

Carolina Farhat

Same patterns, different stories:

the role of Pliocene climatic variations in the diversification  
process of two Amazonian lizards

Mesmo padrão, histórias diferentes:

o papel das variações climáticas do Plioceno no processo de diversificação  
de duas espécies de lagartos Amazônicos

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Ao meu avô Fran,  
que me ensinou a pegar  
lagartixas na parede

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## 1. Introduction

The Amazon rainforest is home to an astounding array of plant and animal species, boasting the highest species diversity in the world (Raven *et al.*, 2020). However, the origin of all this biodiversity and the processes that have shaped its species distributions are still unclear due to the dynamic and complex history of the region (Hoorn *et al.*, 2010, Latrubesse *et al.*, 2010, Turchetto-Zolet *et al.*, 2013). Over the course of the Cenozoic era (66 millions of years ago [mya] to present), the Amazon rainforest underwent significant geomorphological and climatic changes, resulting in major landscape transformations that led to the development of geographical (e.g. mountains or rivers) and/or ecological barriers (e.g. savanna expansion) for forest-dwelling species. Once natural barriers isolate populations and prevent the homogenization of species pools due to the break of genetic flow, these changes favor populational differentiation. Thus, isolated populations accumulate genetic and phenotypic differences over time – a process known as allopatric speciation, which increases the diversity of a region. Therefore, Amazonia is an excellent model to study speciation since it has been suggested that its high biodiversity is largely due to higher diversification rates (Hoorn *et al.*, 2010; Antonelli & Sanmartin, 2011). Exploring the influence of present-day and past abiotic factors on the genetic composition of Amazonian species across space can provide insights into the geological events and demographic processes that drive lineages divergence.

Some distribution patterns are repeatedly recognized for different species across the Amazon Basin and turn out to be indicative of major diversification processes that impacted multiple taxa in the past (Cracraft *et al.*, 2020). Since it is expected that different species accumulate similar geographic genetic signatures in response to a common biogeographic history (Avice *et al.*, 1987), molecular analysis of co-occurring populations can reveal strong correlation with the timing of important geological events (Prates *et al.*, 2016; Baranzelli *et al.*, 2020). Particularly the Amazonian eastern-western pattern of distribution has been recovered for several species – between populations (Vallinoto *et al.*, 2010) and/or sister-clades (Geurgas & Rodrigues, 2010; Miralles & Carranza, 2010; Nunes *et al.*, 2012; Marques-Souza *et al.*, 2022) being most likely related to a wide scale diversification process in Amazonia.

Some primary hypotheses are particularly notable in explaining diversification by allopatric speciation of terrestrial Amazonian species such as: (i) the Pebas System (Wesselingh *et al.* 2010, Hoorn *et al.* 2022), (ii) Riverine barriers (Wallace 1852, Ribas 2012), and (iii) the Plio-Pleistocene climatic variations (Haffer, 1969, Hoorn *et al.*, 2017). All these non-mutually exclusive hypotheses will be explored below, where I present a brief review of Amazonian geological history.

### *Mechanisms proposed to explain origins of diversity in the Amazon rainforest*

During the Neogene period, spanning from about 23 mya to 3 mya, the uplift of the Andes Mountain range led to significant geomorphological modifications in the Neotropics (e.g. Garzzone *et al.* 2008, Mora *et al.* 2010). This long-lasting event caused major changes in the drainage patterns of the Amazon basin. The first stage of Andean uplift took place on the late Oligocene – early Miocene (c.a. 24 mya) and led to the drowning of the Andes foreland basin (Caputo & Soares, 2016) causing a series of marine incursions into the north portion of the Amazon basin, which later led to the formation of the Pebas Lake (Roddaz *et al.* 2010, Hoorn *et al.* 2022). The Pebas Lake was a wetland system that dominated the western Amazon during most of the Miocene (approximately from 23 to 10 mya) and isolated vast tracts of land, particularly the western portion of northern South America (Hoorn, 2010), with alternating periods of fresh and brackish water (Leandro *et al.* 2022). This massive wetland was an important speciation promoter by both facilitating expansion of aquatic taxa (Salas-Gismond, 2015; Wesselingh, 2006) and acting as a physical barrier for land species (Tejada-Lara *et al.*, 2015).

Millions of years later, during the late Miocene (15-8 mya), the increasing elevation of the Andes Mountain range caused the drainage of the Pebas system to the eastern portion of the Amazonia (Hoorn *et al.* 2022). This process changed the water course of the Amazon Basin from northward to eastward and formed the proto-Amazon River and its tributaries (Latrubesse *et al.* 2010; Sioli, 1968). These rivers fragmented the land and created distinct habitats that separated species populations. According to the Riverine barrier hypothesis, rivers served as physical barriers that prevent genetic flow between populations, leading to reproductive isolation and ultimately speciation. This is often used to explain patterns of

species distribution and speciation in regions with complex river systems and high biodiversity, like the Amazon rainforest (Cronemberger *et al.*, 2022; Ribas *et al.*, 2018; Hall & Harvey, 2002).

More recently, in the late Pliocene and Pleistocene period (5 million years ago to 11,700 years ago), Earth experienced glaciation events and other climatic shifts. While in the Neotropics glaciation was less severe than in temperate regions, in Amazonia the Plio-Pleistocene period was marked by periods of drier climate that likely caused forest retractions and change in the predominant vegetation (Hoorn *et al.*, 2017) creating a diverse array of habitats within the Amazon rainforest. This habitat heterogeneity provided isolation of species lineages and promoted diversification (Fuchs *et al.*, 2011; Cronemberger *et al.*, 2022)

All these hypotheses have traditionally been used to explain diversification processes in the Amazonia and could potentially explain east-west disjunct patterns. In this study I compare the phylogeography of two lizard species with disjunct and overlapping distribution in the eastern and western Amazonia: *Anolis trachyderma* and *Potamites eupleopus*.

#### *Distribution and ecology of Anolis trachyderma and Potamites eupleopus*

Both species are strongly associated with rainforest habitats (Ávila-Pires, 1995). *Anolis trachyderma* (Cope, 1875) is a semi-arboreal diurnal species from the speciose Dactyloidae family that can be found mainly on leaf litter and occasionally on tree trunks (Vitt, 2002), where it feeds on spiders (Ávila-Pires, 1995). Its main habitat is pristine shaded forests, avoiding clearings and being often found close to streams (Vitt, 2002). It occurs in Eastern Amazonia albeit with a disjunct pattern with populations in Colombia, Ecuador, Peru and western Brazil (Ávila-Pires, 1995; Ribeiro-Junior, 2015).

*Potamites eupleopus* (Cope, 1875) belongs to the Gymnophthalmidae family and has a similar disjunct distribution pattern (Ávila-Pires, 1995; Ribeiro-Junior & Amaral, 2017). It is strongly associated with aquatic environments such as wetlands, streams, and puddles, to which it actively flees when threatened (Arteaga & Quezada, 2023). It can be found under logs, on leaf litter or on streamside vegetation on well preserved forests. It

widely occurs from the Andes ranges of Colombia, Ecuador, Peru and Bolivia to Eastern Amazonia, also having separate populations in western Brazilian Amazonia (local) (Quezada & Arteaga, 2023).

By having similarities such as being small ectotherms with small home ranges, while having relevant differences in their natural history, these two lizard species are good study models to understand large-scale processes that promote diversification. Furthermore, by belonging to two different lizard groups we are able to understand independently how evolutionary history may lead to present-day overlapping but disjunct patterns.

## 2. Objectives

- To test if the shared distribution patterns of *A. trachyderma* and *P. ecleopus* reflects the same biogeographic history;
- To understand the demographic process that led populations to an eastern-western isolated distribution;
- To generate new data to understand the mechanisms behind the diversification in the Amazon Forest.

## 3. Materials and methods

### 2.1 Molecular sampling

All DNA was extracted from specimens previously collected and stored in the Laboratório de Herpetologia of Instituto de Biociências of Universidade de São Paulo. Tissue samples (muscle or liver) preserved in 100% ethanol were sequenced for four loci, two mitochondrial and two nuclear (see below), using Sanger sequencing method. I obtained molecular data from 65 specimens of *Anolis trachyderma* and 38 specimens of *Potamites ecleopus*. For the *Anolis trachyderma* specimens I sequenced from the mitochondrial genome a 542-bp fragment of NADH dehydrogenase subunit 2 gene (ND2) and a fragment of the 12S gene. From the nuclear genome, I sequenced a 435-bp fragment of the KIAA-2018 (KIAA) like protein gene and a 461-bp fragment of the

megakaryoblastic leukemia 1 (MKL1). From the mitochondrial genome of *P. ecpleopus* specimens we sequenced a 761-base pair (bp) fragment of the NADH dehydrogenase subunit 4 gene (ND4) and a 332-bp fragment of the small subunit of ribosomal RNA 12S gene (12S). From the nuclear genome of *P. ecpleopus* we sequenced a 391-bp fragment of oocyte maturation factor mos (c-mos) gene and a 985-bp fragment of recombination activating protein 1 (RAG1). Primers are listed in Table 1.

