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GENÉTICA DE POPULAÇÕES DE ESPÉCIES INSULARES DE THAMNOPHILIDAE (AVES): UMA ABORDAGEM FILOGEOGRÁFICA NO BAIXO CURSO DO RIO NEGRO.

Érik Henrique de Lacerda Choueri

Manaus – Amazonas

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Sinopse

Estudou-se a dinâmica populacional de quatro espécies da família Thamnophilidae (AVES) em ilhas do baixo Rio Negro, avaliando a relação destas populações com às de outros rios amazônicos. Para se compreender o cenário atual, informações relacionadas ao fluxo gênico e diversidade genética foram acessadas. Estimativas referentes ao número populacional histórico e tempo de divergência entre linhagens foram consideradas para se compreender a influência do paleoambiente sobre estes animais.

Palavras-chave: Anavilhanas, Amazônia central, ilhas fluviais, Jaú, dinâmica de populações

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RESUMO

As planícies alagáveis da Amazônia correspondem à um mosaico de ambientes que ocupam mais de 10% da bacia e abrigam inúmeras espécies de animais e plantas. Neste contexto, a avifauna associada apresenta graus distintos de dependência ao uso de determinados microhabitats. Apesar de sua relevância ecológica, pouco se conhece sobre a dinâmica populacional dos organismos que utilizam estes ambientes, assim como os mecanismos históricos que influenciaram a formação destas paisagens. Desta forma, o presente estudo avaliou a dinâmica populacional de quatro espécies de aves florestais de planícies alagáveis da Amazônia. Dentre estas, foi considerada uma espécie generalista no uso deste habitat (*Hypocnemoides melanopogon*) e outras três que utilizam preferencialmente ilhas fluviais (*Myrmotherula assimilis*, *Myrmoborus lugubris* e *Thamnophilus nigrocinereus*), ambientes dispostos de forma discreta na paisagem. Quando considerados os arquipélagos do baixo Rio Negro, apesar de evidências de fluxo gênico entre ilhas, foram encontrados sinais de distribuição heterogênea da diversidade genética na paisagem. Em escala geográfica mais ampla, linhagens das três espécies especializadas no uso de ambientes insulares apresentaram distinção entre o Negro outros grandes rios amazônicos. Estes resultados são contrastantes com outros estudos que indicaram ausência de estruturação genética em aves de planícies de fluviais amazônicas. Cabe salientar que tal reposta não foi observada para a espécie generalista de áreas alagadas, sugerindo que as características ecológicas dos organismos podem influenciar sua diversidade genética nestes habitats. A congruência temporal das divergências entre estas linhagens dos Negro e Solimões evidencia que processos paleoclimáticos e geológicos relacionados à formação da paisagem no passado recente (Pleistoceno) foram possivelmente responsáveis por reger a organização atual destes organismos. Por fim, as espécies estudadas não apresentaram sinais marcantes de expansão populacional recente, resultado concordante ao obtido para outras aves de planícies alagadas, mas que contrastam com aqueles obtidos para organismos de Terra Firme. Tal resposta pode ser um indicativo de disponibilidade constante dos habitats alagáveis amazônicos durante a transição entre Pleistoceno/Holoceno.

ABSTRACT

Amazon floodplains is a mosaic of environments who occupy more than 10% of basin and harbor countless species of animals and plants. In this context, the associeted avifauna exhibit different degrees of dependence related to use of microhabitats. Despite its ecological importance, the organisms' population dynamics that use these environments are not well known, as well as the historical mechanisms that influenced the formation of these landscapes. Thus, this study evaluated the population dynamics of four forest birds species specialized in the Amazon floodplains use. Among these, were evaluated a generalist specie in the floodplain use (*Hypocnemoides melanopogon*) and three species that use preferably fluvial islands (*Myrmotherula assimilis*, *Myrmoborus lugubris* and *Thamnophilus nigrocinereus*), environments arranged discretely in landscape. When considering archipelagos situated in lower Negro River, despite evidences of gene flow between islands, subtle signals indicate that genetic diversity is heterogeneously distributed across the landscape. In a broader geographic scale, lineages of birds specialists of insular environments exhibited distinctions between Negro and other great rivers of Amazon. These results contrast with other studies that indicated no genetic structure in specialized birds in the amazon's floodplains. It should be noted that such response was not observed in the generalist specie of flooded areas, suggesting that the organisms' ecological characteristics can influence genetic diversity in these habitats. The temporal congruence of divergences between lineages of Negro and Solimões are evidences that paleoclimatic and geologic process related to the formation of landscape in recent past (Pleistocene) were possibly responsible by the current organization of these species. Finally, the species studied does not showed remarkable signals of population expansion, a similar result to that achieved for other floodplains' birds, but are contrasting to those obtained in lowland (*Terra Firme*) organisms. This answer can be an idicative of constant avaliability of floodplains during the transition Pleistocene/Holocene.

