

**Genes, formas e sons revelam estágio incipiente de especiação
alopátrica no anuro amazônico *Allobates tapajos* (Dendrobatidae)**

GABRIELA FARIAS MAIA

Manaus, Amazonas

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Dissertação apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para a obtenção do título de Mestre em Biologia (Ecologia).

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Sinopse:

Foi estudada a variabilidade genética, acústica e morfológica no anuro amazônico *Allobates tapajos* (Dendrobatidae) ao longo de sua distribuição na bacia do rio Tapajós, Pará, Brasil. Com base no efeito da transposição do rio Tapajós sobre as diferentes classes de caracteres, foi avaliado o estágio de diferenciação evolutiva entre populações da espécie.

Palavras chaves: Amazônia, Anura, bioacústica, filogeografia, microevolução, Rio Tapajós.

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“A natureza não faz nada bruscamente”

Jean-Baptiste Lamarck

RESUMO

Na bacia Amazônica, a distribuição de muitas espécies de vertebrados terrestres é delimitada por grandes rios, os quais são frequentemente considerados barreiras biogeográficas fortemente relacionadas à origem e manutenção da alta biodiversidade encontrada na região. No entanto, são poucas as investigações filogeográficas que avaliam o efeito dessas barreiras em múltiplas classes de caracteres genotípicos e fenotípicos. Até o momento, nenhuma investigação multicaráter foi dedicada a um sistema de estudo envolvendo o Rio Tapajós, o qual delimita áreas de endemismo do bioma. Neste estudo testamos a atuação dessa barreira fluvial sobre a variabilidade genética, acústica e morfológica em populações do anuro *Allobates tapajos* (Dendrobatidae). Para isso, amostramos populações ao longo de toda a área de ocorrência conhecida da espécie em ambas as margens das porções média e baixa do rio. Foram obtidos fragmentos de genes mitocondrial (16S) e nuclear (RAG1), bem como medidas morfométricas externas e parâmetros acústicos do canto de anúncio a partir de 48 indivíduos provenientes de seis localidades. Enquanto o marcador nuclear se mostrou monomórfico ao longo da distribuição de *A. tapajos*, o fragmento mitocondrial revelou baixas distâncias genéticas acompanhadas de elevada estruturação espacial, com ausência de compartilhamento de haplótipos entre margens opostas do rio. Os eventos cladogenéticos estiveram concentrados no Pleistoceno, época proposta para o estabelecimento da drenagem do rio Tapajós. Houve divergência em parâmetros acústicos entre margens do rio, o que não foi observado em relação aos marcadores morfológicos analisados. Adicionalmente, não houve correlação entre o padrão de variabilidade das diferentes classes de caracteres entre si nem com a distância geográfica linear entre populações. Desse modo, o conjunto de resultados obtidos suporta o atual status específico das populações de ambas as margens do rio Tapajós e revela um estágio incipiente do processo de especiação em alopatria relacionado com a transposição dessa barreira fluvial.

Palavras-chave: Amazônia, Anura, bioacústica, filogeografia, microevolução, Rio Tapajós.

ABSTRACT

Genes, shapes and sounds reveal an incipient stage of allopatric speciation in the Amazonian frog *Allobates tapajos* (Dendrobatidae)

In the Amazon basin, the distribution of many vertebrate species is delimited by large rivers, which are frequently considered as biogeographical barriers strongly related to the origin and maintenance of the elevated biodiversity found in the region. However, few are the phylogeographical investigations evaluating the effect of such barriers on multiple classes of genotypic and phenotypic characters. To date, no multicharacter investigation has been dedicated to a study system involving the Tapajos River, which delimit areas of endemism in the biome. In this study we tested the effectiveness of this river barrier in the genetic, acoustic and morphological variability in populations of the frog *Allobates tapajos* (Dendrobatidae). For this, we sampled populations throughout the known distributional range of the species on both margins of the middle and lower sections of the river. We obtained fragments of mitochondrial (16S) and nuclear (RAG1) genes, as well as external morphometric measurements and advertisement call acoustic parameters of 48 individuals from six localities. While the nuclear marker was monomorphic across the geographic distribution of *A. tapajos*, the mitochondrial fragment revealed low genetic distances accompanied of high spatial structuring, with no haplotype sharing between opposite river margins. Cladogenetic events were concentrated on Pleistocene, epoch proposed to the establishment of the Tapajós river drainage. Acoustic parameters diverged between river margins, a pattern not observed in relation to the morphological markers analyzed. Additionally, there was no correlation in the variability pattern of the different classes of characters between them or in relation to linear geographic distance among populations. When taken together, the set of obtained results support the current specific status of the populations from both margins of the Tapajós River and reveal an early stage of an allopatric speciation process related to the transposition of this riverine barrier.

Keywords: Amazon, Anura, bioacoustic, phylogeography, microevolution, Tapajós River.

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APRESENTAÇÃO

A presente dissertação é composta por um artigo científico e obedece às normas do periódico internacional *Biological Journal of the Linnean Society*, para o qual será submetida com as alterações necessárias. A dissertação investiga, por meio de múltiplas classes de caracteres, a influência do Rio Tapajós como barreira na variabilidade genotípica e fenotípica de uma espécie de anfíbio anuro. O artigo contém a seguinte estrutura: (1) introdução, abordando principalmente teorias referentes a processos microevolutivos e suas relações com o espaço geográfico; (2) métodos, apresentando a forma como os dados foram coletados e analisados; (3) resultados obtidos; (4) discussão acerca da natureza e das implicações dos resultados; (5) referências citadas ao longo do trabalho. Figuras e tabelas encontram-se inseridas no corpo do manuscrito.

Objetivos

O objetivo desse estudo foi testar a atuação de uma barreira biogeográfica sobre a variabilidade genética, acústica e morfológica em populações de *Allobates tapajos* (Anura, Dendrobatidae). Especificamente, este estudo procurou responder às seguintes questões:

- 1) Existe efeito do Rio Tapajós sobre a distribuição espacial da variabilidade genética, acústica e morfológica em populações de *Allobates tapajos*?
- 2) Em que estágio de diferenciação encontram-se essas populações?
- 3) Qual o status taxonômico das diferentes populações amostradas?

Capítulo 1

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Genes, shapes and sounds reveal an incipient stage of allopatric speciation in the Amazonian frog *Allobates tapajos* (Dendrobatidae)

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Running title: Allopatric speciation in an Amazonian frog

Abstract

*Genes, shapes and sounds reveal an incipient stage of allopatric speciation within the Amazonian frog *Allobates tapajos* (Dendrobatidae).* In the Amazon basin, the distribution of many vertebrate species is delimited by large rivers, which are frequently considered as biogeographical barriers strongly related to the origin and maintenance of the elevated biodiversity found in the region. However, few are the phylogeographical investigations evaluating the effect of such barriers on multiple classes of genotypic and phenotypic characters. To date, no multicharacter investigation has been dedicated to a study system involving the Tapajos River, which delimit areas of endemism in the biome. In this study we tested the effectiveness of this river barrier in the genetic, acoustic and morphological variability in populations of the frog *Allobates tapajos* (Dendrobatidae). For this, we sampled populations throughout the known distributional range of the species on both margins of the middle and lower sections of the river. We obtained fragments of mitochondrial (16S) and nuclear (RAG1) genes, as well as external morphometric measurements and advertisement call acoustic parameters of 48 individuals from six localities. While the nuclear marker was monomorphic across the geographic distribution of *A. tapajos*, the mitochondrial fragment revealed low genetic distances accompanied of high spatial structuring, with no haplotype sharing between opposite river margins. Cladogenetic events were concentrated on Pleistocene, epoch proposed to the establishment of the Tapajós River drainage. Acoustic parameters diverged between river margins, a pattern not observed in relation to the morphological markers analyzed. Additionally, there was no correlation in the variability pattern of the different classes of characters between them or in relation to linear geographic distance among populations. When taken together, the set of obtained results support the current specific status of the populations from both margins of the Tapajós River and reveal an early stage of an allopatric speciation process related to the transposition of this riverine barrier.

Keywords: Amazon – Anura – bioacoustics – phylogeography – microevolution - Tapajós River.

Introduction

Evolutionary divergence between populations isolated by a geographical barrier is a frequently observed phenomenon, and has been widely investigated in the context of allopatric speciation processes (Mayr, 1947; Coyne & Orr, 2004). In isolation, such populations can respond to local interactions with the environment or with other species. This can result in local variation in the type and intensity of selective pressures (Schluter, 2001; Funk, Nosil, & Etges, 2006). In addition, divergence between such populations may occur in the absence of selective factors due to the stochasticity of genetic drift (Avice, 2000). Thus, populations living in allopatry are good candidates for studying genetic and phenotypic differences, since microevolutionary mechanisms may act directly and differentially on such characteristics. This may result in the evolution of differences in traits that contribute to reproductive isolation, with direct implications for the speciation process (Greenberg *et al.*, 2003; Coyne & Orr, 2004; Hoskin & Higgie, 2010).