After amplification, PCR products were purified with Exonuclease I and Alkaline Phosphatase (ExoSAP protocol). Sequencing was performed using the BigDye Terminator 3.1 Cycle Sequencing kit (Applied Biosystems), followed by analysis in ABI Prism 310, 3700 or 3170 Genetic Analyzer Sequencers (Applied Biosystems) at the Instituto de Química, Universidade de São Paulo (IQUSP, São Paulo, Brazil). DNA sequences were combined, edited, aligned and concatenated on Geneious Prime 2022.1.1 software. Sequences were verified for contamination using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), and aligned with the Geneious algorithm using default configurations.

I additionally included available samples from Genebank for the outgroup species. Into the *A. trachyderma* alignments, I implemented sequences of *Anolis carolinensis*, *Anolis ortonii* and *Anolis fuscoauratus* species of all mitochondrial and nuclear markers mentioned above, and only sequences of ND2, KIAA and MKL1 of *Anolis tandai* samples. For *P. ecpleopus* alignments, I implemented sequences of *Loxopholis* and *Neusticurus bicarinatus* species of all mitochondrial and nuclear markers mentioned (12S, ND4, KIAA and RAG1).

**Table 1.** Primers used on sequencing with respective reference authors.

Gene	Type	Primer	Primer sequence (5' -> 3')	Reference
12S	mitochondrial	12SA	CTG GGA TTA GAT ACC CCA CTA	Harris <i>et al.</i> , 1998
		12SB	TGA GGA GGG TGA CGG GCG GT	
ND4	mitochondrial	ND4-R-KL (LEU)	CAT TAG TTT TAC TTG GAT TTG CAC CA	Arévalo <i>et al.</i> , 1994
		ND4-F	CAC CTA TGA CTA CCA AAA GCT CAT	
c-mos	nuclear	G73	GCCGGTAAAGCAGGTGAAGAAA	Saint <i>et al.</i> , 1998
		G74	TGAGCATCCAAAGTCTCCAATC	
RAG1	nuclear	RAG1-AnF1	GAA ATT CAA GCT CTT CAA AGT	Wiens <i>et al.</i> ,

			GAG AT	2008
		RAG1-AnR1	TGT CAA KGA AAG TAA GTG TTG TCT TG	
ND2	mitochondrial	LVT 5617R	CAT TAG TTT TAC TTG GAT TTG CAC CA	Macey <i>et al.</i> , 1997
		LVT MetF	CAC CTA TGA CTA CCA AAA GCT CAT	
KIAA	nuclear	KIAA2018 R1	YTG CCC AGC CAT TTG TGA TAT GCT YTG A	Portik <i>et al.</i> , 2012
		KIAA2018 F1	RCC CAT CCY TAC CTA TGC AGC CAT TA	
MKL1	nuclear	MKL1-f1	GTG GCA GAG CTG AAG CAR GAR CTG	Townsend <i>et al.</i> , 2008
		MKL1-r2	AA GCR CTC TKR TTG GTC ACR GTG AGG	

### 2.3 Phylogenetic reconstruction and divergence time estimation

To test the synchronous eastern-western populational-level diversification between species and which hypothesis of diversification best fits the disjunction of *P. eupleopus* and *A. trachyderma* populations, I performed a phylogenetic reconstruction and estimated divergence the time between east and west populations.

Phylogenetic inference was based on multiple loci, and therefore, I performed a Bayesian phylogenetic reconstruction and a divergence time estimation analysis simultaneously on Beast v2.6.4 (Bouckaert *et al.*, 2014), using a single concatenated alignment. I used a birth-death process tree prior and an uncorrelated lognormal relaxed clock for each gene separately, as we were analyzing population-level divergence. DNA substitution models and the best partition scheme for the Bayesian analysis were inferred using JModelTest 2.1.10 under Bayesian information criterion (BIC). I calibrated the trees with known divergence times of outgroups from previous studies (see table 2) and assumed a normal distribution for these priors.

**Table 2.** Prior used to calibrate tree on Beast. Outgroups and respective nodes location on tree.

Outgroups	Calibrated node	Median	Prior sigma	Prior offset	Reference
<i>Anolis carolinensis</i>	outgroup of Anolis tree	35.0	3.5	3.0	Prates <i>et al.</i> , 2015
<i>Anolis ortonii</i>	MRCA of <i>A. trachyderma</i> specimens and <i>A. fuscoauratus</i>	12.0	1.0	0.5	
<i>Loxopholis sp.</i>	outgroup of Potamites tree	60.0	1.8	2.0	Recoder <i>et al.</i> , 2020
<i>Neusticurus bicarinatus</i>	MRCA of <i>P. eupleopus</i> specimens	46.0	2.5	2.0	

For each species analysis I performed 2 independent runs starting with different random seeds. Each run consisted of 100 million generations sampled every 10 thousand generations. I used TRACER to discard trees sampled before the stability of the log-likelihood values (burn-in of first 20% trees) and to assess proper Markov chain mixing, model parameter stationarity and convergence between runs. All sampled trees that remained were used to estimate branch length, tree topology and values of posterior probability (*pp*) and summarized on TreeAnnotator.

## 2.2 Networks, lineages delimitation and summary statistics

I estimated independently evolving lineages using two algorithms for the molecular species delimitation: *Generalized Mixed Yule-Coalescent* (GMYC; Pons *et al.*, 2006) and *Assemble Species by Automatic Partitioning* (ASAP; Puillandre *et al.*, 2021). GMYC algorithm operates under the assumption that the coalescence rate within a species is significantly higher than the rate at which species diverge. In this method, species assignment was based on the tree topology inferred with BEAST. The ASAP, on the other hand, is the implementation of a hierarchical cluster algorithm based on a multi-step pipeline. In this method, species assignment is based on pairwise genetic distances of a single locus and ranked by their ASAP-score: the lower the score, the better the partition. The GMYC approach was carried out in R version 4.3.0 using packages splits (Species Limits by Threshold Statistics; Ezard *et al.*, 2009) and ape (Analyses of Phylogenetics and Evolution; Paradis *et al.*, 2004) under the “single threshold” model. The ASAP analysis was conducted in the online version (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with default settings.

To visualize genealogical relationships between populations I used Popart 1.7 (Leigh & Bryant, 2015) and determined the number of unique haplotypes, networks and the distribution of shared haplotypes based on a median-joining distance method.

To calculate population genetic and molecular evolutionary statistics I used DNAsp 0.6 software (Rozas *et al.*, 2003). I summarized the genetic diversity of each gene and each species pool (east and west) with mtDNA concatenated datasets and got statistic data for



the following parameters: number of polymorphic sites (S), average number of pairwise nucleotide differences (k), nucleotide diversity ( $P_i$ ), number of haplotypes (h), haplotype diversity (Hd), Watterson estimator per sequence ( $\theta_w$ ) and Fu & Li's intraspecific neutrality test. Using the concatenated mtDNA datasets of each species I also measured population genetic structures and genetic differentiation between east and west populations.

#### 2.4 Testing alternative demographic models of divergence between east-west populations

After getting access to the spatial and temporal histories of each species, I tested which speciation process most likely explains the isolation of the eastern and western populations. To perform this test I conducted an Approximate Bayesian Computation (ABC) with coalescent simulations in DIYABC 2.1 software (Cornuet *et al.*, 2014). The ABC is a statistical method that allows for the estimation of population parameters and inference of demographic histories based on genetic data. This method compares our observed genetic data with simulated data generated using a coalescent framework and to capture the essential patterns of genetic variation, both the observed genetic sequences and the simulated sequences are summarized into informative genetic statistics (Beaumont, 2010).

I tested the isolation process of eastern and western populations under three possible demographic scenarios: (i) vicariance model: an ancestral population with widely distributed across Amazonia, followed by segregation of east and west populations; (ii) east dispersal: ancestral population with restricted distribution on east Amazonia, followed by a dispersal

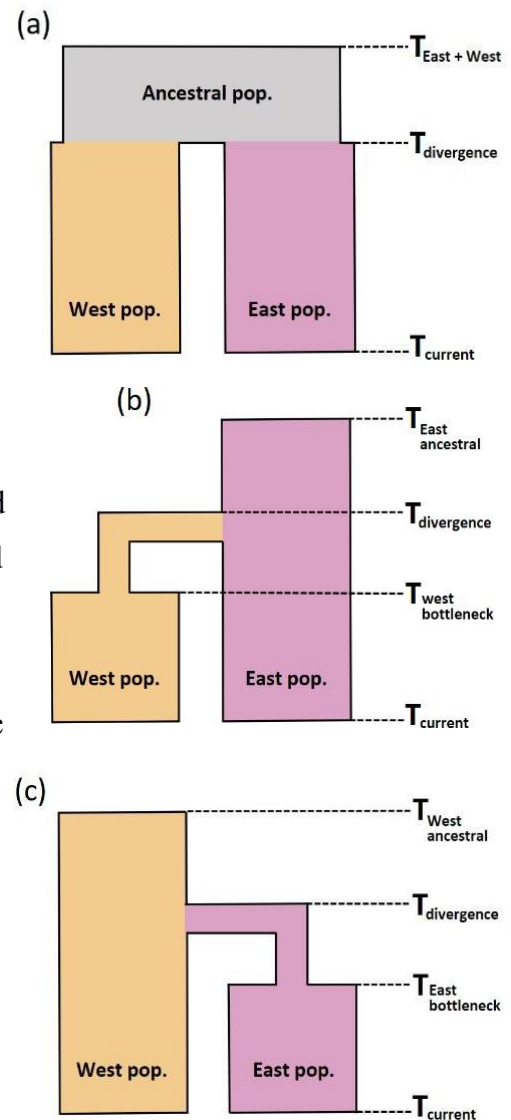


Fig 1. Demographic scenarios tested using coalescent simulations and Approximate Bayesian Computation (ABC). (a) vicariance. (b) east dispersal. (c) west dispersal.



event to the west; and (iii) west dispersal: ancestral population with restricted distribution on west Amazonia followed by a dispersal event to the east (see figure 1).