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INTRODUÇÃO GERAL

A região amazônica é composta por um complexo mosaico de ambientes responsável por moldar a distribuição de vários organismos, sendo que a diferenciação de determinadas unidades evolutivas pode ser relacionada com os processos históricos que formaram a paisagem (Aleixo, 2004; Ribas et al., 2012). Dentre os condutores e mantenedores desta diversidade, os grandes rios são considerados um dos principais agentes, os quais atuaram tanto como barreira vicariante em espécies ancestrais de ampla distribuição (Ribas et al., 2012), quanto como barreiras atuais em zonas de contato secundário (Fernandes et al., 2012). Por esta razão, muitos interflúvios apresentam-se como zonas de endemismo para diversas espécies de aves (Cracraft, 1985; Borges & Da Silva, 2012).

Outros papéis relevantes sobre essas drenagens relacionam-se com seu potencial de influenciar habitats vinculados às suas planícies de inundação, além de atuarem como facilitadores para a dispersão de determinados organismos nessas regiões (Schleuning et al., 2011; Montero et al., 2012). Os ambientes em questão abrigam diferentes fisionomias vegetacionais, desde áreas abertas à florestais, cobrindo cerca de 600.000 km^2 de extensão, o que corresponde a aproximadamente 10% da bacia amazônica (Melack & Hess, 2010; Wittmann et al., 2010). Devido sua magnitude e complexidade, 15% da avifauna do bioma é especializada no uso destes habitats (Remsen & Parker, 1983).

A heterogeneidade ambiental encontrada nas planícies alagadas é reflexo principalmente das características físico-químicas e geológicas dos rios as quais estão associadas (Prance, 1979). Cursos possuindo nascentes próximas às regiões andinas apresentam grande carga sedimentar e resultam na formação dos ambientes de várzea, enquanto que rios que correm pelos antigos escudos cristalinos geralmente transportam um volume sedimentológico menor e estão associados aos ambientes de igapó (Prance 1979). Como consequência desta diferenciação, a produtividade primária e vegetação são distintos entre tais ambientes (Prance 1979). Cabe salientar ainda que os mesmos sofrem influências drásticas relacionadas ao pulso de inundação sazonal dos rios e apresentam evidências de intensa dinâmica geológica (Junk *et al*. 1989; Latrubesse & Franzinelli 2005; Almeida-filho & Miranda 2007; Nogueira *et al*. 2013). O resultado deste conjunto de condições peculiares é a formação de uma ampla gama de habitats ecologicamente diferenciados que incluem florestas em distintos estágios sucessionais, ilhas fluviais, lagos, bancos de areia, entre outros.

Alguns destes ambientes localizados ao longo dos rios amazônicos podem atuar como corredores para o fluxo de indivíduos e genes. De fato, um estudo realizado com uma ave de campos de várzea sazonalmente alagáveis (*Chrysomus icterocephalus*) demonstrou fracos sinais de diferenciação genética ao longo de sua extensa distribuição geográfica (Cadena et al., 2011). Resposta similar foi obtida em um trabalho com o gênero *Xiphorhynchus* onde, apesar de ocorrer diferenciações relacionadas à diversidade genética entre populações, não foram identificadas estruturas filogeográficas distintas dentro da ampla distribuição das espécies abordadas (Aleixo, 2006). Neste contexto, cabe salientar que a presença de populações geneticamente similares nos habitats em questão pode estar relacionada às características ecológicas intrínsecas das espécies avaliadas (Burney & Brumfield, 2009).

Em ambos os casos citados, as aves estudadas ocupam ambientes abertos ou contínuos, os quais podem atuar como facilitadores ao fluxo gênico (Bates et al., 2003). Desta forma, cabe-se questionar se os padrões observados podem também ser aplicados às espécies que ocupam habitats fragmentados inseridos no contexto fluvial. Tal pergunta é apropriada ao se considerar que ilhas são comumente encontradas nos grandes rios amazônicos, exibindo aves especializadas em planícies alagáveis que dependem intimamente destes microhabitats (Rosenberg, 1990). Para estas espécies, aspectos relacionados à dinâmica histórica das ilhas podem ter deixado marcas nas linhagens estabelecidas nestes locais (Avise, 2000). Desta forma, compreender a história dos organismos insulares também seria um modo de entender a evolução destes ambientes (Baker et al., 2014), os quais ainda são motivos de debate no meio geológico.

