

Gregory Thom

Filogeografia comparada de aves de várzea baseada em sequências  
de elementos ultra conservados: reconstruindo padrões  
biogeográficos da Amazônia

Comparative phylogeography of floodplain specialist birds based  
on sequences of ultra conserved elements: inferring Amazonian  
biogeographic patterns

São Paulo

2018

Gregory Thom

Filogeografia comparada de aves de várzea baseada em sequências  
de elementos ultra conservados: reconstruindo padrões  
biogeográficos da Amazônia

Comparative phylogeography of floodplain specialist birds based  
on sequences of ultra conserved elements: inferring Amazonian  
biogeographic patterns

Tese apresentada ao Instituto de  
Biotecnologia da Universidade de São  
Paulo, para a obtenção de Título de  
Doutor em Ciências Biológicas, na  
Área de Biologia – Genética,  
Departamento de Genética e Biologia  
Evolutiva

Orientador(a): Cristina Yumi Miyaki

São Paulo

2018

	<p>Thom, Gregory Filogeografia comparada de aves de várzea baseada em sequências de elementos ultra conservados: reconstruindo padrões biogeográficos da Amazônia 232 páginas</p> <p>Tese (Doutorado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Genética e Biologia Evolutiva.</p> <p>1. Diversificação 2. Biodiversidade 3. Demografia histórica 4. Região Neotropical I. Universidade de São Paulo. Instituto de Biociências. Departamento de Genética e Biologia Evolutiva</p>
--	--

### Comissão Julgadora:

---

Prof.(a) Dr.(a)

---

Prof.(a) Dr.(a)

---

Prof.(a) Dr.(a)

---

Prof.(a) Dr.(a)

---

Profª. Dra. Cristina Yumi Miyaki  
Orientadora

“The Universe is under no obligation  
to make sense to you”  
Neil deGrasse Tyson

Nesta Tese foram inferidos os processos históricos que podem ter influenciado a evolução da comunidade de Aves restritas às planícies alagadas amazônicas baseado na análise de seus padrões de diversificação associados a dados geológicos e climáticos. Foram selecionados três complexos de espécies amplamente distribuídos pela bacia Amazônica e intimamente associados às florestas de beira de rio, *Myrmoborus lugubris*, *Thamnophilus nigrocinereus*/*T. cryptoleucus* e *Myrmotherula assimilis*. Para acessar a diversidade genética e realizar análises filogenéticas e demográficas das espécies foi realizada a captura e sequenciamento de ~2.300 Elementos Ultra Conservados. A Tese foi dividida em três capítulos que abordam aspectos distintos sobre a diversificação desses organismos. No Capítulo 1, *M. lugubris* foi estudado com o intuito de explorar os efeitos de processos microevolutivos, em particular fluxo gênico e sorteamento incompleto de linhagens, na reconstrução filogenética das populações deste complexo. Esse estudo demonstrou a problemática em se estimar árvores de espécies em cenários recentes de diversificação utilizando métodos que não acomodam fluxo gênico como um parâmetro, e explorou o histórico evolutivo desse complexo de espécies, mostrando a presença de fluxo gênico entre populações não irmãs e a presença de uma zona de hibridação com um potencial cenário de heterose. No Capítulo 2 foi realizado um estudo filogeográfico comparado dos três complexos de espécies que sugeriu que ciclos climáticos ocorridos ao longo do Pleistoceno, que parecem ter alterado o padrão de sedimentação e formação de florestas de beira de rio, tiveram importante papel na diversificação deste conjunto de organismos, promovendo períodos de alopatria seguidos de contato secundário. No terceiro Capítulo da Tese exploramos como a atual diversidade genética de populações restritas ao Rio Solimões está distribuída no espaço e se alterações demográficas históricas poderiam estar relacionadas à expansão nas distribuições geográficas. Nesse estudo encontramos sinal de expansão geográfica para *T. cryptoleucus*, mas não para *M. lugubris* e *M. assimilis*, sugerindo que em uma escala intrapopulacional variáveis ecológicas intrínsecas aos táxons estudados devem promover padrões distintos de diversidade genética. Ainda nesse estudo foi encontrado que a atual diversidade genética desses complexos espécies está distribuída de forma heterogênea na paisagem. Os dados apresentados nesta Tese permitiram testar de forma inédita hipóteses de diversificação para a comunidade de Aves que ocorre ao longo de toda a bacia Amazônica, sugerindo que organismos de ambientes pouco explorados por estudos filogeográficos podem revelar novas facetas sobre a diversificação da elevada diversidade Amazônica.

This Ph.D. Dissertation inferred the historical processes that seem to have built the avian community assemblage restricted to the Amazonian floodplains based on their patterns of diversification and geological and climatic data. We analyzed three species complexes widely distributed over the Amazon Basin and specialists of river edge forests, *Myrmoborus lugubris*, *Thamnophilus nigrocinereus*/*T. cryptoleucus*, and *Myrmotherula assimilis*. In order to access their genetic diversity and perform phylogenetic and demographic analyses, we captured and sequenced ~2,300 Ultra Conserved Elements. This Dissertation was subdivided into three chapters that discuss distinct diversification aspects of these taxa. In Chapter 1, we explored the effects of microevolutionary processes in *M. lugubris*, especially gene flow and incomplete lineage sorting in phylogenetic reconstructions of its populations. We demonstrated the potential bias of estimating species tree without accommodating gene flow in recent scenarios of divergence. Additionally, we explored the evolutionary history of this lineage, supporting the presence of gene flow between nonsister populations and a hybrid zone with potential heterosis. In Chapter 2 we performed a comparative phylogeographic study including all three species complexes. The results indicated that climatic oscillations during the Quaternary that altered the pattern of sedimentation and formation of river edge forests seem to have promoted cyclical periods of allopatry and secondary contact. In the third chapter we explored how the genetic diversity of populations restricted to the Solimões river is currently distributed in space and if historical demographic alterations could be related to geographic range expansions. A signal of range expansion was detected only for *T. cryptoleucus* but not for *M. lugubris* and *M. assimilis*, suggesting that at the intra-population scale species-specific ecological variables may promote distinct patterns of genetic diversity. However, despite the absence of a shared pattern of range expansion the genetic diversity of each of the three taxa is heterogeneously distributed in the landscape. The data presented in this Dissertation allowed an unprecedented test of diversification hypotheses for the bird community that occurs throughout the Amazonian floodplains, suggesting that organisms from environments poorly explored by phylogeographic studies may reveal new facets about the diversification of the Amazonian diversity.