In both species, simulations for each scenario were performed considering a generation time of one year and HKY substitution model. I considered the following priors for model parameters of population sizes for both species: actual effective populations sizes in each region ~uniform [0.01, 5] million; effective populations sizes during bottleneck ~ uniform [0.001, 0.1] million; and effective population size of ancestral population prior vicariance ~ uniform  $2 \times [0.01, 5]$  million; keeping the condition size bottleneck < actual size. As for the time priors, I considered for *A. trachyderma*: east and west populations divergence time estimated on BEAST ~ uniform [2.5, 8.7] millions years, bottleneck/expansion time ~ uniform [0, 4.0] millions years and ancestral population time [2.5, 13.0] millions of years; and for *P. ecpleopus*: east and west populations divergence time also estimated on BEAST ~ uniform [5.0, 25.0] millions years, bottleneck/expansion time ~ uniform [0, 15.0] millions years and ancestral population time [15.0, 40.0] millions of years. Keeping the condition bottleneck time < divergence time for both species.

For each species, I selected the most representative mitochondrial fragment to perform the analysis: ND2 for the *A. trachyderma* and ND4 for the *P. ecpleopus*. I conducted 3 million coalescent simulations (1 million for each of the tree scenarios) for each species independently. The following summary statistics were selected for analysis: number of segregating sites, mean pairwise differences of all and between populations, variance of pairwise distances of all and between populations and Tajima's D (Tajima, 1989) and Fst index of differentiation.

## 4. Results

### 3.1 Molecular data

For *A. trachyderma* I sequenced a total of 65 specimens. All the genetic data composed a concatenated matrix of a total 2,147bp (12S = 374bp, ND2 = 663bp, KIAA = 526bp, MKL1 = 584) and 68 specimens including outgroups (12S = 62 successfully sequenced samples, ND2 = 60, KIAA = 60, MKL1 = 29). For *P. ecpleopus* we sequenced a total of 38 specimens. All the genetic data composed a concatenated matrix 2,470bp (12S =

420bp, ND4 = 619bp, c-mos = 400bp, RAG1 = 1,049bp) and 40 specimens including outgroups (12S = 36 successfully sequenced samples, ND4 = 35, c-mos = 37, RAG1 = 23). I used specimens of *A. trachyderma* from 5 distinct localities: Altér do Chão, Pará (3 specimens); Altamira, Pará (1 specimen); Vitória do Xingu, Pará (20 specimens); Rio Içá, Amazonas (11 specimens) and Serra do Divisor, Acre (29 specimens). For *P. eupleopus* we used specimens from 8 distinct localities: Canaã dos Carajás, Pará (2 specimens); Vitória do Xingu, Pará (11 specimens); Altamira (4 specimens); Guarantã do Norte, Mato Grosso (6 specimens); Cusco, Peru (8 specimens); Japurá/Maraã, Amazonas (3 specimens); Serra do Divisor, Acre (3 specimens) and Rio Içá, Amazonas (1 specimen). See Table 3 for further information.

**Table 3.** Sample information

Species	Specimen ID	Locality	12S	ND2	KIAA	MKL1
<i>Anolis trachyderma</i>	916873	Altér do Chão, Pará	x		x	
<i>Anolis trachyderma</i>	916878	Altér do Chão, Pará	x	x		x
<i>Anolis trachyderma</i>	916875	Altér do Chão, Pará	x		x	
<i>Anolis trachyderma</i>	MTR11082	Altamira, Pará	x		x	
<i>Anolis trachyderma</i>	BM 010	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 014	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 136	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 161	Vitória do Xingu, Pará	x	x		x
<i>Anolis trachyderma</i>	BM 186	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 189	Vitória do Xingu, Pará		x	x	
<i>Anolis trachyderma</i>	BM 276	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 279	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 280	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 293	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 364	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 371	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 376	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 419	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 428	Vitória do Xingu, Pará	x	x	x	x
<i>Anolis trachyderma</i>	BM 574	Vitória do Xingu, Pará	x		x	
<i>Anolis trachyderma</i>	BM 583	Vitória do Xingu, Pará	x	x	x	x

<i>Anolis trachyderma</i>	BM 585	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	BM 640	Vitória do Xingu, Pará		x	x	x
<i>Anolis trachyderma</i>	BM 643	Vitória do Xingu, Pará	x	x	x	
<i>Anolis trachyderma</i>	MTR 36063	Rio Içá, Amazonas	x	x		
<i>Anolis trachyderma</i>	MTR 36204	Rio Içá, Amazonas	x	x		
<i>Anolis trachyderma</i>	MTR 36419	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36463	Rio Içá, Amazonas	x	x	x	x
<i>Anolis trachyderma</i>	MTR 36523	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36524	Rio Içá, Amazonas		x	x	
<i>Anolis trachyderma</i>	MTR 36654	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36657	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36692	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36717	Rio Içá, Amazonas	x	x	x	
<i>Anolis trachyderma</i>	MTR 36745	Rio Içá, Amazonas	x	x	x	x
<i>Anolis trachyderma</i>	MBS 003	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 005	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 006	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 009	Serra do Divisor, Acre	x		x	
<i>Anolis trachyderma</i>	MBS 010	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 015	Serra do Divisor, Acre		x	x	
<i>Anolis trachyderma</i>	MBS 018	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 021	Serra do Divisor, Acre	x	x		
<i>Anolis trachyderma</i>	MBS 023	Serra do Divisor, Acre	x			
<i>Anolis trachyderma</i>	MBS 027	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 038	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MBS 040	Serra do Divisor, Acre	x	x		
<i>Anolis trachyderma</i>	MTR 28049	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MTR 28203	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28261	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28262	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28265	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	MTR 28268	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28270	Serra do Divisor, Acre	x		x	
<i>Anolis trachyderma</i>	MTR 28294	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28318	Serra do Divisor, Acre	x	x	x	x
<i>Anolis trachyderma</i>	MTR 28397	Serra do Divisor, Acre	x	x		x
<i>Anolis trachyderma</i>	MTR 28415	Serra do Divisor, Acre		x	x	x
<i>Anolis trachyderma</i>	MTR 28417	Serra do Divisor, Acre	x	x		x

<i>Anolis trachyderma</i>	PRMS 731	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	PRMS 772	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	PRMS 773	Serra do Divisor, Acre	x		x	
<i>Anolis trachyderma</i>	PRMS 804	Serra do Divisor, Acre	x	x	x	
<i>Anolis trachyderma</i>	PRMS 832	Serra do Divisor, Acre	x	x	x	

Species	Specimen ID	Locality	12 S	ND 4	c- mos	RAG 1
Potamites ecleopus	107	Canaã dos Carajás, PA	x	x	x	x
Potamites ecleopus	109	Canaã dos Carajás, PA	x	x	x	
Potamites ecleopus	BM027	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM267	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM284	Vitória do Xingu, PA	x	x	x	
Potamites ecleopus	BM212	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM636	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM362	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM095	Vitória do Xingu, PA	x	x	x	
Potamites ecleopus	BM154	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM269	Vitória do Xingu, PA	x	x	x	x
Potamites ecleopus	BM281	Vitória do Xingu, PA	x		x	x
Potamites ecleopus	BM634	Vitória do Xingu, PA	x	x	x	
Potamites ecleopus	BM600	Altamira, PA	x	x	x	
Potamites ecleopus	BM612	Altamira, PA	x	x	x	x
Potamites ecleopus	BM621	Altamira, PA	x	x		
Potamites ecleopus	BM837	Altamira, PA			x	x
Potamites ecleopus	TM369	Guarantã do Norte, MT	x	x	x	
Potamites ecleopus	TM523	Guarantã do Norte, MT	x	x	x	x
Potamites ecleopus	TM413	Guarantã do Norte, MT	x	x	x	x
Potamites ecleopus	TM370	Guarantã do Norte, MT	x	x	x	x
Potamites ecleopus	TM525	Guarantã do Norte, MT	x	x	x	
Potamites ecleopus	TM524	Guarantã do Norte, MT	x	x	x	
Potamites ecleopus	MYPAlA50	Peru	x		x	
Potamites ecleopus	COGA052	Peru	x		x	
Potamites ecleopus	CORBIDI8331	Peru	x	x	x	
Potamites ecleopus	CORBIDI9775	Peru	x	x	x	x
Potamites ecleopus	CORBIDI9753	Peru	x	x	x	x
Potamites ecleopus	CORBIDI9786	Peru	x	x	x	x
Potamites ecleopus	CORBIDI1000	Peru	x	x	x	
Potamites ecleopus	5					
Potamites ecleopus	CORBIDI9758	Peru				
Potamites ecleopus	MTR33544	Maraã, AM		x	x	x

Potamites ecleopus	MTR33636	Japurá, AM	X	X	X	
Potamites ecleopus	MTR33707	Japurá, AM	X	X	X	
Potamites ecleopus	MBS007	Serra do Divisor, AC	X	X	X	
Potamites ecleopus	MTR28055	Serra do Divisor, AC	X	X	X	X
Potamites ecleopus	MTR28132	Serra do Divisor, AC	X	X	X	X
Potamites ecleopus	MTR36187	Rio Içá, AM	X	X	X	X

Information of sequenced mtDNA and nuDNA fragments for each specimen.