Para tanto, uma das formas mais viáveis e informativas para acessar este tipo de questão se dá através do uso de ferramentas moleculares, sendo tal abordagem utilizada em diversos estudos considerando diferentes escalas geográficas e temporais (Werneck et al., 2012; Woltmann et al., 2012; Wallace et al., 2015). Desta forma, torna-se ideal o uso combinado de marcadores mitocondriais e nucleares por estas moléculas oferecerem informações complementares sobre a dinâmica histórica e recente das populações estudadas (Frankham *et al*. 2008). Neste contexto, pelo fato do DNA mitocondrial apresentar características conhecidas em relação à sua forma de evolução, torna-se possível a compreensão das relações entre linhagens estabelecidas no passado, assim como da dinâmica populacional ancestral (Sorenson et al., 1999; Weir & Schluter, 2008; Capurucho et al., 2013). Para acessar respostas vinculadas à dinâmica populacional em escala temporal recente, é interessante o uso de marcadores vinculados ao DNA nuclear, como os microssatélites. Pelo fato da molécula em questão ser recombinante e de origem biparental, estes marcadores são mais sensíveis para se avaliar o fluxo gênico entre organismos e níveis de diversidade genética das populações avaliadas (Frankham *et al*. 2008).

Além da ferramenta a ser implementada, cabe-se escolher quais organismos serão utilizados como modelo. Neste caso é relevante considerar espécies que fundamentalmente sejam sensíveis à insularidade devido características ecológicas e comportamentais. Para tanto, a família Thamnophilidae é ideal por apresentar diversas espécies florestais sedentárias que geralmente evitam cruzar ambientes abertos (Zimmer & Isler 2003; Fernandes *et al*. 2012). Denta forma, para o presente estudo, foram selecionados representantes apresentando diferenças em relação ao uso dos habitats: *Myrmoborus lugubris* (Cabanis 1847), *Myrmotherula assimilis* (Pelzeln 1868) e *Thamnophilus nigrocinereus* (Sclater 1855), por utilizarem preferencialmente os ambientes insulares foram considerados como "especialistas de ilhas", e *Hypocnemoides melanopogon* (Sclater 1857), uma espécie generalista de áreas alagadas também foi abordada. Tal distinção é relevante, considerando que animais com diferentes hábitos de vida podem apresentar respostas distintas ao uso dos ambientes. Outro ponto importante refere-se à escolha dos arquipélagos trabalhados. Neste caso, as ilhas de Anavilhanas e Parque Nacional do Jaú, situam-se no baixo Rio Negro, região aonde processos tectônicos e sedimentológicos influenciaram a dinâmica recentemente da paisagem (Almeida-filho & Miranda 2007; Latrubesse & Franzinelli 2005). Devida sua complexidade, a formação da área em questão ainda é alvo de debate no meio geológico. Desta forma, estudar a organização dos organismos que habitam estes arquipélagos pode levantar informações relevantes para a melhor compreensão dos mecanismos que atuaram no baixo Rio Negro.

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OBJETIVOS

Compreender as relações encontradas entre populações de quatro espécies florestais de Thamnophilidae com ecologias distintas em fina escala geográfica, com ênfase nas ilhas do curso baixo do Rio Negro; avaliar a relação dos grupamentos destas localidades com os presentes em outros rios amazônicos; e acessar informações relacionadas à história destas espécies nas ilhas do baixo Rio Negro, visando uma melhor compreensão dos ambientes que habitam.

CAPÍTULO 1

Choueri, E.L.; Borges, S.H.; Gubili, C.; Thom, G. & Ribas, C.C. Phylogeography and population dynamics of Antbirds (Thamnophilidae) from Amazonian fluvial islands. Manuscrito em preparação para *Journal of Biogeography*.

Original Article

Population dynamics of Antbirds (Thamnophilidae) in fluvial islands: distinct ecologies influence historical and current patterns of genetic diversity.

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Running head: Population dynamics of Antbirds in fluvial islands

ABSTRACT

Aim To investigate the evolution of the avifauna associated to Amazonian flooded habitats analyzing the genetic diversity of four Thamnophilidae species from the Negro River fluvial islands.

Locations Fluvial islands in the Amazon Basin.