Wild populations are subjected to changes in habitat distribution such as expansions, connections among previously isolated areas, contractions and fragmentations. From an evolutionary perspective, these fluctuations can affect patterns of genetic diversity, population structure and gene flow (Avice 2000). Diversification patterns are influenced by historical events such as climatic and geomorphological changes, current landscape conditions including anthropogenic alterations and/or ecological conditions and intrinsic characteristics of the organisms under study. Hence, understanding the mechanisms of speciation and diversification can assist strategies for biodiversity conservation and to predict the effects of future climatic alteration on natural communities (Moritz and Faith 1998; Moritz 2002; Taberlet and Cheddadi 2002; Williams et al. 2007; Brown et al. 2016; Prates et al. 2016).

Phylogeography aims to understand the principles and processes behind the current geographic distribution and demography of intra-specific lineages or closely related species based on the spatial distribution of genealogies (Avice et al. 1987; Avice 2000). Thus, it can help to reveal mechanisms of speciation and diversification. Historical alteration in climate and landscape may affect genealogical patterns of an entire biological community, and if a process is significant enough, different organisms should present concordant genealogies (Avice et al. 1987; Avice 2000; Soltis et al. 2006; Hickerson et al. 2010). However, variation in the capacity of individuals to respond to changing conditions may increase variation among diversification processes (Smith et al. 2014). Avice (2000) described four levels of genealogical concordance in phylogeography: 1) concordance among sites within a locus indicating genetic structure; 2) concordance among multiple loci providing genomic evidence for a specific pattern; 3) concordance among multiple co-distributed lineages that indicates a similar pattern in the geographic distribution of the genetic structure providing evidence of a shared diversification process; 4) correlation between a genetic

pattern shared by various taxa and previously described biogeographic information supporting historical factors that shaped the distribution of genetic lineages.

Initially, the molecular marker mostly used in phylogeographic studies of animals was the mitochondrial DNA (mtDNA) given its intrinsic characteristics such as the apparent lack of recombination, putative neutrality and haploid matrilineal inheritance with smaller effective population size when compared to autosomal loci leading to more pronounced effects of genetic drift and lineage sorting (Avice 2000). However, the use of a single locus to infer concordance among distinct levels of phylogeography can be compromised as explained as follows (Edwards & Beerli 2002; Edwards 2009; Brito & Edwards 2009). The theoretical basis of phylogeography is anchored on population genetics and the coalescent theory, which mathematically describes the probability of a given group of alleles to coalesce into a common ancestral over the generations backwards in time (Felsenstein 2008). With the continuous development of the coalescent theory, it became possible, based only on sampled alleles, to model virtually any complex diversification scenario and estimate demographic parameters including current and historical population sizes, divergence times and migration rates (Wakeley 2009). However, single locus mtDNA data can dramatically bias and limit demographic parameter estimation using the coalescent (Edwards 2009). For example, analyses performed with a single locus or with multiple concatenated loci can produce statistically supported topologies that are not in agreement with the evolutionary history of a species, especially in recent scenarios of diversification (Kubatko & Degnan 2007; Degnan & Rosenberg 2009). Additionally, it can generate overestimated divergence times since the coalescence process of a gene to a common ancestor usually occurs after the true cladogenetic event backwards in time (Maddison & Knowles 2006; Carstens et al. 2007; Edwards et al. 2007; Heled & Drummond 2010).

The main causes of incongruences among genes' history and species' history are related to the fact that genes can be independently affected by distinct evolutionary processes such as

horizontal gene transfer, duplication/deletion, incomplete lineage sorting (ILS), selection and hybridization (Degnan & Rosenberg 2009; Huang & Knowles 2009). Recent studies have been showing that when natural variation among independent loci is not taken into account statistical power of demographic parameters is reduced and phylogenies with taxa that are not reciprocally monophyletic are hardly resolved (Carstens & Knowles 2007). These reasons resulted in a fast transition from the single locus mtDNA to multilocus data sets (McCormack et al. 2013).

Another important shift in phylogeography is related to the use of coalescent models for statistically test competing hypotheses and estimate demographic parameters, known as statistical phylogeography (Knowles & Maddison 2002; Hickerson et al. 2010). In its initial phase phylogeographic inferences were based on quantitative interpretations such as the shape of a topology, the presence of geographic structured clades, the time of divergence among branches or specific summary statistics that described the data (Slatkin & Maddison 1989; Tajima 1989; Excoffier et al. 1992; Fu & Li 1993). In statistical phylogeography, distinct demographic hypotheses/models are evaluated under Bayesian or likelihood-based approaches and the probabilities of fitting the observed data of each model are compared (Knowles 2009). Under this procedure, models are never treated as the complete history of a taxon but it tests important parameters that describe alternative demographic histories (Anderson 2007; Wakeley 2004). A commonly used approach in statistical phylogeography is the Approximate Bayesian Computation (ABC), which provides a rigorous statistical method to test for competing *a priori* hypotheses, and its use has grown considerably in recent years (Beaumont et al. 2002; Beaumont et al. 2010; Bertorelle et al. 2010; Huang et al. 2011). This Bayesian approach estimates the posterior distribution of model parameters by replacing the likelihood computation by a measure of similarity between observed and simulated data, reducing the complexity of the data to summary statistics, allowing to test complex and more realistic diversification scenarios and also to use large datasets (Beaumont et al. 2010).

The demand for larger multi-loci data sets in order to obtain more robust parameter estimation has encouraged the development and application of new DNA sequencing methods so-called next-generation sequencing (NGS), which is less expensive and laborious than Sanger sequencing that requires isolated amplification and sequencing of each marker for each individual. In NGS platforms it is possible to combine multiple individuals in a massively parallel sequencing of thousands of loci that are later processed with bioinformatic pipelines (McCormack et al. 2013). While whole-genome sequencing techniques are still restrictive for most phylogeographic studies, due to the elevated costs and analytical complexity (McCormack et al. 2013), reduced genomic representation techniques are quickly becoming standard methods in the field (Lerner & Fleischer 2010; McCormack et al. 2013). Despite the quick development and relatively large number of new sequencing methods, there are limitations to be taken into account (Rokas & Abbot 2009; Lerner & Fleischer 2010; Lemmon & Lemmon 2012; McCormack et al. 2012; McCormack et al. 2013). Among the most common limitations are: 1) in general phylogeographic studies are focused on non model organisms (without reference genomes), which makes it difficult to establish homology among loci from different individuals; 2) the need for large numbers of individuals per population, producing intractable data sets for most of the available full-likelihood methods; 3) lack of consensus between protocols for genomic library preparation; and 4) arbitrary threshold for data filtering parameters in bioinformatics pipelines (McCormack et al. 2012, 2013; Harvey et al. 2016).