### 3.1 Phylogenetic reconstruction, lineages delimitation and divergence time estimation

#### 3.1.1 *Anolis trachyderma*

Phylogenetic reconstruction of specimens (see figure 2) recovered highly supported monophyletic clades of east and west populations (pp = 1), with divergence time between the eastern and western populations estimated for Late Pliocene (average 5,5 mya, 95% credibility interval = 2.541 – 8.695 mya). The analysis of model selection for the mitochondrial fragments selected the GTR+I substitution model and for the nuclear fragments the HKY substitution model. The eastern clade includes two sub populations that

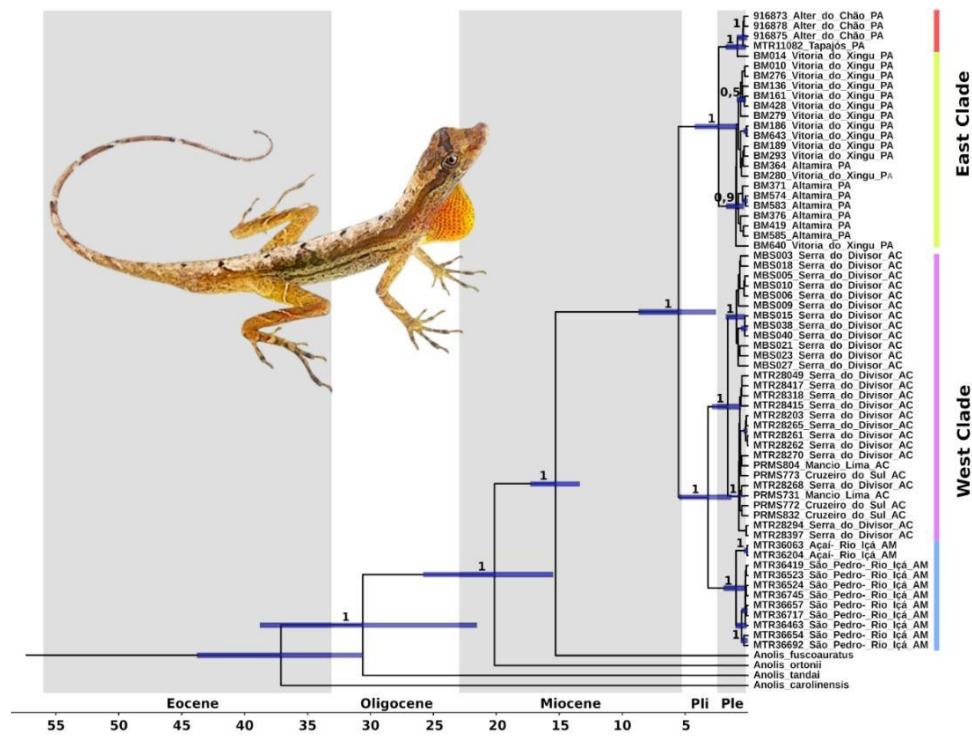


Figure 2. Phylogenetic reconstruction of *Anolis trachyderma*. Posterior probabilities indicated on nodes and 95% confidence interval on purple bars. Photo by 2023 Reptiles of Ecuador.

were recovered with high posterior probability: one with samples from Alter do Chão and the other from Vitória do Xingu and Altamira. The western clade also has two sub populations recovered: one from Serra do Divisor and another from Rio Içá.

The GMYC algorithm identified 5 putative independently evolving lineages within the *A. trachyderma* species. Specimens from western Amazonia were recovered in three different lineages, of which two restricted to Serra do Divisor and one that encompasses samples from Serra do Divisor and São Pedro. Specimens from eastern Amazonia were recovered in two lineages both restricted to Pará, but geographically distinct: one from Vitória do Xingu and Altamira and the other one from Alter do Chão. The best scored partition from ASAP algorithm identified 3 clusters within the *A. trachyderma* species: one composed with samples from eastern Amazonia (Alter do Chão, Vitória do Xingu and Altamira) and two with samples from western Amazonia being one restricted to Serra do Divisor and the other one restricted to São Pedro.

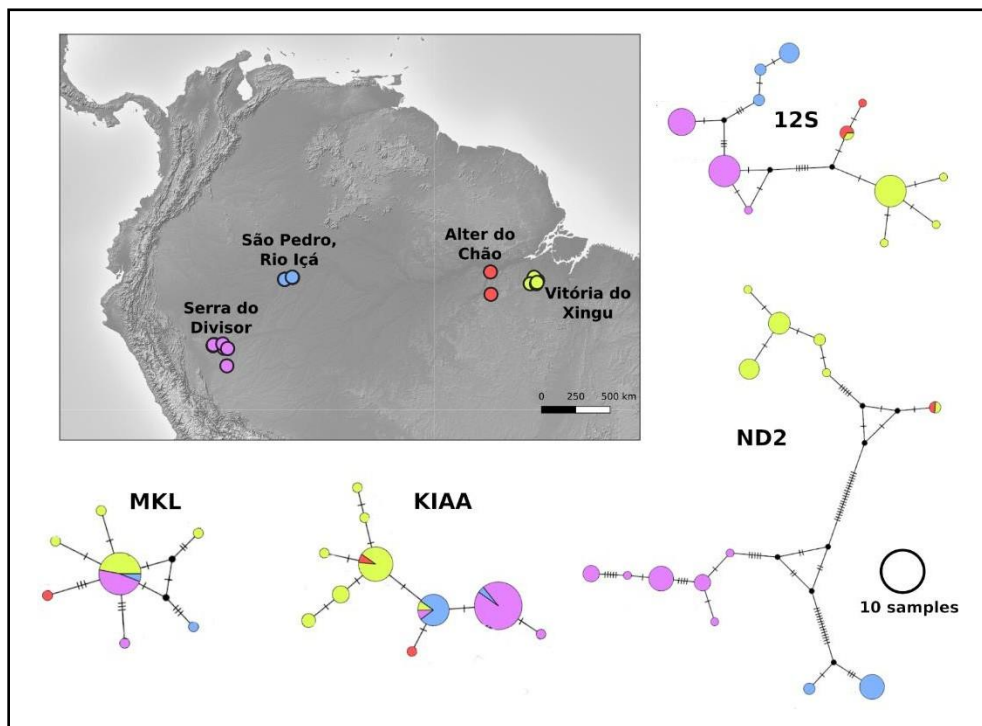


Fig 3. Haplotypes networks of *Anolis trachyderma* and map with respective localities.

Mitochondrial haplotype networks showed separated haplotypes of east and west samples (see figure 3) with ND2 haplotypes separated by more mutational steps than 12S. Both ND2 and 12S networks shows one shared haplotype between Alter do Chão and



Vitória do Xingu groups which may indicate some genetic flow in that region in Pará. As expected, nuclear haplotype networks show less geographical structure, with most haplotypes shared between groups. KIAA network is more structured than MKL probably because of the low number of specimens sequenced for MKL.

### 3.1.2 *Potamites ecleopus*

Phylogenetic reconstruction (see figure 4) recovered two highly supported clades (pp > 0.9): (i) one comprised of specimens from eastern Amazonia (Canaã dos Carajás, Vitória do Xingu, Altamira, and Guarantã do Norte; pp = 1) and (ii) the other comprised of specimens from western Amazonia (Peru and Marañ/Japurá; pp = 0.95). A third clade (iii) was recovered, comprised of specimens also from western Amazonia, but with lower support (Peru, Serra do Divisor and Rio Içá; pp = 0.74). The high genetic distances between (i) + (ii) and (iii) (indicated by the long branches length), suggests clade (iii) to be a distinct species and because of that it was excluded from the divergence time estimation between the eastern and western regions. Time of divergence between eastern and western Amazonia, therefore between clades (i) and (ii), was estimated for the Miocene

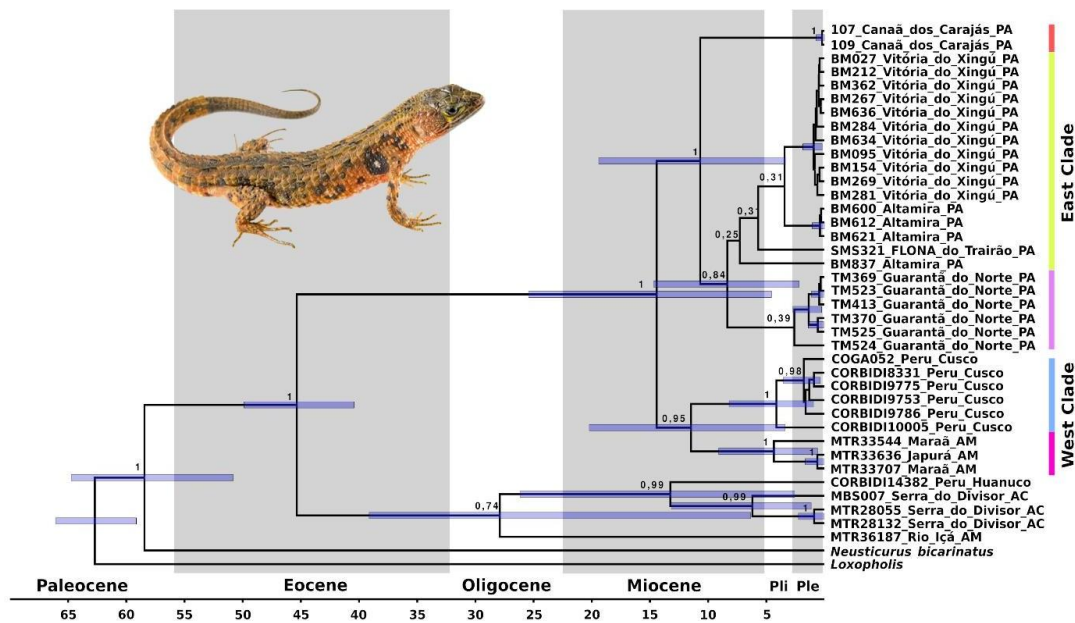


Figure 4. Phylogenetic reconstruction of *Potamites ecleopus*. Posterior probabilities indicated on nodes and 95% confidence interval on purple bars. Photo by 2023 Reptiles of Ecuador.