Methods One generalist floodplain species (*Hypocnemoides melanopogon*) and three river island specialists (*Myrmotherula assimilis*, *Myrmoborus lugubris* and *Thamnophilus nigrocinereus*) were studied. We sequenced two mitochondrial genes and genotyped eight microsatellite loci. Phylogenetic relationships among intraspecific lineages and divergence times were estimated using Bayesian Inference. Haplotype networks, AMOVA and Mantel tests were used to evaluate the spatial organization of genetic diversity. Gene flow and population structure were evaluated using dissimilarity index, Bayesian inference and allele frequencies. Historical demography was inferred through neutrality tests and EBSP.

Results River island specialists present distinct lineages in different Amazonian tributaries, but very weak population structure within the Negro river basin. The floodplain species had no population structure along the Amazon basin or within the Negro river basin. For all species signal of very slight and recent (Pleistocene) population expansion was recovered.

Main conclusions River island species have stronger population structure within Amazonia than floodplain generalists, with a common spatial and temporal pattern of divergence between populations from the Negro islands and from western Amazonia (upper and middle Solimões), which may be related to Amazonian drainage evolution. Island specialists had low genetic diversity within the Negro basin, while the higher and unstructured diversity pattern found in the floodplain generalist species may be a consequence of higher dispersal caused by the seasonal flooding pulse. River island populations have a recent and dynamic history of contact and isolation, but with small historical fluctuation of population sizes, in sharp contrast with the patterns that have been described for upland forest birds.

Keywords: Anavilhanas, central Amazonia, fluvial islands, insular habitats, Jaú, Negro river, population dynamics.

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Main body: 6682 words

INTRODUCTION

Even for species inhabiting the same geographical region, and thus subject to the same landscape history, intrinsic ecological characteristics such as dispersal ability and different strategies of habitat use may influence gene flow and resulting population genetic patterns (Nevo et al., 1984; Burney & Brumfield, 2009). In Amazonia, lineages of several species or species complexes specialized in lowland forest habitats (*terra firme* forests) have their distributions delimited by large rivers (Haffer, 1969; Ayres & Clutton-Brock, 1992; Kaefer et al., 2012; Ribas et al., 2012). These species often have limited dispersal capacity or tend to avoid open areas (e.g. Fernandes et al., 2012, 2014), so that river channels and floodplains are strong barriers to dispersal, favoring genetic structuring of populations (Beja et al., 2010; Fernandes et al., 2014). Paradoxically, these same rivers can facilitate gene flow for floodplain specialists, implying in a lack of genetic structure for such species throughout the basin (Aleixo, 2006; Cadena et al., 2011; Schleuning et al., 2011). Nevertheless, to consider the Amazon river and its tributaries simply as corridors to gene flow can be a biased view, considering the high diversity of habitats found in their floodplains and the diverse ways in which species can use them.

Beyond barriers or corridors, Amazonian rivers are responsible for regulating habitats that occupy approximately 300,000 km^2 of the basin (Wittmann et al., 2010). The physicochemical characteristics of their waters create the environments of *várzea* (in white water rivers) and *igapó* (rivers of black or clear waters) which show distinct physiognomies, primary productivity and plant species diversity and composition (Prance, 1979; Parolin et al., 2004; Wittmann et al., 2010). Amazon rivers floodplains are subjected to strong seasonal cycles whose duration and amplitude are highly spatially and temporally variable through the basin (Junk et al., 2011). Additionally, geological and paleoclimatic evolution are also responsible for shaping these landscapes through time (Franzinelli & Igreja, 2002; Latrubesse & Franzinelli, 2005; Irion et al., 2009).

As a result of these multiple ecological and historical processes, Amazon floodplains harbor a great diversity of habitats, including flooded forests, grasslands, fluvial islands, oxbow lakes, beaches and sandbars (Remsen & Parker, 1983; Parolin et al., 2004;

Wittmann et al., 2006; Albernaz et al., 2007). Consequently, a highly specialized and endemic fauna and flora occupies river-created habitats, with about 15% of non-aquatic Amazonian birds being restricted to floodplains (Remsen & Parker, 1983, Wittmann et al., 2010). Because the seasonal flooding of Amazonian rivers create fleeting habitats, it is expected that these birds have high dispersion potential in order to find new available habitats during flooding peaks (Remsen & Parker, 1983). These two main characteristics of flooded habitats, which are highly dynamic and continuous, could explain the lack of genetic structure found in the few floodplain specialist species that have been studied so far (Aleixo, 2006; Cadena et al., 2011).