Regardless of the evolutionary mechanisms promoting divergence, the longer groups have been separated, the greater the genotypic and phenotypic divergence between them is likely to be due to the accumulation of mutations that become fixed over evolutionary time (Adams *et al.*, 2009). The magnitude of divergence of genetic and phenotypic characteristics between populations can be considered as indicators of their current stage in the speciation process (Coyne & Orr, 1997; Avice, 2000). Such characters can be used as markers in intraspecific studies designed to assess spatial patterns of variation at the population level, as well as investigations of the evolutionary mechanisms underlying such patterns (Santini, Miglietta & Faucci, 2012).

While the genotypic characters used as evolutionary markers in various taxa often focus on DNA nucleotide polymorphisms (Avice, 2004; Bar-Yaacov *et al.*, 2015;

Toews *et al.*, 2016), the phenotypic characters that can be investigated often depend on particularities of the taxonomic group under study. In amphibians, morphological traits have served historically as the most commonly-used markers in the study of between-population variation. This has resulted in great emphasis being placed on morphology when proposing concepts and diagnoses at the species level (Cronquist, 1978; Coyne & Orr, 2004). The validity of this approach is supported by the fact that several studies have shown morphological differentiation, especially in relation to body shapes, among genetically divergent populations of amphibia (Amézquita *et al.*, 2009; Kaefer *et al.*, 2013). Bioacoustic characters are another class of phenotypic characters that, in frogs, are commonly used for population-level studies. Vocalizations, especially the advertisement call, are the main form of communication in frogs, and bioacoustic characters carry a strong phylogenetic signal (Erdtmann & Amézquita, 2009; Goicoechea, De La Riva & Padial, 2010). Their importance in specific recognition means such calls may act as pre-zygotic reproductive isolation barriers, and so may lead to speciation via sexual selection (Gerhardt & Huber, 2002; Wells, 2007).

Rainforests are considered rich areas for cryptic speciation, and so are expected to house a significant amount of as-yet undescribed diversity (Hebert *et al.*, 2004; Funk, Caminer & Ron, 2011). Part of the understanding of evolutionary mechanisms includes the taxonomic challenges posed by the description of amphibian species. Before the developments of molecular techniques, these were largely dependent on morphological data (Funk *et al.*, 2011). However, the use of the evolutionary species concept means that taxonomic decisions concerning species limits should not be based solely on morphological data, since the speciation process is not always accompanied by morphological changes (Fouquet *et al.*, 2007a; Gehara *et al.*, 2014). Thus, an integrative approach to taxonomy has been proposed based on a broad assessment of

morphological, bioacoustic, ecological, behavioral and genetic evidence, and, especially, the accessibility of new tools for differentiating morphologically-similar species (Padial *et al.*, 2010; Pires & Marinoni, 2010). Such approaches reduce bias inherent in the use of single markers, can greatly facilitate the recognition of members of a species complex (Dayrat, 2005; Padial *et al.*, 2008), and consequently, the detection of biogeographic patterns in tropical regions.

Many hypotheses about how species evolve in time and space are tested by phylogeographic approaches, which now integrate genetic markers to a great variety of other characters bearing phylogenetic signals, as well as physical and biological processes in historical time (Brusa *et al.*, 2013; Leite & Rogers, 2013; Maldonado-Coelho *et al.*, 2013; Brunes *et al.*, 2014; Fouquet *et al.*, 2014). In Amazonia, studies of the causes of speciation have focused on the action of rivers in creating allopatric conditions for populations of terrestrial vertebrate (Ron, 2000; Hayes & Sewlal, 2004; Ribas *et al.*, 2011), reducing or fully preventing the dispersal of individual organisms and therefore of gene flow between populations (Peres, Patton & da Silva, 1996; Moritz *et al.*, 2000; Aleixo, 2004; Antonelli *et al.*, 2010). River barriers in Amazonia, especially when the river involved has few meanders, are considered to be one of the major factors affecting the spatial dynamics of species in historical time (Antonelli *et al.*, 2010; Hoorn *et al.*, 2010).

Studies on the Madeira River, a southern-bank tributary of the Amazon, found that it is congruent with a distribution limit for several species of primates, birds and frogs (Ayres & Clutton-Brock, 1992; van Roosmalen *et al.*, 2000; Dias-Terceiro *et al.*, 2015). In contrast, studies conducted in Juruá River found no morphological or molecular differences, or composition differentiation in the frog and mammal species assemblages on either side of the river (Gascon, Lougheed & Bogart, 1996, 1998;

Lougheed *et al.*, 1999; Gascon *et al.*, 2000), suggesting that this river does not act as a barrier to the movement of individuals from one shore to another. Therefore suggests that the particular factors of each river, such as age, width, flow rate and channel dynamics can modify a river's effectiveness as a barrier to the dispersion of organisms over historical time (Ayres & Clutton-Brock, 1992; Leite & Rogers, 2013).

In Amazon, some of the first studies on the diversity and distribution of the flora and fauna were conducted by the British naturalist Alfred Russel Wallace (Wallace, 1852). The patterns he found indicated that several species of vertebrates, notably primates and birds, had their distribution determined by large rivers, with the River Madeira, Solimões/Amazonas and Negro rivers being the principle ones to delimit and separate populations, so creating the main areas of endemism within the Amazonian fauna (Cracraft, 1985; Ron, 2000; Ribas *et al.*, 2011; Silva, 2013). Currently, based on the distribution of birds and primates, nine areas of endemism are recognized for the Amazon Basin (Peres *et al.*, 1996; van Roosmalen *et al.*, 1998): Guyana, Imeri, Napo, Inambari, Rondônia, Rio Negro, Tapajós, Xingu and Belém (Da Silva, Rylands & Da Fonseca, 2005; Borges & Da Silva, 2012; Smith *et al.*, 2014).

Amphibians of the genus *Allobates* (Dendrobatidae) have been used as model in addressing evolutionary questions, especially those relating to geographic patterns of diversification (Lougheed *et al.*, 1999; Amézquita *et al.*, 2009; Tsuji-Nishikido *et al.*, 2012; Simões *et al.*, 2014). This taxon currently includes 51 species distributed from Nicaragua through the Amazon Basin to Bolivia and the Guianas, and into southeastern Brazil (Grant *et al.*, 2006; Frost, 2016). Recent efforts in data collection, combined with integrative analytical approaches, have revolutionized the taxonomic organization of this genus, which had only 21 species before the XXI century began (Simões, 2016). Despite the high number of species observed in *Allobates*, changes in color and

morphological characters of most species are usually subtle, making it difficult to identify and diagnose species on the basis of external morphology (Kaefer *et al.*, 2013; Simões *et al.*, 2013a).

Allobates tapajos (Lima, Simões & Kaefer, 2015; Anura: Dendrobatidae) is a newly-described anuran with a type locality located on the west bank of the Tapajós River. However, Lima *et al.* (2015) suggested that this taxon has geographic distribution along both banks of the middle and lower portions of the river. As the Tapajós River delimits two areas of Amazonian endemism, it is possible that *Allobates tapajos* may have its distribution restricted to the Rondônia area of endemism (that is to say, within the Madeira River/Tapajós River interfluvium), and that, as a result of this, the population of the east bank of the Tapajós River (and so within the Tapajós area of endemism) may represent a separate, and as-yet undescribed, species. In this context, we aimed, in the current study, to test the hypothesis that the biogeographical barrier represented by the Tapajós River, acts on the genetic, acoustic and morphological variability among populations of *Allobates tapajos* (as currently defined). Based on the magnitude and consistency of the effect of this barrier across the different classes of characters, we expected to gain an indication of the stage of differentiation and hence the taxonomic status of the two populations.

Methods

Studied species

Allobates tapajos is distributed in the tropical terra firme (never-flooded) forests on both banks of the middle and lower portions of the Tapajós River, southern Pará State, Brazil (Lima *et al.*, 2015). They are diurnal frogs that occur on the floor of terra-firme close to small waterways. Reproduction occurs during the rainy season and egg

deposition is performed on folded dead leaves within the leaf-litter. Later, tadpoles are transported by one of the parents to water bodies where they metamorphose into adults (Lima *et al.*, 2015).