(approximately 15 mya). Our model selection for the mitochondrial fragments selected the GTR+I substitution model and for the nuclear fragments the HKY substitution model.

As explained above, because they were recovered in a very distinct clade, separated with great support from both eastern and western populations of *P. ecleopus*, the following samples, all from western Amazonia, were disregarded all the following analyses of computation of genetic statistics, species delimitation, network haplotype and demographic model selection: CORBIDI 14382, MBS007, MTR28055, MTR28132 and MTR36187.

The GMYC algorithm identified 11 putative independently evolving lineages within the *P. ecleopus* species. Specimens from western Amazonia were recovered in seven different lineages: two from Garantã do Norte, three from Altamira, one from Vitória do Xingu and one from Canaã dos Carajás. Specimens from eastern Amazonia were recovered in four different lineages: two from Maraã/Japurá and two from Peru. The best scored partition from ASAP algorithm identified 6 subsets within the *P. ecleopus* species geographically restricted: Canaã dos Carajás, Peru, Maraã/Japurá, Garantã do Norte, Altamira and Vitória do Xingu.

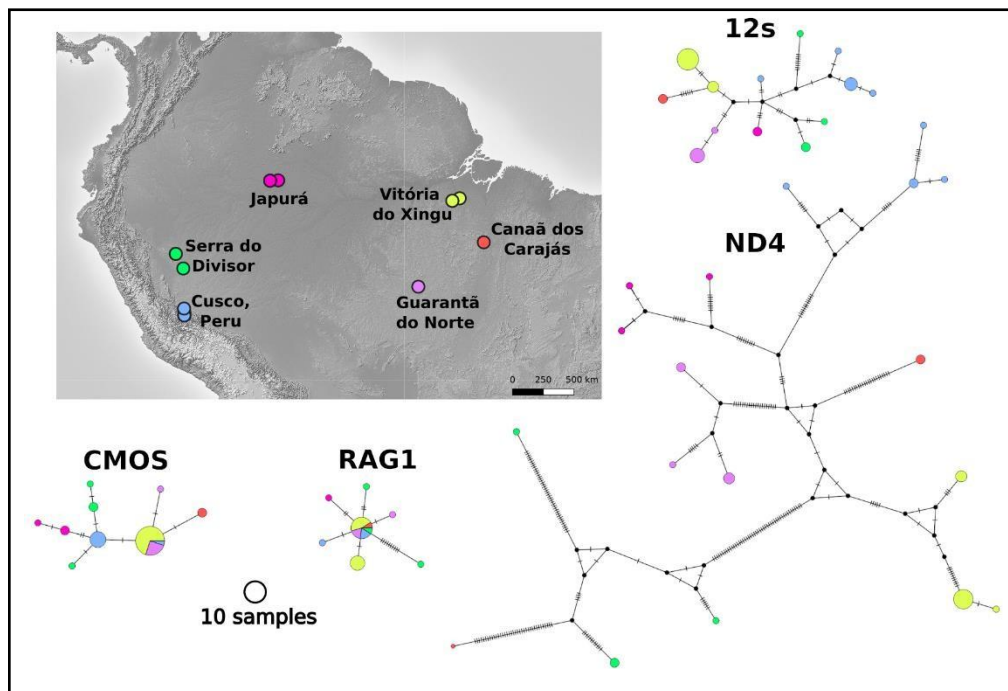


Fig 5. Haplotypes networks of *Potamites ecleopus* and map with respective localities.



Mitochondrial haplotypes networks (see figure 5) show some geographical structure with no shared haplotypes between groups. Both ND4 and 12S networks indicate high genetic distance between localities. The ND4 network has 14 unique haplotypes (29 samples) that are separated by a more mutational step than 12S network. Nuclear haplotypes networks in contrast show no structure and significantly lower number of haplotypes.

### 3.2 Genetic differentiation between populations

For each species, I separated the samples into two populations, based on the clades recovered in the phylogenetic reconstruction (see figures 2 and 4). We considered the western population of *A. trachyderma* specimens from Serra do Divisor and from Rio Içá, and east population specimens from Alter do Chão, Altamira and Vitória do Xingu. For *P. ecpleopus* we considered as western population specimens from Peru, Maraã/Japurá, Serra do Divisor and Rio Içá, and eastern population specimens from Canaã dos Carajás, Vitória do Xingu, Altamira and Garantã do Norte. See table 3 to access summary statistics results.

In relation to the genetic statistics, I detected significant differences when comparing eastern and western populations of both species. The haplotypic diversity (Hd) of mitDNA combined of both *A. trachyderma* and *P. ecpleopus* eastern populations was significantly lower than the Hd of western populations (Table 4). However, the values of Waterson's theta, a measure of genetic diversity which considers the number of segregating sites and the effective population size, did not differ from eastern and western populations of *P. ecpleopus* (21.1 and 22.0 respectively), but was significantly different between eastern and western populations of *A. trachyderma* (4.1 and 10.2 respectively). Similarly, the nucleotide diversity per site, or Pi, which measures the average number of nucleotide differences between two randomly chosen individuals in the population, also followed the same pattern: For *P. ecpleopus* it shows similar values for the eastern and western (2.8 and 2.5 respectively), but highly dissimilar values between the eastern and western populations of *A. trachyderma* (0.3 and 1.8 respectively – Table 3).

**Table 4.** Summary genetic statistics. Bp: base-pair; N: sample number; h: number of haplotype; Hd: haplotype diversity;  $\theta_w$ : Watterson estimator per sequence; Pi(%): percent of nucleotide diversity; k: average number of nucleotide differences; S: number of polymorphic sites; Fu & Li's F\*: Fu and Li's intraspecific neutrality test.

Gene	bp	N	h/Hd	$\theta_w$	Pi(%) / k	S	Fu & Li's F*
<i>Anolis trachyderma</i>							
12S	373	59	12/0.833	3.87	1.454/5.41	18	0.1563
ND2	528	50	12/0.862	11.61	4.242/22.39	50	2.716
KIAA2018 (phased)	435	110	13/0.769	2.086	0.354/1.53	11	0.2853
MKL1 (phased)	301	46	14/0.700	4.551	14/0.706	20	-0.4776
<i>Potamites ecpleopus</i>							
12S	319	31	11/0.845	6.258	1.944/6.183	25	0.28
ND4	582	29	14/0.889	26.737	4.896/28.498	105	0.4385
c-mos (phased)	391	64	6/0.573	1.269	0.255/0.998	6	0.7236
RAG1 (phased)	951	36	5/0.622	1.206	0.089/0.844	5	0.6423
mitDNA combined ( <i>A. trachyderma</i> )	901	46	16/0.900	15.7	3.086/27.77	69	2.0962
mitDNA combined ( <i>P. ecpleopus</i> )	901	28	14/0.884	31.608	3.808/34.27	123	0.7705
<hr/>							
mitDNA polymorphism groups	bp	N	h/Hd	$\theta_w$	Pi(%) / k	S	Fu & Li's F*
<i>Potamites ecpleopus</i>							
East	901	21	8/0.795	21.124	2.886/25.971	76	1.4214
West	901	7	6/0.952	22.041	2.569/23.143	54	0.5601
<i>Anolis trachyderma</i>							
East	901	17	7/0.662	4.141	0.320/2.882	14	-0.5463
West	901	29	9/0.857	10.185	1.873/16.857	40	2.2117

The total fixation index (Fst) for *A. trachyderma* and *P. ecpleopus* was respectively: 0,77380 and 0,48548 which suggests that *Anolis* has less genetic flow between east and west populations than *Potamites*.

### 3.3 Demographic models

Based on the coalescent simulations and the ABC analysis of DIYABC software, our best-fit demographic model for *Anolis trachyderma* was scenario 3, that represents a dispersal with founder event on eastern Amazonia, while for *Potamites ecpleopus* the best scenario was scenario 1, that represents a vicariance event (see Table 5).