Nevertheless, some floodplain resident birds show a high level of microhabitat specialization with a clear preference for using fluvial islands (Rosenberg, 1990). At least 20 Amazonian bird species are recognized to be fluvial island specialists, although some of them can also use disturbed mainland habitats (Rosenberg, 1990; Armacost-Jr & Capparella, 2012). Island specialists are partially or totally dependent on a habitat with spatially limited distribution in the riverine landscape (Rosenberg, 1990; Armacost-Jr & Capparella, 2012), and it is not known if the pattern of little population structure would hold in these cases. In addition, the evolution of Amazonian rivers may have strong effects on the evolution of river island specialists, especially historical changes in sedimentation dynamics and water level (Latrubesse & Franzinelli, 2005; Irion et al., 2009).

In this study, we aimed to examine the genetic diversity and population dynamics of four antbird species (Thamnophilidae) that occur in fluvial islands at the Negro River Basin, one of the largest tributaries of the Amazon River. To assess the influence of ecological characteristics on the genetic patterns, the species chosen include a floodplain generalist and three fluvial islands specialists (Rosenberg, 1990). In addition, to evaluate the relationship between these groups with conspecifics in other Amazonian fluvial islands, we include a more limited sampling obtained from other river systems. Information about geological and paleoclimatic events were also considered, seeking to know how the paleoenvironment affected population dynamics in Negro River archipelagos.

MATHERIALS AND METHODS

Study region

Our study focused in lower course of the Negro River, located in central Amazonia, Brazil (Figure 1). This region includes two archipelagos, one within the Jaú National Park and another one in the Anavilhanas National Park. While the Jaú archipelago is smaller and located in a narrow channel zone, Anavilhanas is a conspicuous landscape feature with approximately 300 islands with distinct geomorphological physiognomies (Latrubesse & Franzinelli, 2005). Other island habitats close to these two archipelagos include a third archipelago, Mariuá, 60km upstream of the Jaú islands, in the middle Rio Negro, and some fluvial islands in the Branco, Solimões, Madeira, Tapajós and Amazonas rivers (Figure 1).

These environments are affected seasonally by the flood pulse, that alters the connectivity among islands and habitat availability (Sioli, 1984; Latrubesse & Franzinelli, 2005). Geologic and climatic historical processes also influenced this region, with evidences of a dynamic palaeoenviroment, with palaeochannels and fluvial deposits being found in interfluvial areas (Almeida-filho & Miranda, 2007; Santos et al., 2015).

Study species and sampling

Antbirds (Thamnophilidae) is one of the most diverse bird families on the Amazon basin (Zimmer & Isler, 2003). Some species occur in fluvial islands, with varying degrees of dependence on these environments (Remsen & Parker, 1983; Cintra et al., 2007). While floodplain generalists use the islands in an opportunistic manner, they are apparently fundamental to maintain populations of island specialists (Rosenberg, 1990).

Four species with different degrees of island specialization were selected. *Hypocnemoides melanopogon* (Sclater, 1857) is a floodplain generalist that uses forest edges close to lakes and rivers, and occurs in a large portion of northern Amazon basin (Zimmer & Isler, 2003). *Myrmoborus lugubris* (Cabanis, 1847), *Myrmotherula assimilis* (Pelzeln, 1868) and *Thamnophilus nigrocinereus* (Sclater, 1855) are island specialists, occasionally found on river margins (Remsen & Parker, 1983; Rosenberg, 1990;

Armacost-Jr & Capparella, 2012). This habitat specialization is reflected in their geographic distribution, which is restricted to large river channels (Zimmer & Isler, 2003). For more information about species characteristics, see Table S1.

To understand population dynamics in the complex insular system of the lower Negro River, dense sampling was conducted on these islands, including six and nine islands of Jaú and Anavilhanas respectively (Table 1). Mist nets were operated in continuous net-lines for eight hours (from 06:00am to 14:00pm). Blood and tissue samples and specimens were deposited in the Genetic Resources and Bird Collections of the National Institute of Amazonian Researches (INPA). In these two archipelagos, 51 samples of *H. melanopogon,* 31 of *M. lugubris,* 32 *M. assimilis* and 27 *T. nigrocinereus* were collected (more details in Table 1 and Supplementary Material Table S2). Samples from other localities were obtained from collections (Table 1, S2).

Molecular data

Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer’s protocol (see Supplementary Information). Mitochondrial DNA genes cytochrome b (*cytb*) and NADH Dehydrogenase 2 (*ND2*) were amplified using primers L14990-H16065 and L5204-H6313 respectively (Sorenson et al., 1999, Table S3).