Study area

The current study was conducted in the state of Pará, Brazil, at six different locations (hereinafter considered separate populations) on both sides of the middle and lower Tapajós River, a major tributary of the Amazon (Figure 1), between the municipalities of Santarém ($2^{\circ}26'22''\text{S}/54^{\circ}41'55''\text{W}$) and Itaituba ($4^{\circ}16'9''\text{S}/55^{\circ}59'23''\text{W}$). The average annual temperature in the region is 27.5°C , and the average annual rainfall is 1950 mm. Rainfall is seasonal, with higher precipitation between the months of December and May (Miranda, 1993; Carvalho *et al.*, 2008).

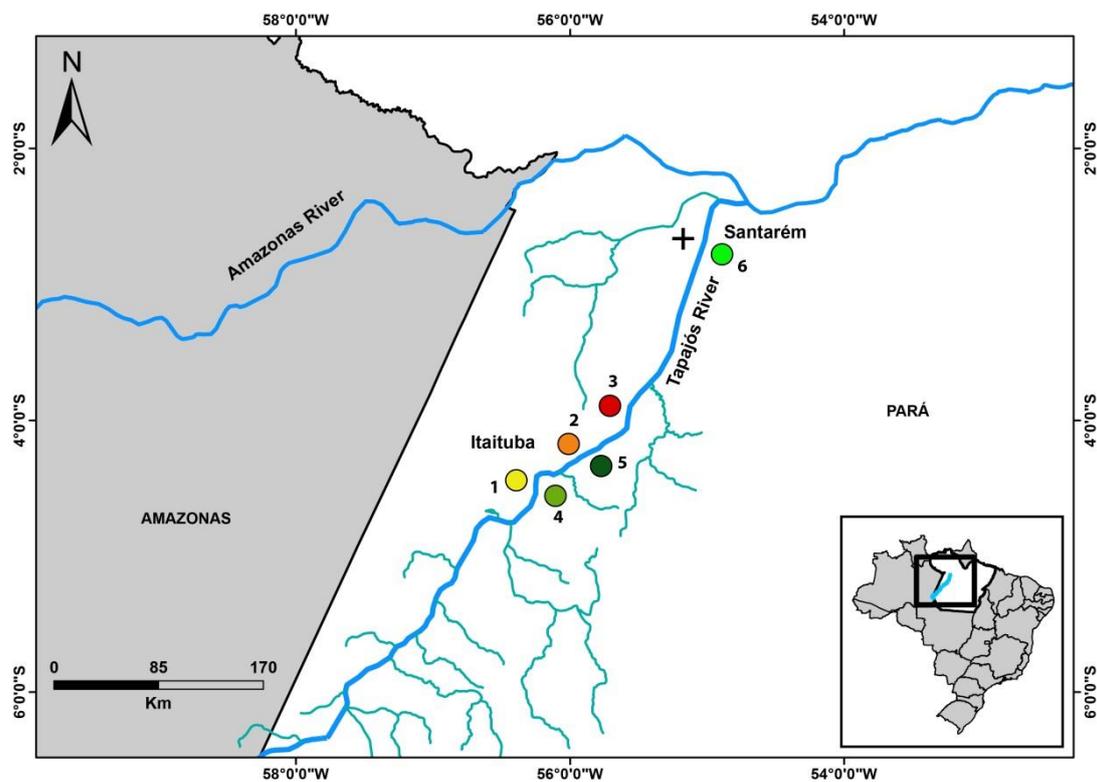


Figure 1. Geographical location of the six *Allobates tapajos* sampling points in the state of Pará. Point 1 (in yellow) is located near the type locality of the species. The sign (+) indicates that the species was not found at the site.

Data collection

Collections were made where calling populations of *Allobates tapajos* were found. Field activities, including the acquisition of acoustic data and specimen collecting, took place from 12 January to 18 March 2015. All activities were conducted during the day.

Table 1. Study sites in the state of Pará, with their geographical coordinates and size of the samples for each data class.

Locality	Coordinates	Morphology	Acoustics	mtDNA	nuDNA
1. Vila Rayol	4°27'30.93"S 56°16'12.85"W	8	8	8	2
2. Itaituba	4°17'14.77"S 56°01'55.00"W	8	8	8	3
3. Brasília Legal	3°56'28.92"S 55°34'56.18"W	8	8	8	4
4. São Luís do Tapajós	4°27'24.645"S 56°14'53.213"W	8	8	8	2
5. Paraná-Mirim	4°19'23.33"S 56°00'39.42"W	7	7	8	2
6. Belterra	2°44'59.112"S 54°57'29.357"W	8	8	8	3
Total		47	47	48	16

Acoustic data

Advertisement calls of *Allobates tapajos* males were recorded with a Marantz PMD660 digital recorder linked to an AKG 568 EB directional microphone, positioned 1.5 m from the calling animal. Each recording was composed of a minimum of three continuous minutes of vocalizations. Since air temperature may have an effect on acoustic properties of frog vocalizations (Gerhardt & Huber, 2002), this variable was measured after each recording using a thermometer.

Recordings of vocalizations were analyzed using the Raven 1.4 program (Charif, Strickman & Waack, 2010). The *A. tapajos* advertisement call has the form of continuous series of notes largely arranged in pairs or trios (Lima *et al.*, 2015). Spectrogram measurements were taken after a Fast Fourier Transform with a Blackman-type window at a resolution of 82 Hz and 2048 points. Temporal (note duration, note repetition rate, between-note interval, and between-call interval) and spectral (maximum, lowest and highest frequencies) parameters were obtained from selections made by applying the tool "Selection spectrum" to the generated oscillograms. Ten songs were analyzed per individual, and the arithmetic mean of the values obtained for each parameter was used as the final value for each specimen.

Morphological data

The collected animals were sacrificed via application of a topical anesthetic cream (5% lidocaine), labelled, fixed in commercial formol diluted to 10%, then preserved in 70% ethanol. Eighteen external morphometric measurements were taken in the laboratory with the aid of optical stereomicroscope coupled ocular micrometer. All measurements were taken from the left side of preserved individuals (Simões *et al.*, 2013b). Specimens were deposited with the Collection of Amphibians and Reptiles of the National Institute of Amazonian Research, Manaus, Brazil (INPA-H) as accession numbers 021245 – 036785.

Molecular data

Muscle tissue was collected from individual *Allobates* and preserved in ethanol prior to the fixation of the donor animal in formaldehyde. Tissues were dissolved in proteinase K/SDS solution, and total genomic DNA was isolated from samples using the Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the

protocols provided by the manufacturer. Segments of 16S rRNA mitochondrial DNA and RAG1 nuclear DNA regions were amplified with the polymerase chain reaction (PCR) using universal primers (oligonucleotide initiators). These segments were chosen due to their ability to reveal phylogeographic patterns. Additionally, the 16S region is widely used as a DNA barcode for amphibians (Vences *et al.*, 2005a; Fouquet *et al.*, 2007b). A PCR was performed for both fragments in a final volume of 15µL. For 16S, the reaction contained 6,2µL of distilled and deionized water; 1.5µL MgCl (25 mM); 1.5 uL of Tris-HCl buffer (10 mM); 1.5 l dNTPs (25 mM); 1.5 uL 16*sar* primer (5'-CGCCTGTTTATCAAAAACAT-3') and 16*sbr* (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi, 1996) at 2 pmol/uL; 0.3 uL of taq DNA-polymerase (5U/uL), and 1 uL of DNA (10ng/uL). For the nuclear gene RAG1, the reaction contained 6.2µL distilled and deionized water; 1.5µL MgCl (50 mM); 1.5 uL of Tris-HCl buffer (10 mM); 1.5 l dNTPs (25 mM), 1.5 uL of each primer Amp_F2 (5'-ACNGGNMGICARATCTTYCARCC-3') and Amp_R1 (5'-AACTACGCTGCATTKCCAATRTCACA-3') (Chiari *et al.*, 2004) at 2 pmol/uL; 0.3 uL of Taq DNA-polymerase (5U/uL), and 1 uL of DNA (10ng/uL). For the amplification reaction, a 16S fragment the following thermocycling program was used: 92°C for thirty seconds for the initial denaturation; 35 cycles of denaturation at a temperature of 92°C for ten seconds, annealing at 50°C for 35 seconds, and extension at 72°C for 90 seconds; the final extension was performed at 72°C for ten minutes. For RAG1: 92°C for thirty seconds for the initial denaturation; 35 cycles of denaturation at a temperature of 92°C for ten seconds, annealing between 55°C and 57°C for 35 seconds, and extension at 72°C for 90 seconds; the final extension was performed at 72°C for ten minutes. A sample of 2 uL of each PCR product was analyzed by electrophoresis of 1% agarose gel stained with ethidium bromide. PCR products were

purified by reaction with EXO-SAP, following the protocol suggested by the manufacturer. Sequencing reactions were performed using the Big Dye kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing products were precipitated by EDTA/ethanol and analyzed in an ABI 3130xl automatic capillary sequencer (Applied Biosystems). These procedures were performed in the Molecular Biology Laboratory (LTBM) of the National Institute for Amazonian Research, Manaus, Amazonas, Brazil.