Among subgenomic sequencing methods currently applied to phylogeography, sequence capture or target enrichment methods have overcome some limitations presented above. This procedure involves the capture of conserved genomic regions by using DNA or RNA probes that hybridize with target DNA enabling the retention of captured fragments that can be sequenced by NGS platforms (Gnirke et al. 2009). This method uses probes that are analogous to primers used in PCR for Sanger sequencing that anneal to specific conserved regions in the DNA of the organisms being studied (eg. mammals and birds; McCormack et al. 2012). When compared to other NGS

methodologies, as restriction digest sequencing (RADseq; Van Tassel et al. 2008), sequence capture does not produce a random genomic representation that demands reference genomes. Among the benefits of sequence capture are: 1) easy selection of locus size; 2) probes are usually widely distributed along the genome; 3) probes usually target regions with low copy number reducing paralogy; 4) it is possible to obtain larger loci by using interconnected probes increasing the information content of each locus and gene tree resolution (McCormack et al. 2013).

Among the targeted genomic regions by sequence capture approaches, ultra conserved elements (UCEs) have shown promising results in distinct timescales (Faircloth et al. 2012; Smith et al. 2014; Manthey et al. 2015; Harvey et al. 2016). UCEs are conserved genomic regions between 50 bp to 200 bp shared among highly divergent lineages (Bejerano et al. 2004). Initially described in the alignment of human and mouse genomes, UCEs were then identified in several groups of vertebrates (Stephen et al. 2008; Janes et al. 2011; Crawford et al. 2012), invertebrates and fungi (Siepel et al. 2005). The functions of UCEs are still poorly understood, but so far studies suggest that part of these markers are related to non coding regions associated to gene regulation and early-stage development (Bejerano et al. 2004; Woolfe et al. 2005; Ahituv et al. 2007; Warnefors et al. 2016; Terracciano et al. 2017). The advantages of using sequence capture of UCEs compared to other markers and sequencing techniques are: 1) reduced overlap with duplicated areas of the genome (Derti et al. 2006); 2) flanking regions progressively less conserved, enabling phylogenetic and phylogeographic studies in distinct time scales (Faircloth et al. 2012); 3) probes are used as reference enabling straightforward identification of orthologous contigs. Even though most of the studies based on UCEs focus on deep phylogenies (classes, orders, families, and genus), recent studies reported well supported intraspecific relationships and demographic parameters (Smith et al. 2013; Manthey et al. 2015; Harvey et al. 2016). Smith et al. (2013) tested the applicability of UCEs in shallow diversification scenarios in five bird lineages with populations occurring in four Neotropical areas of endemism (Central America, Choco, Napo, and south of Amazon River). The

results, revealed a similar diversification pattern to those obtained with mtDNA but with more recent estimated divergence times and a drastic reduction in the posterior distribution of demographic parameters, suggesting a more precise estimation. The results obtained by Smith et al. (2013) indicated that UCEs can be successfully used in phylogeographic studies including demographic parameter estimation increasing the resolution obtained with mtDNA.

### *Amazonian biogeography*

One of the greatest challenges in biogeography is to understand how the high biodiversity of tropical environments was generated and how historical processes and ecological traits acted enabling a large number of species to co-exist (Wiens et al. 2011). Amongst tropical regions, the Amazon (*sensu lato*) stands out as the largest fluvial basin with more than 5.5 million km<sup>2</sup>, around 40% of the remaining tropical forests in the world, as well as one of the greatest biodiversity in the planet (Silva et al. 2005; Irion & Kalliola 2010). Despite the fact that new species are still being described, the Amazon basin harbors at least 7,500 species of Lepidoptera, 1,500 species of birds and around 112,000 species of trees (Hubbell et al. 2008; Hoorn & Wesselingh 2010). The megadiversity of the Amazon fauna has led to the formulation of different biogeographic hypotheses and speciation models (Antonelli et al. 2010), mostly based on shared species distribution patterns and phylogeographic data correlated to historical events (Wallace 1852; Haffer 1969, 2001; Moritz et al. 2000; Ribas et al. 2011). Since the beginning of the 19<sup>th</sup> century, the high diversity associated with complex geographic distributions instigated naturalists and researchers to propose hypotheses that explain the formation of the current Amazonian biodiversity (Wallace 1852; Haffer 1969, 2001). These hypotheses were mostly based on congruent biogeographic patterns, suggesting that certain common cladogenetic events were determinant for the formation of the present diversity (Haffer 2001). The application of molecular biology tools in phylogenetic and population genetics of Amazonian taxa has allowed testing some of these hypotheses, including

Amazonian birds (Aleixo 2004; Fernandes et al. 2012, 2014; d'Horta 2013 et al.; Capurcho et al. 2013). However, the lack of hierarchical division in spatial and temporal assumptions does not allow mutual exclusiveness among most hypotheses, blurring the effects of particular historical events on the formation of the current genetic diversity (Patton & Silva 1998; Hall & Harvey 2002). Additionally, intrinsic characteristics of each organism studied such as ecology and the evolutionary origin tend to produce distinct dispersal probabilities over physical barriers, suggesting an overall complex scenario that cannot be explained by a single generalized hypothesis (Burney & Brumfield 2009; Smith et al. 2014).

Among the most discussed hypotheses for the diversification of terrestrial vertebrates in the Amazon, past forest refugia have long been adopted to explain the origin of the Amazon diversity (refugia hypothesis; Haffer 1969). Evidence supporting this hypothesis have been observed in the Northern Hemisphere (Lessa et al. 2003; Hewitt 2000) and the Neotropical region, in the Atlantic Forest (Carnaval et al., 2009). Although some studies on Amazon birds reported demographic expansions for some populations (Aleixo 2004; Fernandes et al., 2012), a direct relationship between these forest range oscillations and cladogenetic events were not observed, refuting the main assumption of this hypothesis. Phylogeographic studies on Amazonian birds and mammals that refute the refugia hypothesis usually assume that large rivers are effective barriers to gene flow between populations of opposite margins (riverine hypothesis; Wallace, 1854), suggesting that the formation of the current Amazon basin produced the observed distribution patterns (Fernandes et al. 2012; Ribas et al. 2011; Boubli et al. 2015; Thom & Aleixo 2015). Despite the intense debate around the refugia and riverine hypotheses, it is common sense that several other processes may have operated on the formation of the current Amazon diversity (Bush 1994; Haffer 2001; Leite & Rogers 2013).

The increase in the number of phylogeographic studies, as well as the reconstruction of paleobiogeographic models (Aleixo 2004; Solomon et al. 2008; Patel et al. 2011; Weir & Price

2011; Ribas et al. 2011), have shed light on the formation history of the Amazon basin. However, this understanding is still sparse when compared to the high and complex biological diversity in this region. Nevertheless, this complexity in patterns and processes tends to increase since, in its vast majority, phylogeographic studies have been based on organisms restricted to the non-flooded ombrophilous forests (upland forests), the most abundant and diverse environment in the biome (Silva et al. 2005; Capurucho et al.2013).