**Table 5.** Posterior probabilities for three diversification scenarios. Best scenarios indicated with \*

Scenarios	Demographic pattern		Posterior probabilities	
	Western	Eastern	Direct	Logistic
<i>A. trachyderma</i>				
Scenario 1	Stable	Stable	0.3600	0.3659
Scenario 2	Bottleneck followed by expansion	Stable	0.1000	0.1281
Scenario 3*	Stable	Bottleneck followed by expansion	0.5400	0.506
<i>P. ecpleopus</i>				
Scenario 1*	Stable	Stable	0.5400	0.5083
Scenario 2	Bottleneck followed by expansion	Stable	0.3000	0.3336
Scenario 3	Stable	Bottleneck followed by expansion	0.1600	0.1582

Posterior parameters estimated of both species best-fit scenarios support Pliocene divergences time between eastern and western Amazonia (median value *A. trachyderma* = 5.22 and *P. ecpleopus* = 5.54), with 95% credibility intervals of 2.68 - 8.13 mya for *A. trachyderma* and 5.02 - 7.5 mya for *P. ecpleopus*. The *P. ecpleopus* dating, although not consistent with the average divergence time dated in our Bayesian phylogeny (= 15.5 mya), is compatible with the credibility interval (4.557 - 25.435 mya). See Table 6 to access posterior estimated values of the best-fit model parameters. Model validation based on principal component analyses of the summary statistics confirmed that the observed data of both species were contained within the space of simulated data (see Figure 1 of Supplementary material).

**Table 6.** Posterior estimates of time (T) and effective populations sizes ( $N_e$ ) of the best-fit scenario of DIYABC analyses for both species. Credibility intervals of 95% are indicated.

	Mean	Median	Mode	Lower 95% CI	Upper 95% CI
<i>A. trachyderma</i>					
T divergence	5.33	5.22	4.5	2.68	8.13
T east expansion	1.45	1.12	0.613	0.145	3.58
T west ancestral pop.	9.14	9.3	12.5	4.24	12.6
$N_e$ west pop.	2.09	1.93	1.48	0.598	4.09
$N_e$ east po.	1.88	1.61	0.947	0.265	4.27
Nbot east	0.0517	0.0522	0.0912	0.0036	0.0977
<i>P. ecpleopus</i>					
T divergence	5.82	5.54	5.09	5.02	7.5
T W+E ancestral pop.	27.8	28	35.6	15.7	38.8
$N_e$ west pop.	4.08	4.25	4.94	1.67	4.92
$N_e$ east po.	4.12	4.31	4.72	2.62	4.94

## 5. Discussion

Herein, we aimed to test if the shared distribution patterns of *A. trachyderma* and *P. ecpleopus* reflected the same biogeographic history, considering three non-mutually exclusive hypotheses. Our results show that the divergence between the eastern and western populations of both species occurred during the Pliocene, posterior to the Pebas lakesystem. Our demographic and genetic analysis indicate that this divergence resulted from two different processes: vicariance for *P. ecpleopus* and eastward dispersal for *A. trachyderma*, related to climatic oscillations and the subsequent drying of Central Amazonia. This likely reflects how differences in ecology and evolutionary history shaped the diversification of these two overlapping lizard species and helps us understand the mechanisms behind the diversification in the Amazon Forest.

### *Divergence time*

The results of molecular dating suggest a Pliocene divergence between eastern and western populations for both species. Although phylogenetic reconstruction using BEAST recovered an average middle Miocene divergence time for *P. ecpleopus* (15.5 mya), it had a

wide 95% confidence interval (CI), spanning from 4.55 to 25.43 mya, thus we considered the period that the BEAST reconstruction overlaps the narrower diyABC estimated time (95% CI = 5.02 – 7.5 mya) as the most likely divergence time.

Regarding the geological context during the estimated divergence time, there were two main drivers of Amazonian diversification: the climate-mediated landscape change influencing the distribution of forested/open ecosystems; and the fragmentation of these ecosystems mediated by drainage evolution and river channel dynamics.

During the period that preceded the Pliocene (late Miocene, around 10-6 mya), the Pebas system was almost fully drained by the Proto-Amazon basin, with major rivers of the Amazon Basin settled and connecting the Pebas lake system in western Amazonia to the Atlantic Ocean in the east coast (Hoorn, 2010; Haffer, 2008). That draining process caused the sedimentation of the Solimões formation in western Amazonia, which changed the soil and vegetation composition of that region (Hoorn *et al.*, 2017; Nogueira, 2013) to a floodplain forest, contrasting with the older and more stable cratonic formation in eastern Amazonia. Later, in the Pliocene (5.3-2.6 mya), the Amazonian rivers were established as we currently know, and the sedimentary wedge to the east promoted the expansion of *terra firme* environments (Caputo & Soares, 2016; Bicudo et al, 2019). Climate was starting to oscillate with large and sometimes abrupt changes in moisture that caused more intense drying climate in eastern Amazonia (Cheng, 2013; Wang, 2017) and consequent changes in the vegetation composition (Haggi, 2017; Arruda, 2018; van der Hammen, 2000). These environmental factors were important drivers in generating and structuring Amazonian fauna.

The subsequent differences in soil composition, climate, and hydrology between the eastern and western portions probably acted as barriers that promoted allopatric differentiation. Potentially both physical (river dynamics) and ecological barriers (climatic variation) could have shaped the disjunct eastern-western distributions observed today for both *A. trachyderma* and *P. eupleopus*. However, due to major Amazon river orientation being perpendicular to the Andes rather than parallel and that populations are currently distributed in a way that shows no evidence of strong physical barriers separating them, riverine barriers do not solely explain the divergence of eastern and western populations. The interruption of gene flow between these eastern and western populations was thus

likely caused by ecological barriers such as abrupt change associated with variations in the climatic conditions on the onset of the Pleistocene (Hoorn *et al.*, 2017). Therefore, the drier conditions that took place in northern South America during the Plio-Pleistocene led to the isolation of forested portions in eastern Amazonia, fragmenting the Amazon biome and forming a dry corridor (Wuster *et al.*, 2005; Hoorn *et al.*, 2017) connecting the open areas of northern South America (the Guiana Shield and current Venezuelan Llanos) and the Brazilian Shield (mainly the Cerrado savannas) that facilitated the expansion of open area specialists such as South American rattlesnakes (Wuster *et al.*, 2005; Quijada-Mascareñas *et al.*, 2007). This corridor likely posed a potential barrier for forest-dwelling Amazonian lizards such as *Anolis trachyderma* and *Potamites ecpleopus* and could be a major factor in the origin of their disjunct distribution.

*Same divergence times, different evolutionary processes*

Although our dating analysis recovered the same period of divergence time for both species, the demographic model test selected different scenarios of allopatric diversification for *A. trachyderma* and *P. ecpleopus*. This indicates that, even if we assume that the same barrier established both distribution patterns, each species had a different geographic and populational history.

According to our results, *P. ecpleopus* had a widespread ancestral population in the Amazon Basin that was posteriorly separated into the current eastern-western distribution. That vicariant process was also recovered in the patterns of genetic variation between and within populations. We found similar values of genetic diversity within eastern and western clades of *P. ecpleopus*, which is congruent with a demographic history of constant effective population sizes over time. Additionally, *P. ecpleopus* had an overall higher index of DNA polymorphism when compared to *A. trachyderma*, suggesting an older demographic history in the region. This reinforces that before the isolation of the current eastern and western populations *P. ecpleopus* had a widespread distribution across the Amazon Basin with continuous gene flow from west to east. For *A. trachyderma*, our results showed that the ancestral population was originally distributed in the western region and that there was a populational bottleneck constraining the effective number of

individuals that dispersed to the eastern Amazonia. This scenario was also supported by the results of genetic diversity between eastern and western *A. trachyderma* clades, with a notably higher index of DNA polymorphism in the western population. Also, the higher  $F_{st}$  index of *A. trachyderma* indicates a longer period without genetic flow between populations which is most likely due to the fortuitous founder event that originated the disjunct population in Pará.

On the other hand, it is possible that the current disjunct distribution patterns have been shaped by the extinction - an often overlooked process - of populations in central Amazonia. Amazonian avian species such as the Straight-billed Hermit (*Phaethornis bourcieri*) have similar times of divergence between populations of eastern and western Amazonia, albeit with a population still occurring in central Amazonia (in the Imeri, Jaú and Napo basins) (Araújo-Silva *et al.*, 2017). It is therefore possible that *P. ecpleopus* had a similar distribution prior to the undetected extinction of populations across central Amazonia. Due to having a lower vagility than birds, lizards are likely more susceptible to environmental changes and this extinction could have resulted from the well-documented pulses of climatic variation from the onset of the Pleistocene (Cheng, 2013; Wang 2017).

### *Ecology and phylogenetic*

Earth and life evolve together such that the historical biogeography of species is often both the reflection and the consequence of their ecology. Even though both species have a similar disjunct distribution pattern and divergence times, we showed that it may have resulted from different biogeographical and evolutionary processes, and these may be linked with the natural history of species. *Potamites ecpleopus* belongs to a genus that forms a monophyletic clade which originated and diversified in South America around 16.76 mya (Torre-Carvajal *et al.*, 2016). This is congruent with the onset of the Pebas lake system, an important driver of the diversification of aquatic taxa (Albert, 2021; Réjaud *et al.*, 2019; Salas-Gismondi, 2015) including semi-aquatic lizards (Marques-Souza *et al.*, 2018). This lake system may also have facilitated the pre-vicariance expansion of *P. ecpleopus* across the Amazon basin since it is a species strongly associated to riverine ecosystems (Arteaga & Quezada, 2023). On the other hand, *Anolis* lizards are a recent

addition to the South American fauna (Poe *et al.*, 2017). This speciose genus originated in Central America and likely arrived with the closure of the Isthmus of Panama (~10 mya) along with several other groups during the Great American Biotic Interchange (Bacon, *et al.* 2015). *Anolis trachyderma* thus likely diversified after the onset of the formation of the Pebas lake system, which may have also posed an ecological barrier to its expansion across central Amazonia. Since it occurs restricted to *terra firme* forested habitats, the changes experienced by the Amazon sedimentary basin could have directed the population dispersal from western to eastern Amazonia through the higher-elevated southern arc. This dispersal route has also been suggested for a *terra firme* Amazonian hummingbird which dispersed eastwards from the Andes foothills to the Tapajós basin, likely via a southern Amazonian corridor (Araújo-Silva, 2017). Thus, our results shed light on the importance of ecological barriers in the speciation of Amazonian lizards and on the importance of considering species ecology to interpret biogeographical and diversification processes.