Homologous regions of nucleotide sequences were automatically aligned using the MUSCLE algorithm (Edgar, 2004) using the MEGA 6.6 program (Tamura *et al.*, 2013), which makes sequences available for visual inspection of errors, and for coding any insertions or deletions. Sequences were manually checked in Geneious 5.3.4 (Kearse *et al.*, 2012). Final alignments had a length of 517 basepairs (bp) for the mtDNA 16S fragment and 555 bp for nuDNA RAG1. Sequences of representative haplotypes were deposited in the GenBank database.

Data analysis

Population analysis

Geneological relations between individual samples, taking into consideration the source populations, were estimated by means of a haplotype network using Haploview 4.2 program (Barrett *et al.*, 2005). For this, a maximum likelihood tree (Supplemental Material 1) was generated in RaxML 7 (Stamatakis, 2006), in accordance with a nucleotide substitution pattern preset in jModelTest 2 (Guindon & Gascuel, 2003; Durrin *et al.*, 2012). The relative partitioning of the genetic variation (within populations, between populations and between river banks) was established by standard molecular analysis of variance (AMOVA; Excoffier, Smouse & Quattro, 1992) using

the Arlequin version 3.5 program (Excoffier & Lischer, 2010). The relative genetic structure between sampling locations was estimated by calculating distance-based fixation indexes (F_{ST} : Wright, 1951), which is equivalent to the N_{ST} of Lynch & Crease (1990). Statistically significant F_{ST} values were obtained after 1023 haplotype permutations in Arlequin. Pairwise genetic differentiation between sampling sites was estimated with the Kimura-2 distance parameter (Kimura, 1980) in Mega 6 (Tamura *et al.*, 2013). The most probable number of genetic clusters formed by the sampled mtDNA sequences was inferred by Bayesian analysis of population structure using BAPS version 6 (Corander *et al.*, 2008). Based on nucleotide frequencies, this model seeks to generate k groups of individuals, so that those assigned to the same group are as genetically similar to each other as possible. The upper limit for the number of groups was set from the number of sampling sites ($n = 6$). Log-likelihood values for the best models were used to select the arrangement of the most likely groups.

Divergence time estimation

Unique haplotypes were analyzed from populations recovered from the analyzes carried out in Arlequin 3.5. Available 16S sequences of *A. gasconi* (GenBank KJ747333; GenBank KJ747334) were used as the outgroup since this is the most closely-related species in relation to *A. tapajos* (Lima *et al.*, 2015). Additionally, the co-sequence-specific 16S *Allobates tapajos* (GenBank KR047027; GenBank KR047028; Type-location) and *Allobates* "aff. *machesianus*" (GenBank EU342545; GenBank EU342546; Location Curuá-Una) were used as an internal group. Since the origin of these species are not known from fossils, we used a calibration method based on mitochondrial DNA substitution rates to provide an idea about the relative divergence times. The time sequence of diversification was estimated via BEAST 1.7 (Drummond

& Rambaut, 2007; Drummond *et al.*, 2012), using the settings uncorrelated relaxed clocks and simple birth process prior - Yule (Aldous, 2001). As a divergence rate we used the 0.0069 substitutions/million years proposed for amphibian mitochondrial DNA by Macey *et al.* (1998). An initial tree was generated randomly, with a chain length of 10 million generations, with samples taken every 10 thousand, discarding 10% of the trees as burn-in, resulting in 900 trees sampled in a Monte Carlo Markov Chain (MCMC). The stationarity of the posterior distributions for all model parameters, including medians and ranges from 95% (HPD) of the nodes, was found using Tracer 1.5 (Drummond & Rambaut, 2007). From the MCMC output, a final consensus tree was generated using Tree Annotator 1.6.2 (Drummond & Rambaut, 2007).

Phenotypic differentiation

Differentiation in acoustic and morphological characters on either side of the Tapajós River was tested using two generalized linear models (GLM, one for each character class) using the first two components generated by a principal component analysis (PCA). The characters were considered as the dependent, and river banks as the independent, variables. Discriminant function analysis (DFA) was used to test whether the sampled populations differed phenotypically, with acoustic and morphometric characters as predictor variables. Discriminant function was used to compute the probability of assigning each individual to its population (variable group) by a Jackknife classification matrix. This method included all male specimens for which measurements were available. Adjustment of the bioacoustic data by linear regression was not needed as neither temperature (23.7-28.5°C) nor body size (SVL, 14.3-18.3 mm) had an effect on the acoustic variables (Kaefer *et al.*, unpublished data). In order to minimize the effect of body size on morphometric measurements a series of 12 morphological

measurements were used as predictor variables (Verdade & Rodrigues, 2007): LL/SVL, HAND3/SVL, FL/SVL, HW/HL, EN/HL, EL/HL, TYM/HL, TYM/EL, IN/HW, HAND1/HAND2, HAND2/HAND3, HAND4/HAND1 (See supplemental material 2 for acronym meanings).

Correlation between geographic, phenotypic and genetic differentiation

We tested the correlations between linear geographic distances (measured in km from GPS coordinates), genetic and phenotypic distances between populations by applying Mantel tests using distance matrices (Mantel, 1967). We also performed partial Mantel tests to assess the correlation of genetic/phenotypic distances between populations by controlling the effects of the river transposition and geographical distance between sampling sites (Smouse, Long & Sokal, 1986; Telles *et al.*, 2001). A binary correlation matrix was constructed by assigning the values '0' and '1' to, respectively, pairs of locations within and between opposing sides of the river.

The average genetic distances (16S) between the sampling locations were calculated with a Kimura 2-parameter model (K2P), using the program MEGA version 6. A Matrix of acoustic distances was obtained using Euclidean distances between all possible population pairs using the scores for the means of acoustic measurements of the first and second components produced by a PCA. The major components were obtained from the arithmetic mean of the advertisement call parameters between the sampled specimens from each sample location, and were used to reduce the number of independent phenotypic variables. The same process was used to generate an array of morphological distances. Mantel tests were conducted using the ZT program (Bonnet & Van de Peer, 2002) via permutation of null models (Anderson & Legendre, 1999) with 10,000 randomizations.

Results

Population analysis

We obtained 48 16S mtDNA sequences corresponding to 20 different haplotypes, and 16 RAG1 nuDNA sequences corresponding to a single haplotype (Supplemental Material 3 and 4). Most of the mitochondrial DNA haplotypes were restricted to a single location and there was no sharing of haplotypes between the two river banks.

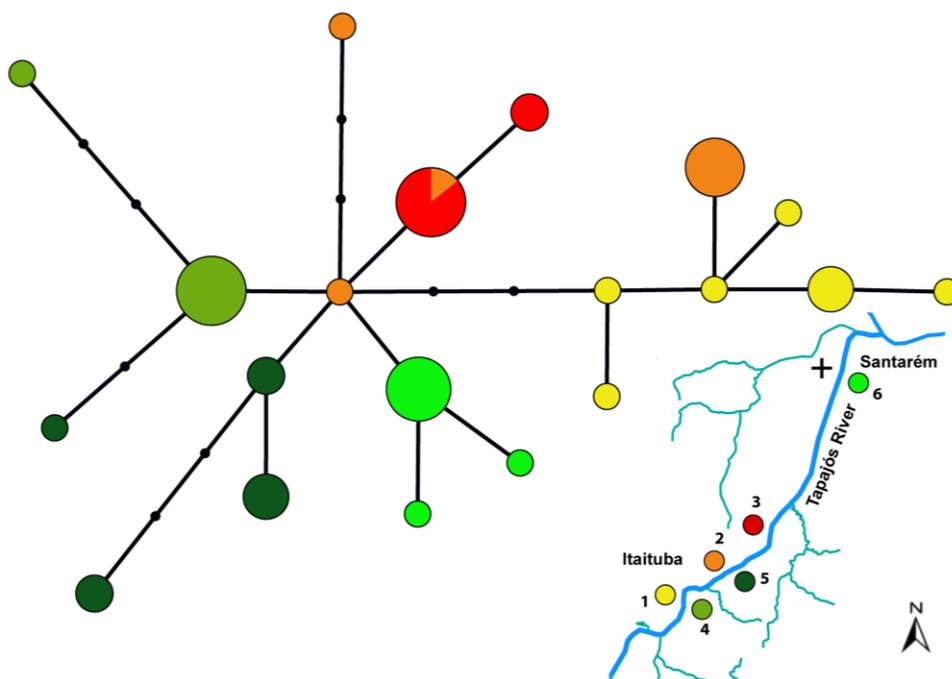


Figure 2. Haplotype network with 48 sequences of 16S mtDNA for *Allobates tapajos*. The size of each ellipse indicates the relative haplotype frequency and the color indicates the origin of individuals according to the geographical map.