### *Biogeography of floodplain forest species*

Studies of upland species of vertebrates, mainly birds and mammals, set the basis for the current knowledge on Amazonian biogeography (Haffer 1969; Cracraft 1985; Ribas et al. 2011; D’Horta et al. 2013). However, organisms occurring in other kinds of environments such as open areas (eg. meadows and campinaranas) and floodplains have rarely been subjects of phylogeographic studies. These organisms tend to respond differently to the same historical events or could be affected by processes that did not act on upland forest species (Capurucho et al. 2013).

The Amazonian floodplains house the most diverse and largest flooded forests in the world, covering more than 300,000 km<sup>2</sup>, with high levels of species endemism - approximately ten percent of tree species and 15 percent of non aquatic bird species are endemic (Remsen & Parcker 1983; Wittmann et al. 2006; Junk et al. 2011; Wittmann et al. 2012). Despite its large diversity and relatively easy access, endemic terrestrial species are poorly represented in phylogeographic studies and the potential diversification processes shaping the current genetic diversity are poorly understood resulting in unknown levels of cryptic diversity (Aleixo 2006; Cadena et al. 2011; O’Neil et al. 2011; Harvey et al. 2017). This is critical since this is a highly threatened environment due to habitat conversion for agriculture and energy production by hydroelectric power plants (Albernaz 2011; Latrubesse et al. 2017) with several endangered endemic species (BirdLife international 2016).

The linear connectivity of floodplains and other flooded habitats along Amazonian rivers potentially allows adapted species to disperse freely, forming panmictic populations (Aleixo et al., 2006). Annual fluctuations in the level of the rivers in Amazonian floodplains resulted in cycles characterized by intense sediment and nutrient transportation resulting in constant changes in river channels and river-created environments (Wittmann et al. 2006; Junk et al. 2011). Thus the dynamics of this environment may lead to selection for better dispersion abilities (Remsen & Parker et al., 1983). For these reasons, it is more likely that taxa adapted to floodplains do not have the same population structure as upland species, whose distributions are generally subdivided by large rivers (Ribas et al. 2011; Smith et al., 2014). The few phylogeographic studies of terrestrial vertebrates occupying flooded environments supported this scenario of lack of genetic structure between populations distributed throughout the entire Amazon basin with lower levels of genetic diversity when compared to upland forest species (Aleixo et al. 2006; Cadena et al. 2011; Harvey et al. 2017). However, when Cohn-Haft et al. (2007) characterized the geographical distribution of *várzea* bird, they described three zones of endemism for the Amazonian floodplains, suggesting that the region separating the Solimões and Amazonas rivers is a suture zone with a gradual transition between distinct communities. Similarly, community turnovers based on alpha diversity were described in several taxonomic groups including fishes, spiders, and plants, suggesting a shared pattern of diversification that so far was not properly explored with molecular tools (Hubert & Renno 2006; Albernaz et al. 2011; Vinticinque et al. 2007).

Choueri et al. (2017), while describing the landscape genetics of four antbird species along the Negro river archipelagos, included individual samples from other large Amazonian rivers (Madeira, Solimões, and Amazonas) and found a considerable level of divergence between samples restricted to distinct rivers. Despite the low number of samples used by Choueri et al. (2017), this geographically structured pattern suggested that historical events might have affected the diversification of distinct lineages of these lowland bird species. Thus, comparative

phylogeographic studies focusing on taxa restricted to Amazonian floodplains with phenotypic structure along the main Amazonian tributaries (as the ones used by Cohn-Haft et al. [2007] and Choueri et al. [2017]) can reveal new scenarios and drivers for the diversification of the large Amazon biodiversity.

### *Study groups*

In the present study, we focus on the patterns and processes related to the diversification of birds restricted to Amazonian floodplains, specifically Antbirds (Thamnophilidae - Aves). South America concentrates the highest diversity of birds, with more than 2,700 species (del Hoyo et al. 2018) and the Amazon basin concentrates more than half of this number (Hubbel et al. 2008; Hoorn & Wesselingh 2010). Antbirds are a Neotropical radiation with approximately 241 species (Zimmer & Isler 2017), considered as good models for phylogeographic studies in the Amazon due the high diversity in the biome - more than 40 species can be found syntopically -, intimate relationship with specific environments, and high intraspecific genetic structure possibly related to poor dispersal abilities (Thom & Aleixo 2015; Fernandes et al. 2012; Fernandes et al. 2014; Zimmer & Isler 2017). Here we selected three lineages (species complexes) of the family Thamnophilidae, intimately related to river-created environments widely distributed over the main Amazonian rivers such as Amazon, Solimões, Madeira, Negro, Branco and Tapajós rivers. The selected species complexes and their distribution areas are described below (Figure 1).

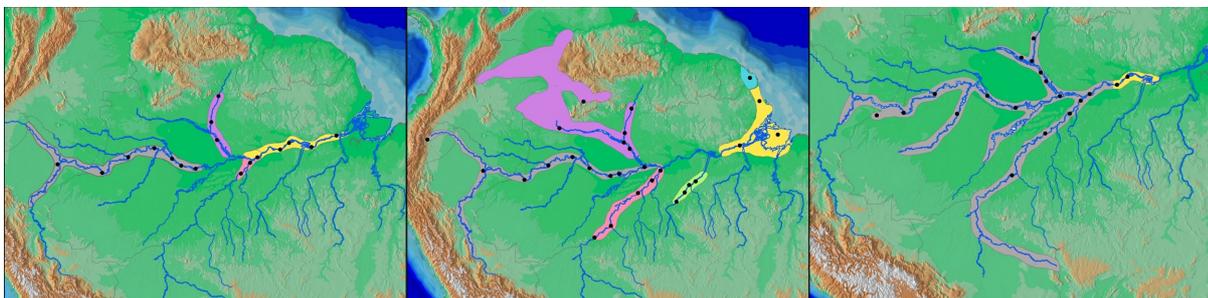


Figure 1: Geographic distribution of the three taxa studied. Left - *Myrmoborus lugubris* (gray - *M. l. berlepschi*; purple - *M. l. stictopterus*; pink - *M. l. femininus*; yellow - *M. l. lugubris*). Center - *Thamnophilus nigrocinereus/cryptoleucus* (gray - *T. cryptoleucus*; purple - *T. n. cinereoniger*; pink -

*T. n. tschudii*; green - *T. n. huberi*; yellow - *T. n. nigrocinereus*; blue - *T. n. kulczynskii*). Right - *Myrmotherula assimilis* (grey - *M. a. assimilis*; yellow - *M. a. transamazonica*).