Eight microsatellite heterologous primer pairs developed for Thamnophilidae were used to assess variability in nuclear DNA (Ágreda et al., 2006; Barnett et al., 2007; Feldheim et al., 2010; Table S4). These were submitted to transferability tests with temperature gradient to verify their amplification success (for more details, see Supplementary material). Allele sizes were determined on ABI-3130 DNA Sequencer (Applied Biosystems). Genotypic data were acquired using GeneMarker 2.6 (http://www.softgenetics.com/GeneMarker.html)

Nuclear DNA analyses

The occurrence of null alleles was verified with MicroChecker 2.2.3 (Oosterhout et al., 2004). Deviation from Hardy-Weinberg's equilibrium (HWE), estimates of inbreeding coefficient (*Fis*) and Linkage Disequilibrium (LD) were detected using the GenePop online version (http://genepop.curtin.edu.au/). Bonferroni corrections were applied for HWE and LD multiple comparison analysis (Rice, 1989).

To access information about genetic diversity, indexes of observed and expected heterozygosities (*Ho* and *He*, respectively) were inferred using Arlequin 3.5 (Excoffier & Lischer, 2010). The first index regards the estimated fraction of heterozygotes on the populations while the second is the real frequency of heterozygotes detected. Allele richness (*Ar*) was calculated using Fstat 2.9.3.2 (Goudet, 2001). A hierarchical analysis of molecular variance (AMOVA) was used to examine how the genetic variability of each species is distributed across the landscape in Arlequin 3.5 (Excoffier & Lischer, 2010). Here, we assumed three geographic hierarchical scales for comparison: individuals within islands, between islands within archipelagos and between archipelagos. We also verified correlations between genetic and geographic distances using Mantel tests.

To evaluate gene flow, pairwise F_{ST} comparing archipelagos were employed per species using FreeNA with 10,000 randomizations (Chapuis & Estoup, 2007). This software calculates corrected F_{ST} values considering occurrence of null alleles, a common artifact when heterologous primers are used Chapuis & Estoup (2007).

Population structure based on allele frequencies was inferred using Structure 2.3.4 (Pritchard et al., 2000) with 100,000 generations, 10% burn-in and five iterations. Plots of the absolute values of ln $\Pr(X|K)$ were generated by Structure Harvester 0.6.93 (Earl & vonHoldt, 2012).

Mitochondrial DNA analyses

The model of sequence evolution per codon was defined by Bayesian Information Criterion in PartitionFinder 1.1.0 (Lanfear, 2012). Bayesian phylogenetic analysis was performed in MrBayes 3.2 (Ronquist et al., 2012) to evaluate relationships of lineages from the Negro basin (Anavilhanas, Jaú, Mariuá and Branco) to samples from other Amazonian rivers. For these phylogenetic analyses we used closely related species as outgroups. We used four parallel MCMC (*Markov Chain Monte Carlo*) chains and 1,000,000 generations sampling every 100 generations. Initial 10% of generations were discarded as burn-in.

We used a coalescent approach to estimate the timing of origin of the Negro basin clades using BEAST 1.8 (Drummond & Rambaut, 2007). For this analysis, *Hypocnemoides maculicauda* was used as the outgroup for *H. melanopogon,* while for the other three species conspecific lineages from other Amazonian rivers were used as outgroups, based on well supported clades found in the previous phylogenetic analysis (MrBayes). The Yule speciation process was selected and a rate of evolution of 2.1% divergence per million years for *cytb* (Weir & Schluter, 2008) was employed with an uncorrelated lognormal relaxed clock (Drummond et al., 2006). We ran MCMC with 10,000,000 of generations, discarding 10% as burn-in. Convergence of the runs was confirmed in TRACER 1.6 (Rambaut et al., 2014). FigTree 1.4.2 (Rambaut, 2012) was used to visualize the phylogenetic trees.

The genealogy of haplotypes was evaluated in Network 4.6 (http://www.fluxus-engineering.com/sharenet.htm) (Bandelt et al., 1999), including samples from the Negro basin (Anavilhanas, Jaú, Mariuá and Branco). To verify the occurrence of population structure, we estimated the number of clusters (*k)* using Bayesian posterior probability in BAPS 6.0 (Corander et al., 2013). The mixture model was applied to test for groups of individuals using four iterations of *k* ranging from one to ten. No prior information about sampling localities was provided.

Summary statistics related to number of haplotypes (*Hn*), haplotype diversity (*Hd*) and nucleotide diversity (*π)* were calculated in DnaSP 5.0 (Librado & Rozas, 2009). To verify hierarchical structure of the genetic variation, a hierarchical AMOVA was performed using the same scenarios evaluated for the nuclear markers, based on global values of genetic distance-based *Φst*. Also in this context, a pairwise matrix of dissimilarity index *Φst* comparing the different Negro basin archipelagos was built. To evaluate the relationship between geographic and genetic distances, Mantel tests were employed. These analyses were computed in Arlequin 3.5 (Excoffier & Lischer, 2010).