The AMOVA indicated that most of the total genetic variation was found between populations present on the same river bank (49.88%). River banks were responsible for 17.34% of the genetic variation (Table 1). F_{ST} values showed a high and significant level of general population structuring (Table 2). The highest genetic distances were observed between populations on opposite banks of the river.

Table 1. Analysis of molecular variance (AMOVA) based on fragments of the mitochondrial *16S* DNA. The relative distribution of genetic variability of *Allobates tapajos* is presented according to hierarchical levels.

Source of variation	Percentage of variation
Between riversides	17.34
Among populations within riversides	49.88
Within populations	32.77

Table 2. Pairwise F_{ST} fixation indexes (lower left matrix) and average (%) Kimura 2-parameter genetic distances (upper right matrix). Populations west (W) and east (E) from Tapajós river are indicated. Values calculated between the sampling localities for each *Allobates tapajos* population. Significant F_{ST} values are indicated with asterisks. Locality numbers are shown according to Figure 1.

Locality	Vila	Itaituba	Brasília	São Luís	Paraná	Belterra
1. Vila (W)	-	0.7	1.1	1.2	1.3	1.1
2. Itaituba (W)	0.231*	-	0.9	1.0	1.1	0.9
3. Brasília (W)	0.814*	0.555 *	-	0.5	0.7	0.5
4. São Luís (E)	0.791*	0.557*	0.775*	-	0.7	0.5
5. Paraná (E)	0.707*	0.472*	0.620*	0.561*	-	0.7
6. Belterra (E)	0.808*	0.571*	0.814 *	0.761*	0.610*	-

Bayesian analysis of population structure supported the partition of individuals of *A. tapajos* into three genetic groups, two of them restricted to interfluvial regions (ML value = -280.7419; probability = 0.95 or 0.04149). A co-occurrence of genetic groups within localities was observed (Figure 3). Four locations (1, 3, 4 and 6) each contained individuals from only one genetic group.

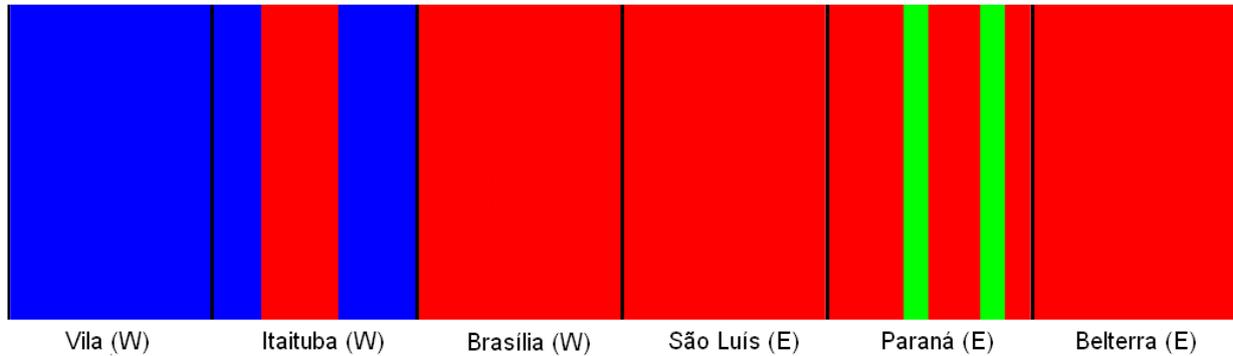


Figure 3. Barplot from the Bayesian Analysis of population structure of 48 individuals of *Allobates tapajos*. Distinct colors represent each of the three estimated genetic clusters. Individuals are displayed according to sampling localities identified in the lower panel.

Divergence Times Estimation

Bayesian analysis indicated *A. gasconi* as the sister species of *A. tapajos*. For *A. tapajos*, there was weak support for intraspecific cladogenetic events, with the exception of one clade composed exclusively of West Bank haplotypes, which includes the type locality of the species. The most basal division in *A. tapajos* was assigned to the Pleistocene (median = 1.58 million years (mya) and HPD = 0.79, 2.42), with most of the diversification events occurring over the last million years (Figure 4).

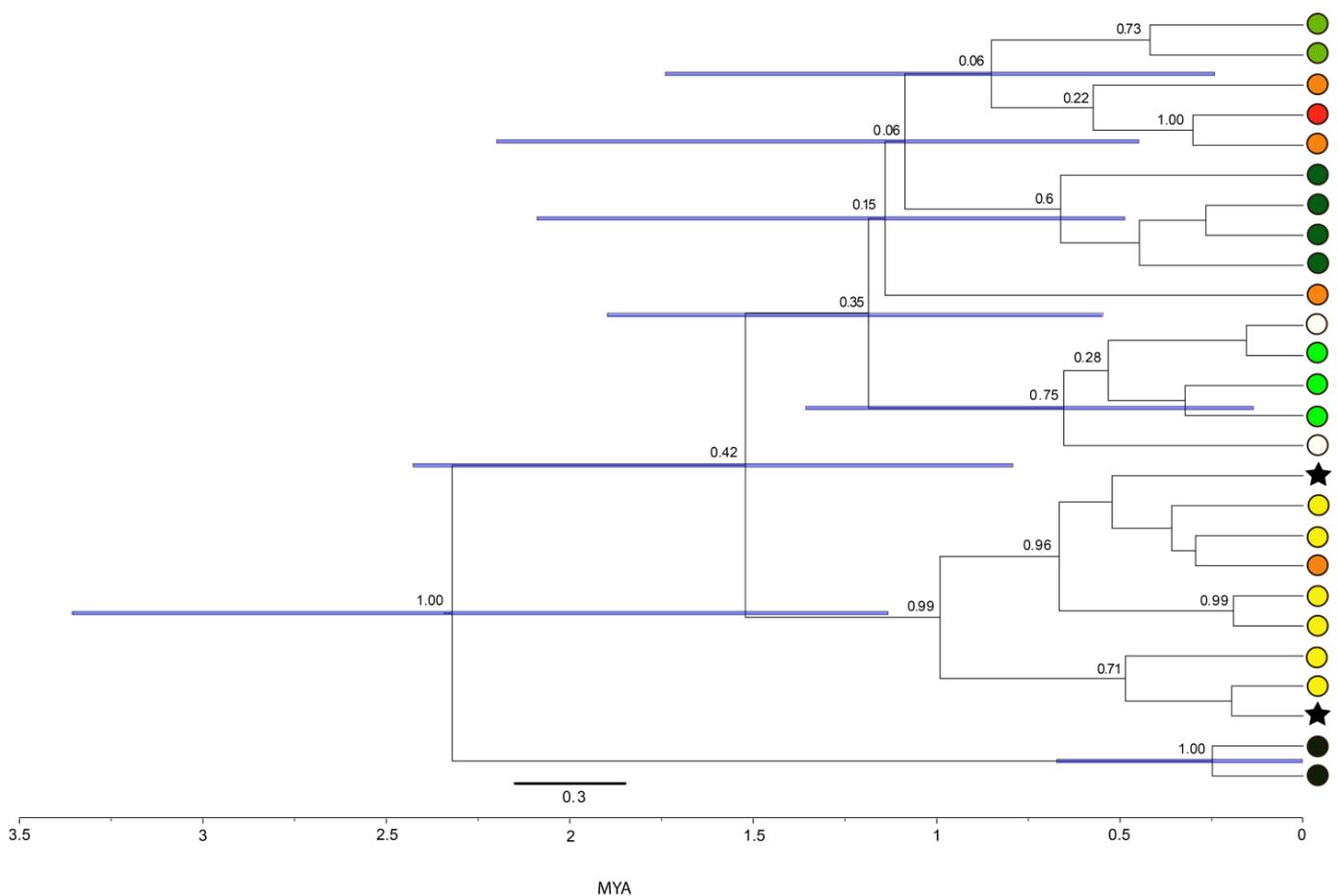


Figura 4. Timetree of *Allobates tapajos* based on fragments of the 16S rDNA gene. Only unique haplotypes were included. Age estimates and confidence intervals (horizontal bars) of the divergences were obtained via BEAST (Bayesian Evolutionary Analysis Sampling Trees). Posterior probabilities of the older nodes are presented. Terminals were colored according to their localities of origin, which are depicted in Figure 1. White terminals represent Genbank sequences from the east margin of the river (Curuá-Una). Black circles represent the outgroup. The stars depicts a Genbank sequence from the type locality of the species in the west margin of the Tapajós River.