#### *Myrmoborus lugubris* (Cabanis, 1847)

Occurs in the understory of river-created environments in *várzea* forests, mainly in islands of whitewater rivers (Amazon, Branco, and Solimões rivers) usually in marshy forests dominated by *Cecropia* sp. and *Heliconia* sp. (Zimmer & Isler 2017). Four subspecies are recognized (Figure 1): *M. l. lugubris* occurs in the Amazon River west of the Madeira river; *M. l. femininus* is restricted to the lower course of the Madeira river; *M. l. stictopterus* is distributed in the Negro and Branco rivers; *M. l. berlepschi* is restricted to the Solimões Basin. The taxa *M. l. stictopterus* and *M. l. female*, resemble intermediate phenotypes between the nominal form and *M. l. berlepschi*, located in the distal portions of the geographic distribution (Haffer & Fitzpatrick 1985). Thus the morphological structure of the complex could be related to a clinal variation with a continuous transition between taxa (Zimmer & Isler 2017). The sister species of *M. lugubris* complex is *M. leucophrys* (Bravo 2012).

#### *Thamnophilus nigrocinereus* Sclater, 1855

Distributed over the entire Amazon river and some large tributary rivers such as Negro (Branco), Madeira, Tapajós, Xingu, and Tocantins rivers (Figure 1). It occurs in the understory and medium strata of floodplain forests, gallery forests, mangroves and savanna shrubs, always close to large rivers. However, in most of the distribution, it prefers islands and borders of *igapós* forest (Zimmer & Isler 2017). This species complex is composed of two species, *T. cryptoleucus*, which is monotypic, and *T. nigrocinereus*, which groups five subspecies. *T. n. nigrocinereus* is distributed in the lower Amazon river; *T. n. kulczynskii* occurs in French Guiana and northeast Amapá state in Brazil mainly in mangroves; *T. n. huberi* is restricted to the medium and upper Tapajós river; *T. n. cinereoniger* occurs in the Negro and Branco rivers as well as part of the Orinoco basin; *T. n.*

*tschudii* is endemic to the lower and middle courses of the Madeira river. Zimmer & Isler (2017) reported that the morphological and vocal variability within *T. nigrocinereus* suggests that a taxonomic revision is needed as some of these taxa should be recognized as full species. Brumfield & Edwards (2007) supported the reciprocal monophyly of *T. nigrocinereus* and *T. cryptoleucus*, with low genetic distance in mitochondrial markers (ND2: 1.1% and cyt b: 1.3%), proposing a recent scenario of diversification or introgression, since these species occur in sympatry at the confluence of the Madeira and Solimões rivers. These authors did not recover with high statistical support the sister group of *T. nigrocinereus* complex, but this complex is in a clade composed by *T. punctatus*, *T. stictocephalus*, *T. caerulescens*, *T. unicolor*, *T. aroyae*, and *T. aethiops*.

#### *Myrmotherula assimilis* Pelzeln, 1868

It is distributed over the Solimões, Madeira, Purus, Jurua, Negro, and upper Amazon rivers, occupying a wider range of environments than the two previous study groups, including areas of tall *várzea* and *igapó* forests (Figure 1). Two subspecies are recognized: *M. a. assimilis* occurs along most part of the distribution, and *M. a. transamazonica* is restricted to the Amazon River in Pará state, Brazil (Zimmer & Isler 2017). The sister species is *Myrmotherula menetriesii* (Bravo et al. 2012).

#### *Objectives*

The aim of this doctoral Dissertation was to produce new evidence about the processes shaping the current genetic diversity of Amazonian floodplain communities to contribute with knowledge on the evolution of this ecosystem. We analyzed the molecular systematics, phylogeographic structure, and historical demography of endemic Amazonian floodplain forest birds to understand the diversification patterns of this group of organisms. The specific objectives are presented in each chapter of the Dissertation.

## General Discussion and Conclusions

---

In this Dissertation we described diversification patterns of three bird species complexes restricted to the Amazonian floodplains, *Myrmoborus lugubris*, *Thamnophilus nigrocinereus*/*T. cryptoleucus*, and *Myrmotherula assimilis*, which enabled us to infer potential historical processes that shaped current genetic diversity and geographic distribution of this community. In Chapter 1 the pattern of genetic structure and phylogenetic relationships of *Myrmoborus lugubris* populations were studied revealing a complex scenario of diversification. In Chapter 2 the patterns of co-divergence and co-demography of three studied species were explored. Finally in Chapter 3 the dynamics of geographic range expansion of populations restricted to the Solimões river was analyzed.

### *Diversification of Amazonian floodplain forest organisms*

The diversification patterns observed here supported that Amazonian floodplains are a highly dynamic environment over time producing complex scenarios of diversification by modulating microevolutionary processes such as gene flow and genetic drift. The effects of gene flow and incomplete lineage sorting observed here support the demand for larger datasets as those obtained with next-generation sequencing and the application of methods that can handle both processes to estimate the phylogenetic relationship of species and populations occurring in this dynamic environment. Similarly, given the discrepancy between divergence times from sister species and within species complexes, this dynamism may promote high extinction rates constantly vanishing genetic diversity.

The phylogeographic patterns observed here revealed an interesting and unexplored facet for the diversification of the large Amazonian biodiversity. On the opposite to the expectation of the rivers as barriers hypothesis - where large Amazonian tributaries are expressive barriers to gene

flow for upland forest organisms - the floodplain forest species studied in this Dissertation are compartmentalized over the main Amazonian sub-basins such as Solimões, Negro, Madeira, Tapajós and Amazonas, without signs of isolation on opposite margins of large rivers. The results obtained were in disagreement with previous phylogeographic studies that supported widely distributed populations over the entire Amazon basin, suggesting that more specialized species in terms of habitat tend to be more affected by historical events. Hence supporting that the diversification processes of the floodplain community cannot be explained by a single generalized model, even considering that some taxon present similar histories. This complex biogeographical history is also observed for upland Amazonian forest species.

The results presented in this Dissertation support that the central Amazonian basin is a suture zone splitting populations and species occurring in distinct sub-basins with variable levels of gene flow. Additionally, we suggest that climatic oscillations around 0.2 Mya produced events of isolation and secondary contact following river level fluctuations that altered their sedimentation patterns, mainly in white-water rivers. However, given potential high extinction rates in this environments it is possible that the effect of older historical processes have been erased and are no longer detectable by the methods we applied. Hence it is possible that multiple events operated in the current genetic diversity of distinct taxa that may have responded differently given intrinsic ecological attributes producing a complex scenario. In this sense, the Amazonian floodplains are still an overlooked environment by phylogeography and landscape genetics approaches, given its huge diversity and specific habitats with exclusive evolutionary histories. Thus the continuity and accumulation of studies regarding the diversification of floodplain organisms have the potential to expose new scenarios and drivers of speciation for the Amazonian biodiversity. Similarly, the association between genetic and environmental data such as niche modeling and remote sensing can yield a better understanding of what physical features are more effective shaping the observed genetic diversity.