To assess information about historical population demography within the Negro river basin, Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) were used based on 10,000 coalescent simulations in DnaSP 5.0 (Librado & Rozas, 2009). Also in this context, Extended Bayesian Skyline Plots (EBSP) were generated to infer variations on effective population size through time using BEAST 1.8 (Drummond & Rambaut, 2007). Selection of evolutionary models and evolutionary rates used were the same used to estimate divergence times (described above). Setting of priors, operators and demographic indicators followed recommendations of Heled & Drummond (2008). The Bayesian MCMC was performed for 100,000,000 generations sampling every 100 generations, whilst discarding the initial 10% of samples as burn-in. Finally, the convergence of runs was evaluated on TRACER 1.6 (Rambaut et al., 2014).

RESULTS

Phylogeographical context of the Negro basin samples

As all studied species have distributions that are larger than the Negro basin, phylogeographical analyses were performed using the mtDNA dataset to determine the evolutionary relationships of the samples from this region on a larger spatial context. In *H. melanopogon* samples from the western portion of the Amazon basin (upper Solimões) appeared within the Negro river basin clade (Figure S1). Within *M. assimilis* samples from western Amazon (upper Solimões) appeared as the sister group to an eastern clade including samples from the Negro basin and from the lower Solimões and the Amazon/Madeira/Tapajós rivers (Figure S1). Divergence between western and eastern Amazonia was dated to about 400 ky (Figure 3). For both *T. nigrocinereus* and *M. lugubris* the Negro river basin samples formed a monophyletic clade (Figure S1) that originated at about 500 ky and 600 ky, respectively (Figure 3).

Population structure at the Negro basin

Microsatellite data

Eight microsatellite loci were polymorphic for *H. melanopogon* and *T. nigrocinereus*, while five exhibited polymorphism in *M. lugubris* and *M. assimilis* (Table 2). Linkage disequilibrium was not detected in any species. Considering differences between observed and expected heterozygosity, several loci in all species were not in HWE after Bonferroni correction, with some deviations being restricted to specific archipelagos (Table S5). The possible occurrence of null alleles was indicated for these loci. The mean observed heterozygosity ranged between 0.18 (*M. lugubris*) and 0.53 (*T. nigrocinereus*) (Table 2).

Most nuclear genetic diversity has no correlation with geography, with 77.51% (*M. lugubris)* to 92.05% (*T. nigrocinereus)* of the molecular variation occurring within islands (Table 3). The absence of geographic structure within the Negro river basin was also evident for *T. nigrocinereus* by a significant correlation between genetic and geographic distances found in the Mantel test (Figure S2). Accordingly, despite STRUCTURE results indicated *k=5* for *H. melanopogon* and *M. lugubris,* and *k*=2 for *M. assimilis* (Figure S3), the small difference between likelihood values and *barplots* interpretation indicates the occurrence of just one population (Pritchard et al., 2000). Pairwise F_{ST} matrix between archipelagos ranged from -0.07 (tending to zero) to 0.03 (comparison between Jaú and Anavilhanas to *M. assimilis*), indicating genetic similarity among islands of lower Negro and, consequently, gene flow (Table S6).

Mitochondrial data

Sequences of *cytb* (911 to 1020bp) and *ND2* (944 to 1027bp) were obtained for 175 individuals of the four studied species. The generalist floodplain species *H. melanopogon* exhibited 29 haplotypes within the Negro River basin, with nucleotide diversity varying from 0.4% in Anavilhanas to 0.7% in Jaú (Table 4). In contrast, the number of haplotypes found for the specialist species in the same region ranged from 17 (*M. lugubris*) to 19 (*T.

nigrocinereus), with lower nucleotide diversities when compared to H. melanopogon (Table 4).

Accordingly, *H. melanopogon* haplotypes were differentiated by several mutational steps, did not exhibit any relationship with geographic regions and only one population was identified in the BAPS analysis (Figure 2). Within island specialist species some geographic signal was present, but there were shared haplotypes between Anavilhanas and Jaú (*M. lugubris* and *M. assimilis*) and among Anavilhanas, Jaú and Branco river (*T. nigrocinereus*) (Figure 2). Population structure analysis identified two lineages for *M. lugubris* and *T. nigrocinereus*, and three for *M. assimilis* (dashed lines, Figure 2).

