	<i>Thecadactylus rapicauda</i>	Esmeraldas, Reserva Tesoro Escondido/ECU	-79.144170	0.539544
QCAZR15072	<i>Thecadactylus rapicauda</i>	Esmeraldas, Reserva Tesoro Escondido/ECU	-79.138240	0.498188
QCAZR15391	<i>Thecadactylus rapicauda</i>	Esmeraldas, Durango/ECU	-78.752790	1.182966
HQ426509	<i>Thecadactylus solimoensis</i>	Madre de Dios/Peru	-73.006617	-10.022521
<b>AY604487</b>	<i>Thecadactylus solimoensis</i>	Porto velho/RO	-72.138500	-8.851574
<b>AY604488</b>	<i>Thecadactylus solimoensis</i>	Madre de Dios/Perú	-73.006617	-10.022521
<b>AY604483</b>	<i>Thecadactylus solimoensis</i>	Sucumbios, Reserva Faunistica Cuyabeno/ECU	-75.968826	0.013300
<b>AY604484</b>	<i>Thecadactylus solimoensis</i>	Parque Estadual Guajara-Mirim/RO	-64.309243	-10.524195
<b>AY604486</b>	<i>Thecadactylus solimoensis</i>	Loreto, San Jacinto/Peru	-74.591331	-3.667505
<b>AY604485</b>	<i>Thecadactylus solimoensis</i>	Amazonas	-59.903333	-3.515555
<b>AY604494</b>	<i>Thecadactylus rapicauda</i>	CEIBA Biological Center, Demerara/Guiana	-55.651226	5.647947
<b>AY604496</b>	<i>Thecadactylus rapicauda</i>	CEIBA Biological Center, Demerara/Guiana	-55.649639	5.621125
<b>AY604502</b>	<i>Thecadactylus rapicauda</i>	Fazenda Nova Esperança/RR	-61.883024	2.581377
<b>AY604509</b>	<i>Thecadactylus rapicauda</i>	CEMEX, Agropecuaria Treviso, Santarém/PA	-54.842472	-3.150667
<b>EU293734</b>	<i>Thecadactylus solimoensis</i>	Madre de Dios/Peru	-73.006617	-10.022521
<b>EU293725</b>	<i>Phyllopezus pollicaris</i>	Parque Nacional da Serra das Confusões/PI	-43.4920006	-9.2228317
<b>EU293723</b>	<i>Phyllopezus maranjonensis</i>	Balsas, Amazonas/Peru	-78.0312645	-6.8448898

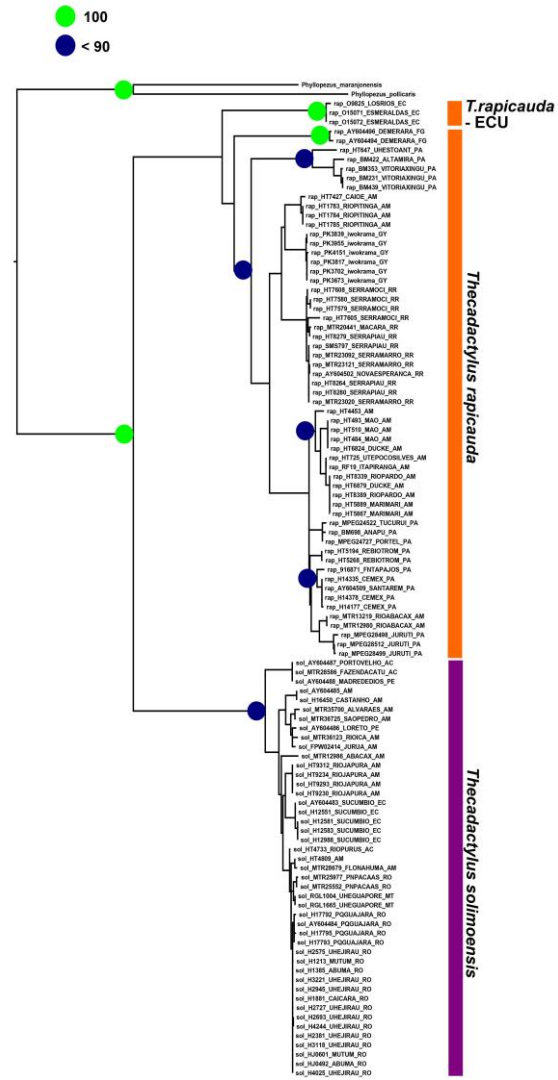
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**Table S 2. Primers used for amplification and sequencing in this study, with details of the PCR conditions used.**

Locus	Primer	Reference	T (°C)
CYTB	TRA3f 5'GTAAT(AG)GCCAC(AC)GCATTCGT3'	Kronauer <i>et al.</i> , 2005	53
	TRA3R 5' GGGTCTTCTAC(CT)GG(CT)TG(AG)CC 3'		
PRLR	PRLR_f1 5'GACARYGARGACCAGCAACTRATGCC3'	Townsend <i>et al.</i> , 2008)	52
	PRLR_r3 5' GACYTTGTGRACTTCYACRTAATCCAT 3'		
MXRA5*	MXRA5_F2 5'KGCTGAGCCTKCCTGGGTGA 3' MXRA5_R2 5' YCTMCGGCCYCTGCAACATTK 3'	Portik <i>et al.</i> , 2012)	<b>touchdown</b>
RAG2	PY1f 5'CCCTGAGTTTGGATGCTGTACTT 3'	Gamble <i>et al.</i> , 2008	55
	PY1r 5' AACTGCCTRTTGTCCCCTGGTAT 3'		