Phenotypic differentiation

Discriminant function analysis correctly identified 70.0% of the source locations of individual *A. tapajos* based on acoustic characters. When using morphological characters, 66% of *A. tapajos* individuals were allocated correctly to their populations of origin (Supplemental Material 5-7). For the acoustic data, the two main components together explained 70% of variability in *A. tapajos*, while the first two morphological

components explained 43.34% of the data variability. The results of the generalized linear models showed significant difference in acoustic characteristics between the two river banks ($F = 5.485$ and $P = 0.007$, Figure A), while individuals from different sides of the river did not differ in morphometric characters ($F = 1.397$ and $P = 0.258$, Figure B).

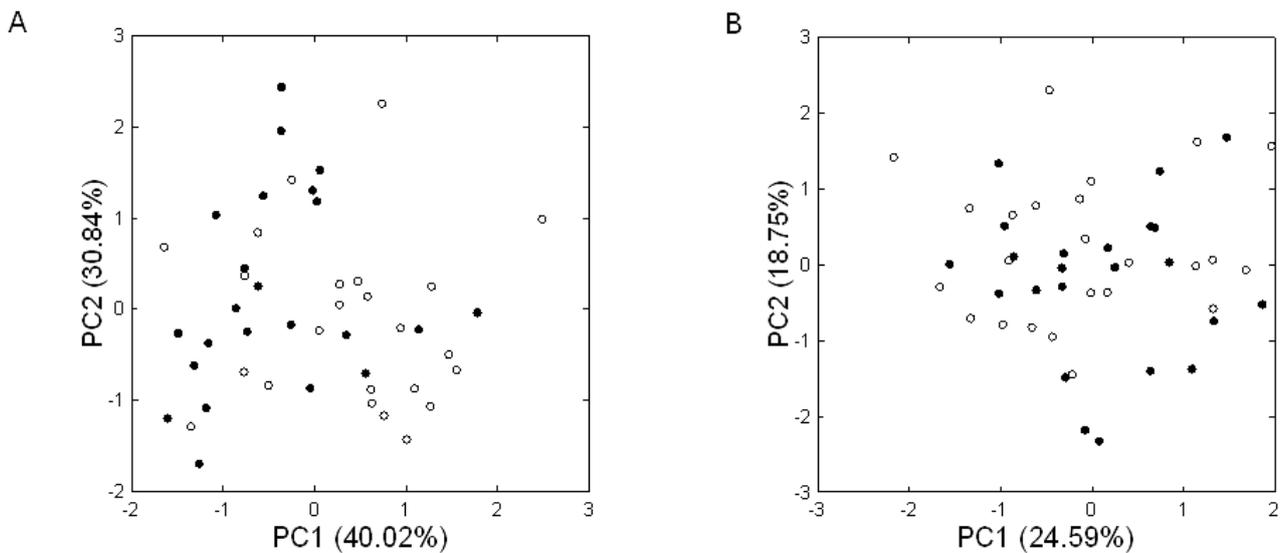


Figure 4. Distribution of individual scores of *Allobates tapajos* along the first and second principal components according to acoustic (A) and morphological (B) characters open and closed circles indicate individuals from the west and east riverbanks, respectively.

Correlation between geographical, phenotypic and genetic distances

Mantel tests showed no correlation between the different sets of tested data, even when the effects of the river and distance were controlled via partial models. The single exception was the correlation between the binary variable side of the river and acoustic distance (Table 3).

Table 3. Simple and partial Mantel tests evaluating correlations among geographic, phenotypic and genetic distances of *Allobates tapajos* from the sampled localities.

Model	<i>Allobates tapajos</i>	
	r	P
GenD X MorD	0.404	0.130
GenD X AcoD	0.434	0.055
MorD X AcoD	-0.167	0.216
GenD X GeoD.Riv	0.483	0.083
MorD X GeoD.Riv	0.108	0.350
AcoD X GeoD.Riv	-0.180	0.358
GenD X Riv.GeoD	0.393	0.095
MorD X Riv.GeoD	0.003	0.433
AcoD X Riv.GeoD	0.875	0.043*

Simple Mantel tests are presented as “Matrix 1” x “Matrix 2” and partial Mantel tests are presented as “Matrix 1” x “Matrix 2”. “Covariate matrix”. GenD genetic distance, GeoD geographic distance, MorD morphological distance, AcoD acoustic distance. Riverside (binary variable) is indicated as Riv. Significant correlations are indicated with asterisks.

Discussion

Overall, our results revealed a subtle, but spatially structured differentiation process in the analyzed character classes. While morphological and nuclear DNA characters exhibited, respectively, low and no variability over the study area, acoustic characters and mitochondrial DNA showed moderate spatially-structured differentiation, both among populations and between the spatial assemblages separated by the Tapajós River, the main geographical barrier in the study area.

The differing results obtained from nuclear and mitochondrial DNA markers reflect their different diversification rates (slower in nuclear DNA: Brown, Jr., & Wilson, 1979; Moritz, Dowling & Brown, 1987). Thus, the nuclear DNA is generally uninformative for the resolution of intraspecific phylogenies, sometimes even being monomorphic as in the current study. In contrast, mitochondrial DNA is widely used in

phylogeographic studies and barcode approaches in amphibians (Vences *et al.*, 2005b) and, in this study, was highly variable despite the low degree of divergence among populations and between the river banks. High levels of genetic structure in mitochondrial DNA, such as observed within the distribution *A. tapajos*, have often been reported in studies involving frogs (Carnaval & Bates, 2007; Hurzaid *et al.*, 2014), particularly in small-sized and territorial Neotropical species, which gene flow between sites is probably restricted due to low individual dispersion capacity (Kaefer *et al.*, 2013; Fouquet *et al.*, 2015). In *A. tapajos* effects of such high philopatry could be observed in: 1) the results of the Bayesian analysis of genetic structure where most of the locations did not show a mixture of gene clusters, 2) the significance levels of the F_{ST} values for all pairs of locations, and 3) the haplotype network in which there was restrict and no gene sequence sharing between localities and between opposite banks of the Tapajós River, respectively.

The influence of the Tapajós River on genetic variability of *A. tapajos* could be seen in the absence of haplotype sharing between populations on the two banks of the river, restriction of two BAPS groupings to different interfluvial regions, and in how the greatest values of genetic divergence were obtained between population pairs located on opposite sides of the river. The restriction of gene flow by Amazonian rivers is probably the biogeographic pattern most commonly reported in studies involving terrestrial vertebrates (Antonelli *et al.*, 2010; Ribas *et al.*, 2011; Leite & Rogers, 2013), including frogs (Kaefer *et al.*, 2013; Simões *et al.*, 2014; Fouquet *et al.*, 2015). The current study is the first to investigate the effect of the Tapajós River on genetic variability in a species of frog. Although the development of the Tapajós River drainage system is considered recent compared to others in the Amazon basin (1.3 - 0.8 mya; Ribas *et al.*, 2011), this river is widely known as a barrier that delimits areas of endemism in

Amazonia (Cracraft, 1985; Borges & Da Silva, 2012), phylogeographic patterns at both genus and species levels (Ribas *et al.*, 2011; Simões *et al.*, 2014), and even the distribution of terrestrial animal assemblages (Moraes *et al.*, 2016).

Analyzed phenotypic traits varied less between the sampled populations than did mtDNA. However, generalized linear models and the Mantel test showed variation in the analyzed acoustic characters between the banks of the Tapajós River. This result may be related to the high levels of morphological conservatism reported in amphibians (Schonrogge *et al.*, 2002; Bickford *et al.*, 2006), which has been held responsible for the likely high proportion of as-yet undetected species that are part of cryptic species complexes (Padial & De La Riva, 2009; Funk *et al.*, 2011). When analysed on single framework, it is often observed that acoustic characters show greater between-populations variability than do morphological markers (Simões *et al.*, 2008; Tsujii-Nishikido *et al.*, 2012). In fact, acoustic characters are reported to promote speciation processes in anurans via mate recognition and sexual selection (Boul *et al.*, 2007; Guerra & Ron, 2008).

Testing the correlation between different character classes and geographic factors can contribute to the understanding of the roles of stochastic (drift) and deterministic (selection) forces in differentiation processes. Often the correlation between the variation in any class of character and geographical distance (isolation by distance) is attributed to the effect of drift on the evolution of these characteristics (Hutchison & Templeton, 1999). In the current study, unlike the findings of larger-scale investigations on Amazonian frogs (Amézquita *et al.*, 2009; Kaefer *et al.*, 2013), no studied markers showed variation patterns that correlated with the linear distance between geographic locations even when the effect of the river was considered as a covariate. This suggests that the chosen markers may be under locale-specific selective

pressure. Similarly, Mantel tests detected no relationship between the variability of the analyzed characters and the transposition of the Tapajós River. A notable exception was the significant effect of the riverine barrier on the acoustic interpopulation distances, which supports the idea that such characters have greater evolutionary plasticity than morphological ones. Indeed, advertisement calls have been proposed as phylogeographical markers (Wycherley, Doran & Beebee, 2002a) for showing correlation with genetic distances between populations (Wycherley, Doran & Beebee, 2002b; Kaefer *et al.*, 2013). Failure to find a correlation between genetic and acoustic distances in *A. tapajos* is probably due to the low range of variation, especially of the mitochondrial marker, which showed maximum distance of only 1.3%.