## 6. Conclusion

Our study showed an interesting example of how the evolutionary history of two disjunct co-distributed lizards can differ according to their ecology, even when exposed to a similar biogeographical barrier (Rull, 2011; Smith *et al.*, 2014; Rull & Carnaval 2020), in our case, the climate fluctuations in the Plio-Pleistocene. Despite having the same distribution patterns, divergence times, and the same biogeographical event behind the disjunct distributions of *A. trachyderma* and *P. epleopus*, the demographic histories and speciation processes of each species are different. *Potamites epleopus* was probably widely distributed in the Amazon Basin during the Pliocene, and the development of a “dry diagonal” on Central Amazonia promoted the vicariance event between east and west populations. However, the same climatic barrier prevented *A. trachyderma* to colonize the Central Amazonia, and a long-range dispersal event from west to east, which also took place on the Pliocene, is probably responsible for the current disjunct distribution of this species.

Furthermore, our results supported the climatic variations of Plio-Pleistocene as an important driver of Amazonian diversification processes, reinforcing the role of “soft”



ecological barriers in the break of genetic flow between populations in this biodiverse region. Finally, our results shed light to the importance of an integrative approach that considers the ecological and evolutionary context of species as models to understand the drivers of different speciation processes. Thus, following recent studies (e.g. Antonelli et al., 2018) we believe that an accurate model to understand the diversification in the Neotropics can only be achieved through a multidisciplinary approach, considering contributions from geomorphology, systematics, biogeography, ecology, among others.

## **Abstract**

The Amazon rainforest is the most biodiverse region in the world yet the drivers that generate such biodiversity are still underexplored. Among those drivers there are some significant geomorphological processes that, throughout time, shaped space and species distributions. Some primary hypotheses are particularly notable in explaining the diversification and subsequent biogeographical patterns: the Pebas System (a mega-wetland during the Miocene), the Riverine barriers (following the drainage of the Pebas System), and the Plio-Pleistocene climatic variations. In this study, I aimed to test which of these three biogeographical events could have led to the isolation of the currently disjunct overlapping eastern-western populations of two lizard species *Anolis trachyderma* and *Potamites ecpleopus*. To do so, I sequenced samples of both species for 2 mitochondrial and 2 nuclear markers and generated a dated phylogeny to estimate the divergence time between eastern and western clades. Furthermore, I tested three possible demographic models of speciation: vicariance, eastward dispersal and westward dispersal. My results showed that the east-west divergence of both species occurred during the Pliocene (approximately 5 millions of years ago), likely caused by climatic variations that formed a dry ecological barrier separating eastern and western forests. Each species went through a different diversification process: while *A. trachyderma* had an ancestral western population with a posterior eastward dispersal, *P. ecpleopus* had a widespread ancestral distribution followed by vicariance. These processes may be related to their different ecology and evolutionary history. Overall, my results offer a baseline to understand the diversification and biogeographical patterns of the most biodiverse forest in the world.

## Resumo

A floresta amazônica é a região com a maior biodiversidade do mundo, mas os fatores que geram essa biodiversidade ainda são pouco explorados. Entre esses fatores, estão processos geomorfológicos significativos que, ao longo do tempo, moldaram o espaço e a distribuição das espécies. Algumas hipóteses primárias são particularmente notáveis na explicação da diversificação e dos padrões biogeográficos subsequentes: o Sistema Pebas (uma megapântano formado durante o Mioceno), as barreiras fluviais (após a drenagem do Sistema Pebas) e as variações climáticas do Plio-Pleistoceno. Neste estudo, meu objetivo foi testar qual desses três eventos biogeográficos poderia ter levado ao isolamento das populações leste-oeste, atualmente disjuntas e sobrepostas, de duas espécies de lagartos, *Anolis trachyderma* e *Potamites eupleopus*. Para isso, sequenciei amostras de ambas as espécies para dois marcadores mitocondriais e dois marcadores nucleares e gerei uma filogenia datada para estimar o tempo de divergência entre os clados leste e oeste. Além disso, testei três possíveis modelos demográficos de especiação: vicariância, dispersão para o leste e dispersão para o oeste. Meus resultados mostraram que a divergência leste-oeste de ambas as espécies ocorreu durante o Plioceno (aproximadamente 5 milhões de anos atrás), provavelmente causada por variações climáticas que formaram uma barreira ecológica seca separando as florestas do leste e do oeste. Cada espécie passou por um processo de diversificação diferente: enquanto *A. trachyderma* teve uma população ancestral ocidental com uma dispersão posterior para o leste, *P. eupleopus* teve uma distribuição ancestral ampla seguida de vicariância. Esses processos podem estar relacionados às ecologias e histórias evolutivas diferentes de cada espécie. Em geral, meus resultados oferecem uma base para entender a diversificação e os padrões biogeográficos da floresta mais biodiversa do mundo.

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ANEXO

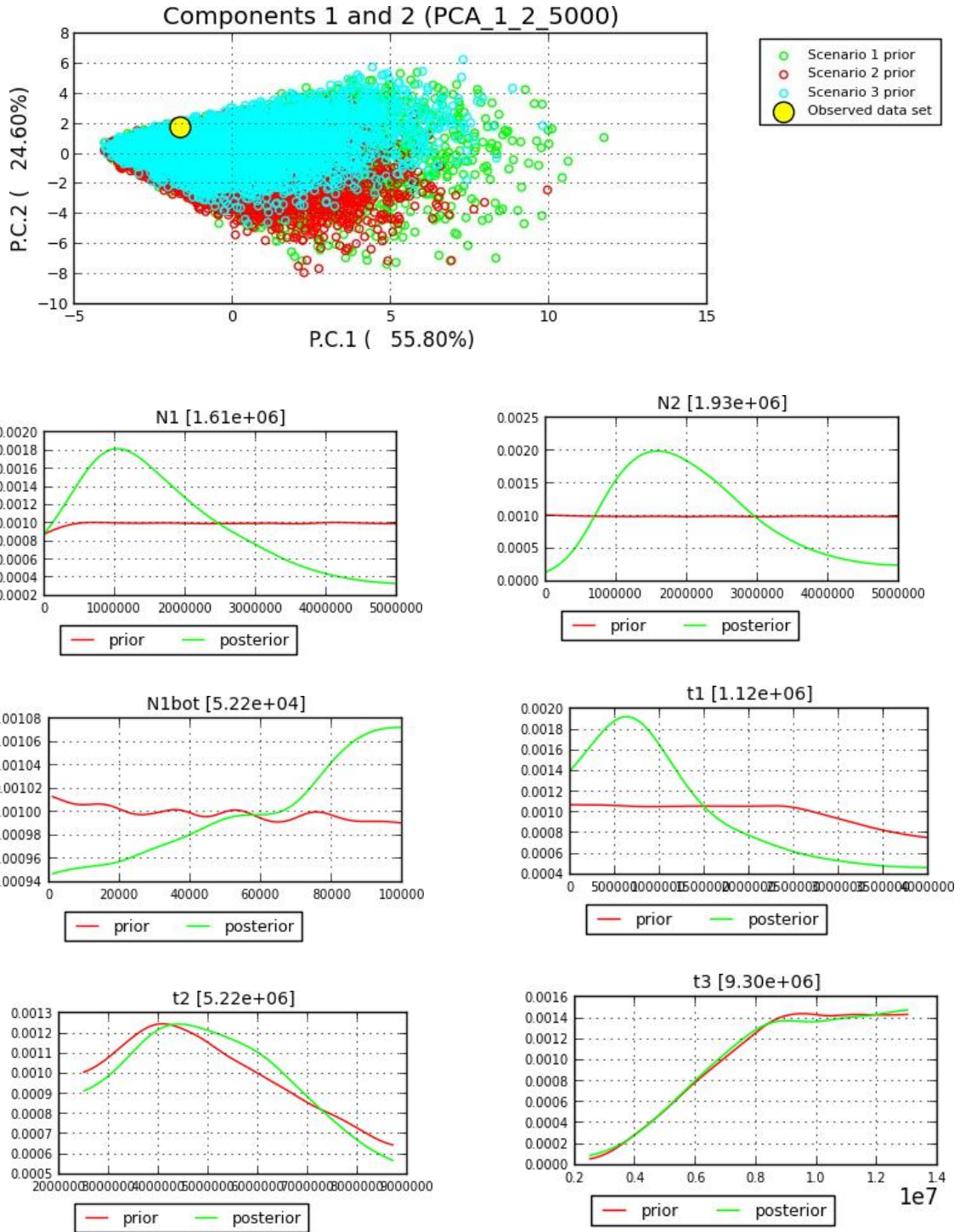


Figure 1. Anolis trachyderma principal component analysis (PCA) of simulated data generated by diyABC above and prior and posterior probabilities graphs of each parameters below.