- Ahituv N., Zhu Y., Visel A., Holt A., Afzal V., Pennacchio L.A., Rubin E.M. 2007. Deletion of ultraconserved elements yields viable mice. *PLoS Biology*. 5.
- Albernaz A.L., Pressey R.L., Costa L.R., Moreira M.P., Ramos J.F., Assunção P.A., Franciscon C.H. 2011. Tree species compositional change and conservation implications in the white-water flooded forests of the Brazilian Amazon. *Journal of Biogeography*. 39:869–883.
- Aleixo A. 2004. Historical diversification of a terra-firme forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. *Evolution*. 58:1303.
- Aleixo A. 2006. Historical diversification of floodplain forest specialist species in the Amazon: a case study with two species of the avian genus *Xiphorhynchus* (Aves: Dendrocolaptidae). *Biological Journal of the Linnean Society*. 89:383–395.
- Anderson D.R. 2008. *Model-based inference in the life sciences: a primer on evidence*. Springer, New York.
- Antonelli A., Quijada-Mascareñas A., Crawford A. J., Bates J. M., Velazco P. M., Wüster W. 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. Pp. 387-404. In: Hoorn C., Wesselingh E. P. (Eds.) *Amazonia, Landscape, and Species Evolution: A Look into the Past*. Wiley-Blackwell, London.
- Beaumont M.A., Zhang W., Balding D.J. 2002. Approximate Bayesian computation in population genetics. *Genetics* 162: 2025–2035.
- Beaumont M.A. 2010. Approximate Bayesian computation in evolution and ecology. *Annual Review of Ecology, Evolution, and Systematics*. 41:379–406.

- Beaumont M.A., Nielsen R., Robert C., Hey J., Gaggiotti O., Knowles L., Estoup A., Panchal M., Corander J., Hickerson M., Sisson S.A., Fagundes N., Chikhi L., Beerli P., Vitalis R., Cornuet J.-M., Huelsenbeck J., Foll M., Yang Z., Rousset F., Balding D., Excoffier L. 2010. In defense of model-based inference in phylogeography. *Molecular Ecology*. 19:436–446.
- Bejerano G. 2004. Ultraconserved elements in the human genome. *Science*. 304:1321–1325.
- Bertorelle G., Benazzo A., Mona S. 2010. ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology*. 19:2609–2625.
- BirdLife International. 2016. The IUCN Red List of Threatened Species 2016: e.T22680736A92875297.<http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22680736A92875297.en>. Downloaded on 20 November 2017.
- Boubli J.P., Ribas C., Alfaro J.W.L., Alfaro M.E., Silva M.N.F.D., Pinho G.M., Farias I.P. 2015. Spatial and temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. *Molecular Phylogenetics and Evolution*. 82:400–412.
- Bravo G.A. 2012. Phenotypic and niche evolution in the antbirds (Aves, *Thamnophilidae*). Ph.D. Dissertation, Louisiana State University, Baton Rouge.
- Brown J.L., Weber J.J., Alvarado-Serrano D.F., Hickerson M.J., Franks S.J., Carnaval A.C. 2016. Predicting the genetic consequences of future climate change: The power of coupling spatial demography, the coalescent, and historical landscape changes. *American Journal of Botany*. 103:153–163.
- Brumfield R.T., Edwards S.V. 2007. Evolution into and out of the Andes: a Bayesian analysis of historical diversification in *Thamnophilus* antshrikes. *Evolution*. 61:346–367.

- Burney, Brumfield. 2009. Ecology predicts levels of genetic differentiation in neotropical birds. *The American Naturalist*. 174:358.
- Bush M.B. 1994. Amazonian speciation: a necessarily complex model. *Journal of Biogeography*. 21:5-17.
- Cadena C.D., Gutiérrez-Pinto N., Dávila N., Chesser R.T. 2011. No population genetic structure in a widespread aquatic songbird from the Neotropics. *Molecular Phylogenetics and Evolution*. 58:540–545.
- Capurucho J.M.G., Cornelius C., Borges S.H., Cohn-Haft M., Aleixo A., Metzger J.P., Ribas C.C. 2013. Combining phylogeography and landscape genetics of *Xenopipo atronitens* (Aves: Pipridae), a white sand campina specialist, to understand Pleistocene landscape evolution in Amazonia. *Biological Journal of the Linnean Society*. 110:60–76.
- Carnaval A.C., Hickerson M.J., Haddad C.F.B., Rodrigues M.T., Moritz C. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*. 323:785–789.
- Carstens B.C., Knowles L.L., Collins T. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. *Systematic Biology*. 56:400–411.
- Choueri É.L., Gubili C., Borges S.H., Thom G., Sawakuchi A.O., Soares E.A.A., Ribas C.C. 2017. Phylogeography and population dynamics of antbirds (Thamnophilidae) from Amazonian fluvial islands. *Journal of Biogeography*. 44:2284–2294.
- Cohn-Haft M., Naka L.N., Fernandes A.M. 2007. Padrões de distribuição da avifauna da várzea dos rios Solimões-Amazonas. Pp. 287-324. In: Albernaz A.L. (Eds). *Conservação da Várzea, Identificação e Caracterização de Regiões Biogeográficas*. IBAMA/ ProVárzea/INPA, Manaus.

- Cracraft J. 1985. Historical biogeography and patterns of differentiation within the South American avifauna: areas of endemism. *Ornithological Monographs*:49–84.
- Crawford N.G., Faircloth B.C., McCormack J.E., Brumfield R.T., Winker K., Glenn T.C. 2012. More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biology Letters*. 8:783–786.
- Degnan J.H., Rosenberg N.A. 2009. Gene tree discordance, phylogenetic inference, and the multispecies coalescent. *Trends in Ecology and Evolution*. 24:332–340.
- Derti A., Roth F.P., Church G.M., Wu C.-T. 2006. Mammalian ultraconserved elements are strongly depleted among segmental duplications and copy number variants. *Nature Genetics*. 38:1216–1220.
- D’Horta F.M., Cuervo A.M., Ribas C.C., Brumfield R.T., Miyaki C.Y. 2012. Phylogeny and comparative phylogeography of *Sclerurus* (Aves: Furnariidae) reveal constant and cryptic diversification in an old radiation of rainforest understorey specialists. *Journal of Biogeography*. 40:37–49.
- Edwards S.V., Liu L., Pearl D.K. 2007. High-resolution species trees without concatenation. *Proceedings of the National Academy of Sciences of the USA*. 104:5936–5941.
- Edwards S.V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution*. 63:1–19.
- Edwards S.V., Beerli P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*. 54:1839.
- Excoffier L., Smouse P.E., Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