In turn, correlation tests between different classes of genotypic and phenotypic markers can be used to provide clues about the relative evolution of different attributes in study populations (Pröhl *et al.*, 2006). However, except for a marginally significant relationship between genetic and acoustic distances, the current study did not find correlations of this nature. This result reinforces the utility of advertisement calls as phylogeographic markers and the great potential of this class of characters in studies addressing the role of sexual selection in speciation processes. Advertisement calls function as sexual signals, and thus can act as pre-zygotic barriers, via reduced preference and recognition between individuals from different populations should they ever meet (Ryan, 1988).

With regard to the specific status of the different sampled populations, the extent of haplotype restriction, genetic groupings and significant acoustic differentiation between the two banks of the river was not sufficient to delimit distinct taxonomic units under any operational concept of species requiring diagnostic character recognition (Goldstein & DeSalle, 2011). Even under theoretical species concepts, such as the

lineage concept (de Queiroz, 1998, 2007), the low genetic differentiation between populations from opposite sides of the river, and the non-monophyly of the clades that represent them suggest the occurrence of an incipient speciation process in the study system. This assumption is supported by estimates of a recent establishment of the Tapajós River drainage (Ribas *et al.*, 2011), as well as by the divergence times estimated in this study between the clades now occurring on each side the river, which proved to be congruent. Thus, the characterization of the spatial distribution of genotypic and phenotypic variability of *A. tapajos* illustrates a stage within the process of divergence that can serve as a model for investigating the early stages of speciation process.

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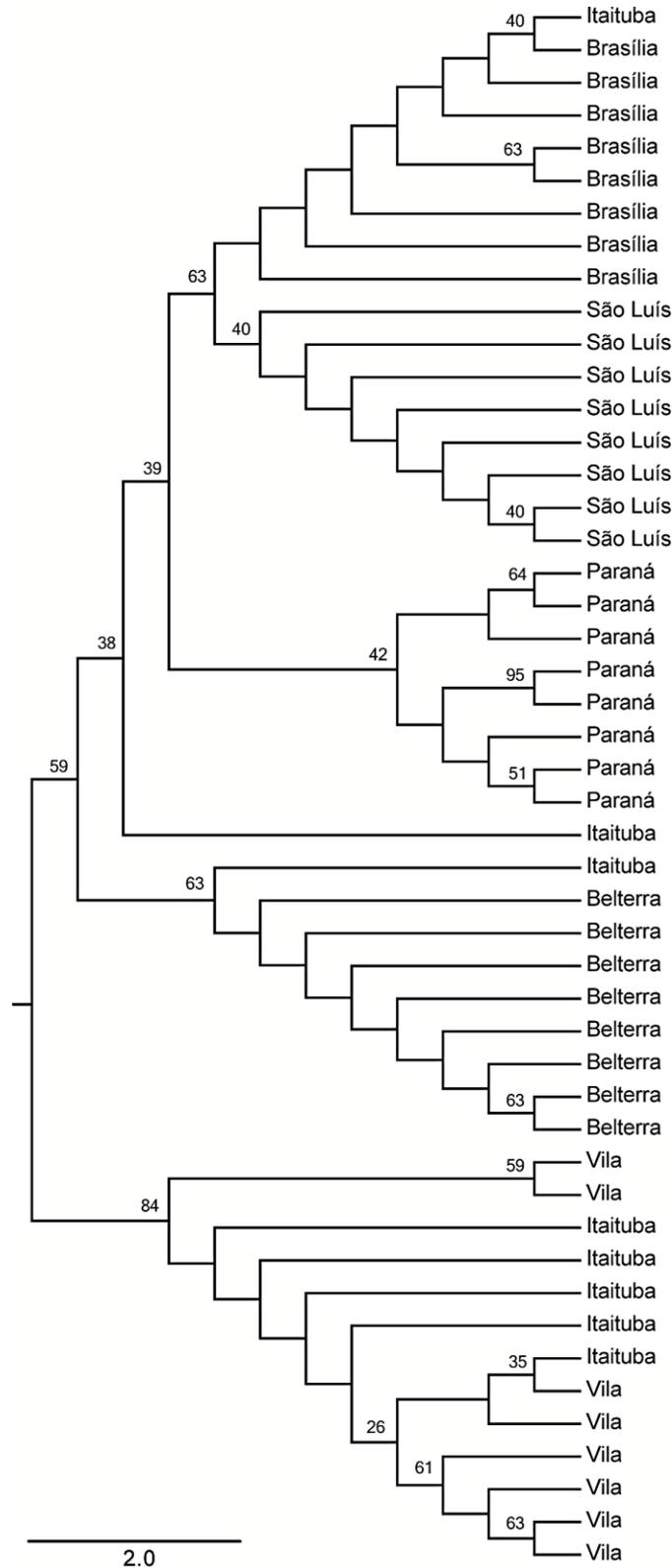
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SÍNTESE

- O padrão de diferenciação congruente entre os marcadores de DNA mitocondrial e bioacústicos sugerem que os trechos médio e baixo do Rio Tapajós exercem efeito sobre a distribuição espacial da variabilidade genotípica e fenotípica de *Allobates tapajos*.
- Ao considerarmos que marcadores de DNA nuclear e morfológicos, os quais apresentam taxas evolutivas reconhecidamente lentas, não responderam à transposição da barreira geográfica, é possível inferir que o processo de diferenciação entre populações de margens opostas do rio encontra-se em estágio inicial.
- A não-correlação entre os padrões de variabilidade espacial das diferentes classes de marcadores genotípicos e fenotípicos indica que estes podem ser sujeitos a pressões seletivas distintas. Alternativamente, podem simplesmente não ter desenvolvido divergência por conta da idade recente da barreira geográfica considerada, estimativa corroborada pela datação dos eventos de diversificação intraespecífica em *A. tapajos*.
- Reconhecendo-se os moderados níveis de variabilidade nas classes de caracteres investigadas, bem como a necessidade de caracteres diagnósticos para a designação de táxons em nível específico, observamos que populações de ambas as margens do rio pertencem à mesma espécie nominal.

SUPPLEMENTAL MATERIAL

SM1. Maximum likelihood tree generated on RaxML 7, using 48 individual fragments of 16S mtDNA (517 pb) from *Allobates tapajos*.



SM2. Morphometric variables measured from *Allobates tapajos*.

Morphometric variables	Acronym
Snout-vent length	SVL
Head length from angle of jaws to tip of snout	HL
Head width at level of angle of jaws	HW
Snout length from anterior corner of eye to tip of snout	SL
Eye to nostril distance from anterior corner of eye to centre of nostril	EN
Inter-nostril distance	IN
Eye length from anterior to posterior corner	EL
Inter-orbital distance	IO
Diameter of tympanum	TYM
Forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow	FAL
Upper arm length from trunk insertion to outer edge of flexed elbow	UAL
Hand length from proximal edge of palmar tubercle to tip of fingers I, II, III and IV	HAND1, HAND2, HAND3, HAND4
Tibia length from outer edge of flexed knee to heel	TL
Foot length from proximal edge of outer metatarsal tubercle to tip of toe IV	FL
Femur length	LL

SM3. Distribution of *16S* rDNA haplotypes of *Allobates tapajos* among 6 sampled localities sampled in Brazilian Amazonia. Collection numbers of vouchers (INPA-H) and GenBank accession numbers are provided concerning representative sequences.

Haplotype	Locality						Voucher	GenBank
	1	2	3	4	5	6		
H01		5					INPA-H036565	KX524470
H02		1	6				INPA-H036533	KX524471
H03		1					INPA-H036519	KX524472
H04		1					INPA-H036577	KX524473
H05					1		INPA-H036528	KX524474
H06					3		INPA-H036570	KX524475
H07					2		INPA-H036559	KX524476
H08					2		INPA-H036526	KX524477
H09				7			INPA-H036537	KX524478
H10				1			INPA-H036525	KX524479
H11	3						INPA-H036560	KX524480
H12	1						INPA-H036587	KX524481
H13	1						INPA-H036515	KX524482
H14	1						INPA-H036573	KX524483
H15	1						INPA-H036549	KX524484
H16	1						INPA-H036542	KX524485
H17			2				INPA-H036562	KX524486
H18						6	INPA-H036544	KX524487
H19						1	INPA-H036584	KX524488
H20						1	INPA-H036568	KX524489

SM4. Haplotype distribution RAG1 nuDNA of *Allobates tapajos* between 6 sampled locations.