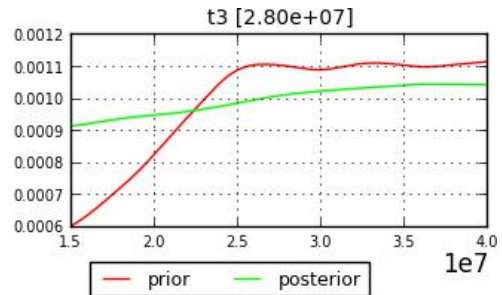
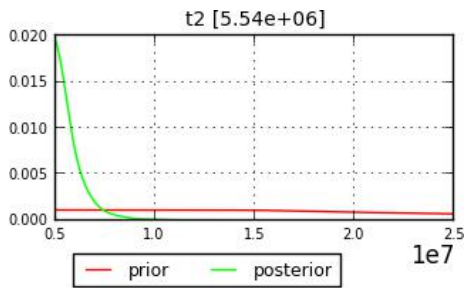
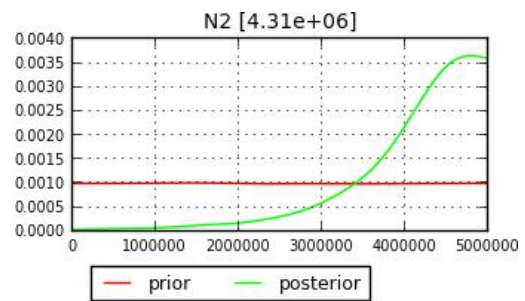
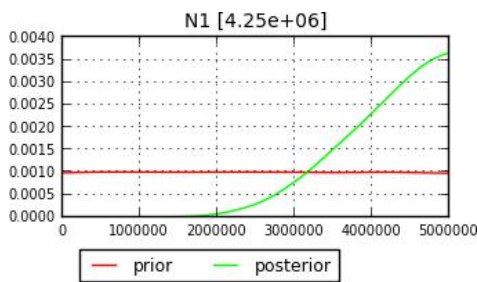
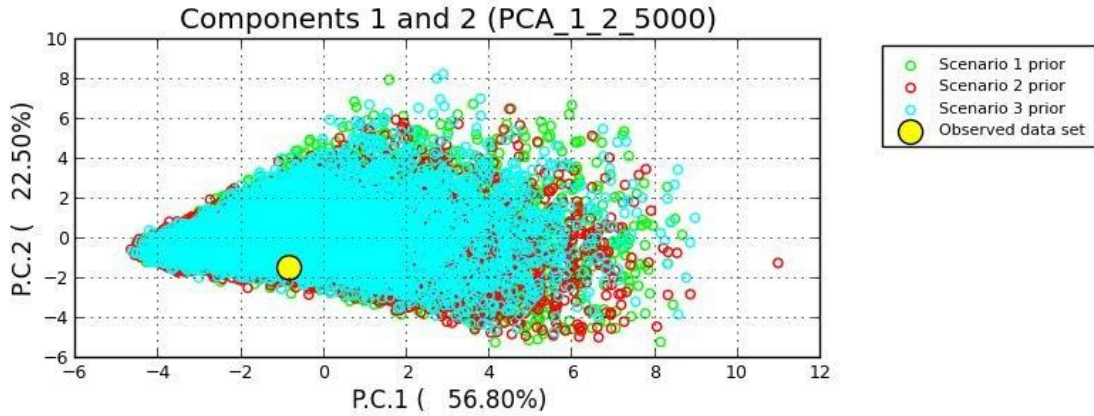


Figure 2. *Potamites ecleopus* principal component analysis (PCA) of simulated data generated by diyABC above and prior and posterior probabilities graphs of each parameters below.



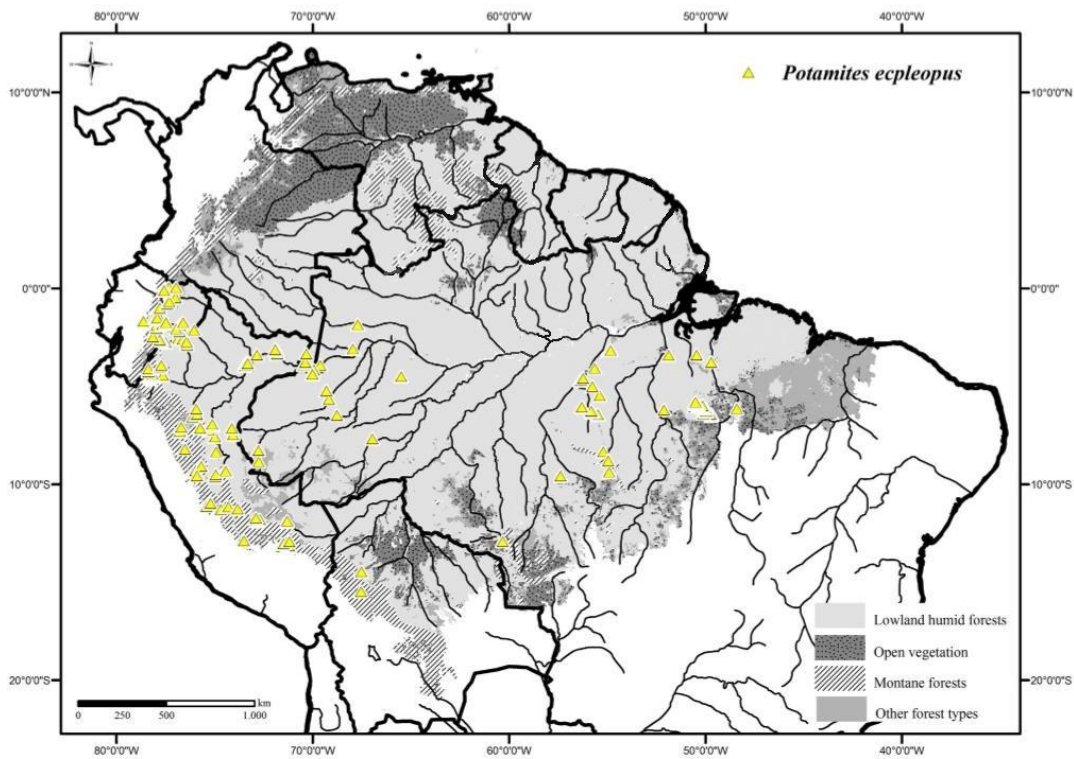
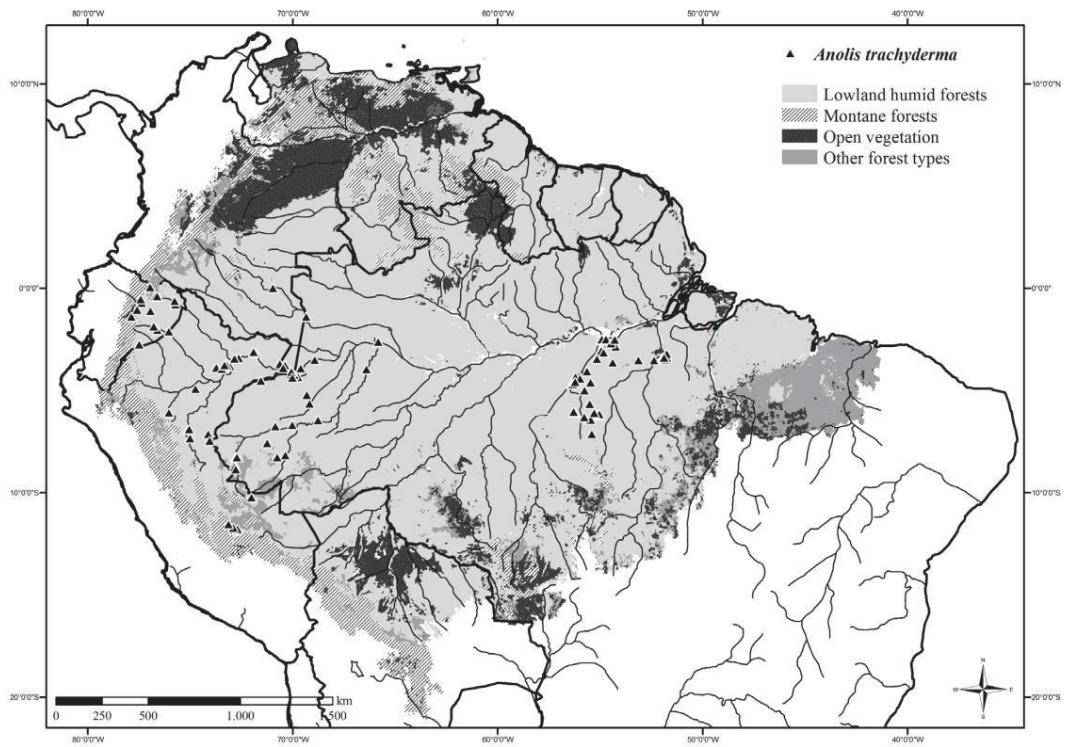


Figure 3. Distribution maps of *A. trachyderma* above and *P. ecleopus* below. Adapted from Ribeiro-Júnior 2015 and Ribeiro-Junior & Amaral 2017.