- Faircloth B.C., McCormack J.E., Crawford N.G., Harvey M.G., Brumfield R.T., Glenn T.C. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*. 61:717–726.
- Felsenstein J. 2008. *Inferring Phylogenies*. Sinauer Associates, Inc., Sunderland.
- Fernandes A.M., Wink M., Sardelli C.H., Aleixo A. 2014. Multiple speciations across the Andes and throughout Amazonia: the case of the spot-backed antbird species complex (*Hylophylax naevius/Hylophylax naevioides*). *Journal of Biogeography*. 41:1094–1104.
- Fernandes A.M., Wink M., Aleixo A. 2012. Phylogeography of the chestnut-tailed antbird (*Myrmeciza hemimelaena*) clarifies the role of rivers in Amazonian biogeography. *Journal of Biogeography*. 39:1524–1535.
- Gnirke A., Melnikov A., Maguire J., Rogov P., Leproust E.M., Brockman W., Fennell T., Giannoukos G., Fisher S., Russ C., Gabriel S., Jaffe D.B., Lander E.S., Nusbaum C. 2009. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nature Biotechnology*. 27:182–189.
- Haffer J. 1969. Speciation in Amazonian forest birds. *Science*. 165:131–137.
- Haffer J., Fitzpatrick J.W. 1985. Geographic variation in some Amazonian forest birds. *Ornithological Monographs*. 36:147–168.
- Haffer J. 2001. Hypotheses to explain the origin of species in Amazonia. Pp. 45-118. In: Viera I., D'Incao M.A., Silva J.M.C., Oren D. (Eds.) *Diversidade Biológica e Cultural da Amazônia*. Museu Paraense Emilio Goeldi, Belém.
- Hall J.P.W., Harvey D.J. 2002. The phylogeography of Amazonia revisited: new evidence from riodinid butterflies. *Evolution*. 56:1489.

- Harvey M.G., Aleixo A., Ribas C.C., Brumfield R.T. 2017. Habitat association predicts genetic diversity and population divergence in Amazonian birds. *The American Naturalist*. 190:631–648.
- Harvey M., Aleixo A., Ribas C.C., Brumfield R.T. 2016. Habitat preference predicts genetic diversity and population divergence in Amazonian birds. *The American Naturalist* 190: 631-648
- Harvey M.G., Smith B.T., Glenn T.C., Faircloth B.C., Brumfield R.T. 2016. Sequence capture versus restriction site associated DNA sequencing for shallow systematics. *Systematic Biology*. 65:910–924.
- Heled J., Drummond A.J. 2009. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*. 27:570–580.
- Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature*. 405:907–913.
- Hickerson M., Carstens B., Cavender-Bares J., Crandall K., Graham C., Johnson J., Rissler L., Victoriano P., Yoder A. 2010. Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*. 54:291–301.
- Hoorn C., Wesselingh F.P. 2010. Amazonia landscape and species evolution: a look into the past. Wiley-Blackwell, London.
- Huang H., Knowles L.L. 2009. What is the danger of the anomaly zone for empirical phylogenetics? *Systematic Biology*. 58:527–536.
- Huang W., Takebayashi N., Qi Y., Hickerson M.J. 2011. MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC Bioinformatics*. 12:1.
- Hubbell S.P., He F., Condit R., Borda-De-Agua L., Kellner J., Steege H.T. 2008. How many tree species are there in the Amazon and how many of them will go extinct? *Proceedings of the National Academy of Sciences of the USA*. 105:11498–11504.

- Hubert N., Renno J.-F. 2006. Historical biogeography of South American freshwater fishes. *Journal of Biogeography*. 33:1414–1436.
- Irion G., Kalliola R. 2010. Long-term landscape development process in Amazônia. Pp. 185-197. In: Hoorn, C., Wesselingh, E.P. (Eds.) *Amazonia, Landscape, and Species Evolution: A Look into the Past*. Wiley-Blackwell, London.
- Janes D.E., Chapus C., Gondo Y., Clayton D.F., Sinha S., Blatti C.A., Organ C.L., Fujita M.K., Balakrishnan C.N., Edwards S.V. 2010. Reptiles and mammals have differentially retained long conserved noncoding sequences from the amniote ancestor. *Genome Biology and Evolution*. 3:102–113.
- Junk W.J. 2011. *Amazonian Floodplain Forests: Ecophysiology, Biodiversity, and Sustainable Management*. Springer, Berlin.
- Junk W.J., Piedade M.T.F., Schöngart J., Cohn-Haft M., Adeney J.M., Wittmann F. 2011. A classification of major naturally-occurring Amazonian lowland wetlands. *Wetlands*. 31:623–640.
- Knowles L.L. 2009. Statistical phylogeography. *Annual Review of Ecology, Evolution, and Systematics*. 40:593–612.
- Knowles L.L., Maddison W.P. 2002. Statistical phylogeography. *Molecular Ecology*. 11:2623–2635.
- Kubatko L.S., Degnan J.H., Collins T. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology*. 56:17–24.
- Latrubesse E.M., Arima E.Y., Dunne T., Park E., Baker V.R., D’Horta F.M., Wight C., Wittmann F., Zuanon J., Baker P.A., Ribas C.C., Norgaard R.B., Filizola N., Ansar A., Flyvbjerg B., Stevaux J.C. 2017. Damming the rivers of the Amazon basin. *Nature*. 546:363–369.
- Leite R.N., Rogers D.S. 2013. Revisiting Amazonian phylogeography: insights into diversification hypotheses and novel perspectives. *Organisms, Diversity and Evolution*. 13:639–664.

- Lemmon A.R., Lemmon E.M. 2012. High-throughput identification of informative nuclear loci for shallow-scale phylogenetics and phylogeography. *Systematic Biology*. 61:745–761.
- Lerner H., Fleischer R. 2010. Prospects for the use of next-generation sequencing methods in ornithology. *The Auk*. 127:4–15.
- Lessa E.P., Cook J.A., Patton J.L. 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences of the USA*. 100:10331–10334.
- Fu Y.X., Li W.H. 1993. Statistical tests of neutrality of mutations. *Genetics* 133: 693–709.
- Maddison W.P., Knowles L.L., Collins T. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology*. 55:21–30.
- Manthey J.D., Campillo L.C., Burns K.J., Moyle R.G. 2016. Comparison of target-capture and restriction-site associated DNA sequencing for phylogenomics: a test in cardinalid tanagers (aves, genus: *Piranga*). *Systematic Biology*. 65:640–650.
- Mccormack J.E., Hird S.M., Zellmer A.J., Carstens B.C., Brumfield R.T. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*. 66:526–538.
- Mccormack J.E., Maley J.M., Hird S.M., Derryberry E.P., Graves G.R., Brumfield R.T. 2012. Next-generation sequencing reveals phylogeographic structure and a species tree for recent bird divergences. *Molecular Phylogenetics and Evolution*. 62:397–406.
- Moritz C., Patton J.L., Schneider C.J., Smith T.B. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*. 31:533–563.
- Moritz C., Faith D.P. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*. 7:419–429.