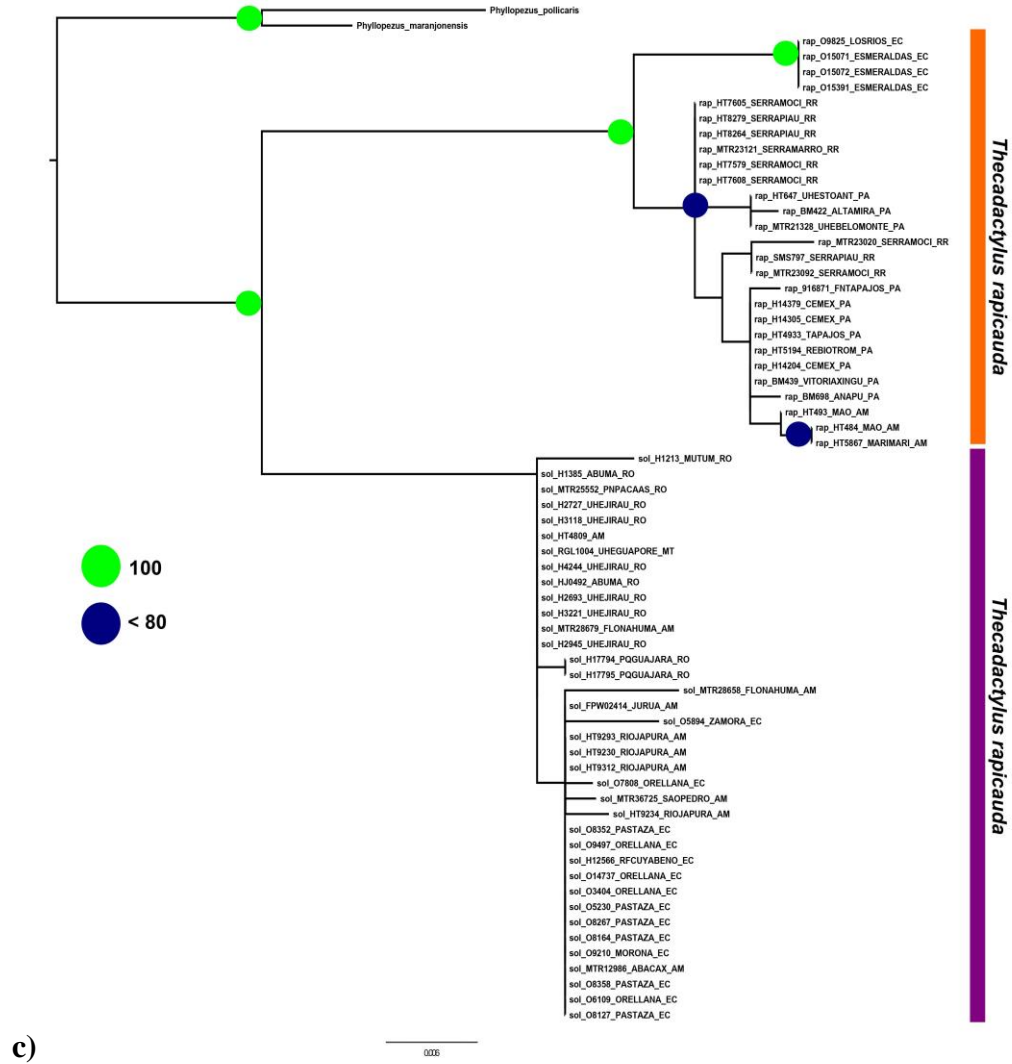
\*Standard touchdown cycles: 95°C–1:30min, 10X [95°C–35s, 63°C–35s (-0.5°C/cycle), 72°C–1min], 10X (95°C–35s, 58°C–35s, 72°C–1min), 15X (95°C–35s, 52°C–35s, 72°C–1min; final rest at 10°C. **PCR amplification conditions:** Initial denaturation at 94°C 5min, followed by 35 cycles consisting of 94°C denaturation (1 min); T °C annealing (0:30s), 72 °C extension (1 min), and a final extension at 72 °C (5 min) and final rest at 20°C.

**Figure S1 1. Maximum likelihood genetic trees for markers (a) Cytb, b) PRLR, c) MXRA5, d) RAG2. The green circles indicate the probabilities 100% and the blue circles the probabilities <90% and <80%.**



a)





c)





d)

**Figure S1 2. Estimated haplotype networks for *Thecadactylus* species. (A) *T. rapicauda*, haplotype locality: M - Amazonas; VT - Vitória do Xingu ; PA - Pará ; SR - Serra da Mocidade; RC - Rio Pitinga; GY - Guiana. (B) *T. solimoensis*. AB - Rio Abacaxis; AM - Amazonas ; AV - Alvarães ; CATU - Fazenda catuaba; CT - Castanho ; FH - Flona Humaitá; ICA - Rio Içá ; JAPU - Rio Japurá ; JIRA - UHE Jirau; PD - São Pedro; PN - Parna Pacaas; PORE - UHE Guaporé; PQ - Pq. Guarajá; PURU - Rio Purus; MU - Mutum.**