Haplotype	Locality						Voucher	GenBank
	1	2	3	4	5	6		
H01	2	3	4	2	2	3	INPA-H036549	KX524492

SM5. Morphometric measurements (in mm) of individuals of *Allobates tapajos* in each study locality in Brazilian Amazonia. Values are presented as mean (above) and standard deviation (below). Morphometric traits are described in ME2.

Locality	SVL	HL	HW	SL	EN	IN	EL	IO	TYM
Vila	15.50 ± 0.74	4.23 ± 0.06	5.11 ± 0.06	1.78±0.15	1.38±0.1	2.21±0.05	2.71±0.06	4.42±0.09	0.83±0.04
Itaituba	15.85 ± 0.44	4.41 ± 0.08	4.98 ± 0.18	1.79 ± 0.08	1.34 ± 0.07	2.13±0.07	2.63±0.07	4.32±0.12	0.81±0.05
Brasília	15.08 ± 0.38	4.29 ± 0.08	4.91 ± 0.14	1.78±0.08	1.32±0.07	2.09±0.06	2.64±0.08	4.25±0.11	0.84±0.06
São Luiz	15.13 ± 0.43	4.29 ± 0.11	4.93 ± 0.14	1.74±0.12	1.36±0.07	2.08±0.09	2.7±0.12	4.27±0.14	0.8±0.06
Paraná	15.24 ± 0.61	4.29 ± 0.09	5.04 ± 0.06	1.75 ± 0.14	1.31±0.09	2.09±0.09	2.71±0.11	4.29±0.08	0.85±0.05
Belterra	15.40 ± 0.52	4.17 ± 0.15	5.03 ± 0.09	1.85±0.10	1.41±0.1	2.09±0.08	2.68±0.11	4.26±0.11	0.77±0.07

Locality	FAL	UAL	HANDI	HANDII	HANDIII	HANIV	TL	FL	LL
Vila	3.81±0.22	3.96±0.22	3.55±0.16	3.33±0.24	3.73±0.11	2.37±0.17	9.14±0.26	7.50±0.29	6.59±0.023
Itaituba	3.67±0.22	3.90±0.30	3.03±0.28	3.09±0.34	3.65±0.08	2.25 ±0.10	9.05±0.18	7.16±0.34	6.77±0.25
Brasília	3.67±0.16	3.98±0.21	3.25±0.19	3.08±0.23	3.56±0.15	2.17±0.19	8.95±0.33	7.34±0.22	6.61±0.20
São Luiz	3.75±0.12	3.93±0.19	3.27±0.21	3.11±0.16	3.52±0.14	2.25±0.12	8.95±0.22	7.34±0.14	6.98±0.26
Paraná	3.65±0.20	3.91±0.12	2.96±0.25	2.79±0.28	3.60±0.10	2.25±0.09	8.85±0.27	6.87±0.65	6.77±0.18
Belterra	3.75±0.18	3.94±0.32	3.43±0.19	3.24±0.26	3.62±0.11	2.34±0.13	9.08±0.20	7.20±0.14	6.83±0.23

SM6. Advertisement call measurements of individuals of *Allobates tapajos* in each study locality in Brazilian Amazonia. Values are presented as mean (above) and standard deviation (below).

Locality	Note duration	Note interval	Call interval	Lowest frequencies	Highest frequencies	Maximum frequencies	Note repetition rate
Vila	0.032 ± 0.004	0.133 ± 0.008	0.458 ± 0.057	5134.669 ± 169.219	6012.491 ± 138.768	5743.913 ± 201.02	6.136 ± 0.41
Itaituba	0.032 ± 0.003	0.150 ± 0.02	0.471 ± 0.1	5189.903 ± 208.092	6093.966 ± 203.883	5759.529 ± 163.42	5.613 ± 0.62
Brasília	0.033 ± 0.003	0.130 ± 0.014	0.443 ± 0.048	5155.679 ± 97.081	5977.086 ± 130.575	5691.429 ± 193.13	6.199 ± 0.58
São Luiz	0.031 ± 0.002	0.159 ± 0.027	0.413 ± 0.071	5020.258 ± 104.902	5870.823 ± 144.939	5571.984 ± 206.74	5.407 ± 0.69
Paraná	0.034 ± 0.002	0.158 ± 0.015	0.450 ± 0.046	4988.171 ± 106.884	5889.621 ± 120.791	5635.196 ± 165.00	5.262 ± 0.454
Belterra	0.030 ± 0.003	0.132 ± 0.012	0.429 ± 0.054	5040.444 ± 168.556	5913.470 ± 188.836	5543.593 ± 227.85	6.243 ± 0.384

SM7. Classification matrix based on the discriminant function analysis, using phenotypic data from males of *Allobates tapajos* from the east and west banks of the Tapajos River, Brazil.

Jackknife classification			
	East	West	% correct
Bioacoustics			
East	17	6	74
West	8	16	67
Total	25	22	70
Morphology			
East	15	8	65
West	8	16	67
Total	23	24	66

APÊNDICES


 Ministério da
Ciência, Tecnologia
e Inovação


AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): **GABRIELA FARIAS MAIA**
 Curso: ECOLOGIA
 Nível: Mestrado
 Orientador(a): Dr. Igor Luis Kaefer (UFAM)
 Coorientador(a): Dra. Albertina Pimentel Lima (INPA)

Título

“Diversificação evolutiva em *Allobates* sp. (Anura: Aromobatidae) no Rio Tapajós”

BANCA JULGADORA:

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 Rafael do Nascimento Leite (INPA)

SUPLENTE:

Camila Cherem Ribas (INPA)
 Sérgio Henrique Borges (FVA)

PARECER

ASSINATURA

MARCELO MENIN	<input checked="" type="checkbox"/> Aprovado () Reprovado	<i>Marcelo menin</i>
FERNANDA DE PINHO WERNECK	<input checked="" type="checkbox"/> Aprovado () Reprovado	<i>Fernanda Werneck</i>
RAFAEL DO NASCIMENTO LEITE	<input checked="" type="checkbox"/> Aprovado () Reprovado	<i>Rafael Leite</i>
CAMILA CHEREM RIBAS	() Aprovado () Reprovado	_____
SÉRGIO HENRIQUE BORGES	() Aprovado () Reprovado	_____

Manaus(AM), 10 de Abril de 2015

OBS: _____

INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA INPA
 PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA PPG-ECO
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Ministério da
Ciência, Tecnologia
e Inovação



ATA DA DEFESA PÚBLICA DA
DISSERTAÇÃO DE MESTRADO DO
PROGRAMA DE PÓS-GRADUAÇÃO EM
ECOLOGIA DO INSTITUTO NACIONAL
DE PESQUISAS DA AMAZÔNIA.

Aos 12 dias do mês de julho do ano de 2016, às 09h00min, no Auditório dos PPG's ATU/CFT/ECO - INPA/Campus III/V8. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Marcelo Menin**, da Universidade Federal do Amazonas - UFAM, o(a) Prof(a). Dr(a). **Marina Anciães**, do Instituto Nacional de Pesquisas da Amazônia - INPA e o(a) Prof(a). Dr(a). **Sérgio Henrique Borges**, do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes o(a) Prof(a). Dr(a). Rafael do Nascimento Leite, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a). Dr(a). Jansen Alfredo Sampaio Zuanon, do Instituto Nacional de Pesquisas da Amazônia - INPA, sob a presidência do(a) primeiro(a), a fim de proceder a arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **GABRIELA FARIAS MAIA**, intitulado: "**Genes, formas e sons revelam estágio incipiente de especiação alopatrica no anuro amazônico *Allobates tapajos* (Dendrobatidae)**" Orientado pelo(a) Prof(a). Dr(a). Igor Luís Kaefer, da Universidade Federal do Amazonas - UFAM e co-orientado pelo(a) Prof(a). Dr(a). Albertina Pimentel Lima, do Instituto Nacional de Pesquisas da Amazônia - INPA .

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

APROVADO(A)

REPROVADO(A)

POR UNANIMIDADE

POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Marcelo Menin

Marcelo Menin

Prof(a).Dr(a). Marina Anciães

Marina Anciães

Prof(a).Dr(a). Sérgio Henrique Borges

Sérgio Henrique Borges

Prof(a).Dr(a).Rafael do Nascimento Leite

Prof(a).Dr(a). Jansen Alfredo Sampaio Zuanon

Rosalei A. Galvão Leite
p/ Coordenação PPG-ECO/INPA