- Moritz C., Funk V., Sakai A.K. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*. 51:238–254.
- Nei M., Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*. 76:5269–5273.
- O’Neill J.P., Lane D.F., Naka L.N. 2011. A cryptic new species of thrush (Turdidae: *Turdus*) from western Amazonia. *The Condor*. 113:869–880.
- Patel S., Weckstein J.D., Patané J.S., Bates J.M., Aleixo A. 2011. Temporal and spatial diversification of *Pteroglossus araçaris* (AVES: Ramphastidae) in the Neotropics: Constant rate of diversification does not support an increase in radiation during the Pleistocene. *Molecular Phylogenetics and Evolution*. 58:105–115.
- Patton J.L., da Silva M.N.F. 1998. Rivers, refuges, and ridges. The geography of speciation of Amazonian mammals. Pp. 202-213 In D.J. Howard, S.H. Berlocher (Eds.) *Endless Forms: Species and Speciation*. Oxford University Press, New York.
- Prates I., Xue A.T., Brown J.L., Alvarado-Serrano D.F., Rodrigues M.T., Hickerson M.J., Carnaval A.C. 2016. Inferring responses to climate dynamics from historical demography in neotropical forest lizards. *Proceedings of the National Academy of Sciences of the USA*. 113:7978–7985.
- Remsen J.V., Iii T.A.P. 1983. Contribution of river-created habitats to bird species richness in Amazonia. *Biotropica*. 15:223.
- Ribas C.C., Aleixo A., Nogueira A.C.R., Miyaki C.Y., Cracraft J. 2011. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society B: Biological Sciences*. 279:681–689.
- Rokas A., Abbot P. 2009. Harnessing genomics for evolutionary insights. *Trends in Ecology and Evolution*. 24:192–200.

- Siepel A. 2005. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Research*. 15:1034–1050.
- Silva J.M.C.D., Rylands A.B., Fonseca G.A.B.D. 2005. The fate of the Amazonian areas of endemism. *Conservation Biology*. 19:689–694.
- Slatkin M., Maddison W.P. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* 123: 603–613.
- Smith B.T., Harvey M.G., Faircloth B.C., Glenn T.C., Brumfield R.T. 2013. Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Systematic Biology*. 63:83–95.
- Smith B.T., McCormack J.E., Cuervo A.M., Hickerson M.J., Aleixo A., Cadena C.D., Pérez-Emán J., Burney C.W., Xie X., Harvey M.G., Faircloth B.C., Glenn T.C., Derryberry E.P., Prejean J., Fields S., Brumfield R.T. 2014. The drivers of tropical speciation. *Nature*. 515:406–409.
- Solomon S.E., Bacci M., Martins J., Vinha G.G., Mueller U.G. 2008. Paleodistributions and comparative molecular phylogeography of leafcutter ants (*Atta* spp.) Provide new insight into the origins of Amazonian diversity. *PLoS ONE*. 3:e2738.
- Soltis D.E., Morris A.B., Mclachlan J.S., Manos P.S., Soltis P.S. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*. 15:4261–4293.
- Stephen S., Pheasant M., Makunin I.V., Mattick J.S. 2008. Large-scale appearance of ultraconserved elements in tetrapod genomes and slowdown of the molecular clock. *Molecular Biology and Evolution*. 25:402–408.
- Taberlet P. 2002. Ecology: enhanced: quaternary refugia and persistence of biodiversity. *Science*. 297:2009–2010.

- Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.
- Tassell C.P.V., Smith T.P.L., Matukumalli L.K., Taylor J.F., Schnabel R.D., Lawley C.T., Haudenschild C.D., Moore S.S., Warren W.C., Sonstegard T.S. 2008. SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature Methods*. 5:247–252.
- Terracciano D., Terreri S., Nigris F.D., Costa V., Calin G.A., Cimmino A. 2017. The role of a new class of long noncoding RNAs transcribed from ultraconserved regions in cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*. 1868:449–455.
- Thom G., Aleixo A. 2015. Cryptic speciation in the white-shouldered antshrike (*Thamnophilus aethiops*, Aves – Thamnophilidae): The tale of a transcontinental radiation across rivers in lowland Amazonia and the northeastern Atlantic Forest. *Molecular Phylogenetics and Evolution*. 82:95–110.
- Venticinque E.M., Rego F.N.A.A., Brescovit A.D., Rheims C.A., Ruiz G.R.S. 2007. A araneofauna (Arachnida, Araneae) das várzeas do Rio Amazonas: padrões de distribuição e estado do conhecimento atual. Pp. 179-198. In: Albernaz A.L. (Eds). *Conservação da Várzea, Identificação e Caracterização de Regiões Biogeográficas*. IBAMA/ ProVárzea/INPA, Manaus.
- Wakeley J. 2004. Recent trends in population genetics: more data! More math! Simple models? *Journal of Heredity*. 95:397–405.
- Wakeley J. 2009. *Coalescent Theory: an Introduction*. Roberts & Co. Publishers, Greenwood Village.
- Wallace A.R. 1854. On the monkeys of the Amazon. *Journal of Natural History Series 2*. 14: 451–454.

- Warnefors M., Hartmann B., Thomsen S., Alonso C.R. 2016. Combinatorial gene regulatory functions underlie ultraconserved elements in *Drosophila*. *Molecular Biology and Evolution*. 33:2294–2306.
- Weir J.T., Price M. 2011. Andean uplift promotes lowland speciation through vicariance and dispersal in *Dendrocincla* woodcreepers. *Molecular Ecology*. 20:4550–4563.
- Wiens J.J., Pyron R.A., Moen D.S. 2011. Phylogenetic origins of local-scale diversity patterns and the causes of Amazonian megadiversity. *Ecology Letters*. 14:643–652.
- Williams J.W., Jackson S.T., Kutzbach J.E. 2007. Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Sciences of the USA*. 104:5738–5742.
- Wittmann F., Householder E., Piedade M.T.F., Assis R.L.D., Schöngart J., Parolin P., Junk W.J. 2012. Habitat specificity, endemism and the neotropical distribution of Amazonian white-water floodplain trees. *Ecography*. 36:690–707.
- Wittmann F., Schongart J., Montero J.C., Motzer T., Junk W.J., Piedade M.T.F., Queiroz H.L., Worbes M. 2006. Tree species composition and diversity gradients in white-water forests across the Amazon Basin. *Journal of Biogeography*. 33:1334–1347.
- Woolfe A., Goodson M., Goode D.K., Snell P., McEwen G.K., Vavouri T., Smith S.F., North P., Callaway H., Kelly K., Walter K., Abnizova I., Gilks W., Edwards Y.J.K., Cooke J.E., Elgar G. 2004. Highly conserved non-coding sequences are associated with vertebrate development. *PLoS Biology*. 3.
- Zimmer K., Isler M.L. 2017. Typical Antbirds (Thamnophilidae). In: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (Eds.). *Handbook of the Birds of the World Alive*. Lynx Edicions, Barcelona. (retrieved from <https://www.hbw.com/node/52291> on 20 November 2017).