Although most of the mitochondrial genetic diversity was within islands for all species (i.e., no geographic structure), a considerable proportion of genetic diversity was attributed to comparisons between archipelagos for *M. lugubris* (24.04%) and *M. assimilis* (21.55%) and between islands for H. melanopogon (29.03%) (Table 3). Pairwise dissimilarity index between Jaú and Anavilhanas archipelagos was significant for *M. lugubris* (Table S6), suggesting restricted gene flow.

Historical demography at the Negro basin

Demographic analyzes based on summary statistics detected signal of population expansion in the Negro river basin for all the species (Table 4). When the archipelagos were analyzed independently, demographic expansion was detected for *H. melanopogon* in both Anavilhanas and Jaú. Among the island specialists, the signal of demographic change were subtle or inexistent: in *M. lugubris*, just Tajima's *D* in Jaú was significant (*D*=-2.07). *T. nigrocinereus* exhibited expansion in Anavilhanas (*Fs*=-3.82) and *M. assimilis* did not present signal of population demographic changes in any archipelago.

Bayesian Skyline Plots corroborate the trends of population expansion within the Negro river basin (Figure 4). Expansion events are not very pronounced and occurred at approximately 50,000 years in *H. melanopogon* and *M. lugubris*, while in *M. assimilis* and *T. nigrocinereus*, the expansion events were older, happening at 80,000 and 100,000 years before present, respectively.

DISCUSSION

Historical connections among Amazonian insular birds

This study is one of the first efforts to understand population dynamics in Amazonian fluvial island birds. These naturally fragmented environments represent a significant area of floodplains and harbor specialized fauna and flora communities, which exhibit mechanisms for dealing with seasonal changes in flooding level (Piedade et al., 2005; Cintra et al., 2007). For this reason, different patterns recovered in species with distinct ecological characteristics are possibly related to differing strategies to use these flooded habitats.

In a broader geographic scale, the identification of distinct lineages within *M. lugubris*, *M. assimilis* and *T. nigrocinereus* associated to different Amazonian rivers may be an indication of higher restriction to gene flow in islands specialist species when compared to floodplain generalist species. The discrete distribution of genetic diversity in these floodplain species contradicts the proposal of river-created habitats as “corridors” to gene flow. Indeed, Aleixo (2006) found a lack of population structure in two Dendrocolaptidae species that occupy floodplains. *Xiphorhynchus obsoletus* and *X. kienerii* exhibited one haplotype widely shared throughout the species distributions, and AMOVA results indicated high genetic variation within populations (Aleixo, 2006). These patterns may be due to the use of continuous environments at the rivers banks in a generalist manner by these species. A similar pattern was observed in *Chrysomus icterocephalus* (Icteridae), with one ancestral haplotype represented throughout its whole distribution (Cadena et al., 2011), but in this case, besides habitat connectivity, the use of open vegetation areas by these birds may also promote gene flow (Bates et al., 2003).

On the other hand, considering the high microhabitat specialization of island specialist Antbirds, it may be expected that there are limitations to dispersal in regions where islands are rare or absent. The results obtained for *T. nigrocinereus* agree with the hypothesis that microhabitat continuity rules gene flow in specialist species. In the lower Negro River archipelagos, where distances among islands are smaller, the genetic differentiation of nuclear DNA correlates with geographic distances, a pattern that is expected in continuous habitat conditions. Besides, AMOVA results for both markers in this species exhibited more than 90% of variation occurring without relationship to geographic structure<distance?>, similar to the result obtained in *X. obsoletus* and *X. kienerii* (Aleixo, 2006). In contrast, different lineages of *T. nigrocinereus* were found in islands from other Amazonian rivers (Solimões, Madeira, Tapajós and Amazonas). The absence of islands in the final stretch of lower Negro may act as a barrier to dispersal for this species. Another peculiarity of this region that may reduce gene flow are the poorly developed floodplains in river banks (Franzinelli & Igreja, 2002). Therefore, even the occasional use of the river margins by *T. nigrocinereus* is impaired, which may have interrupted connectivity and, consequently, isolated populations from the Negro basin.

Population dynamics of Negro river basin insular birds

Even considering the restricted mobility and territorialism typical of the Thamnophilidae, we found evidence of varying degrees of gene flow among islands and archipelagos of the Negro river basin for the four studied species. Most of the genetic variation occurred without relationship to geography, pairwise dissimilarity indexes comparing Anavilhanas and Jaú were not significant in most cases, nuclear DNA variation indicates one single population within each species, and there were shared haplotypes among areas.

Despite this lack of population structure related to the distinct archipelagos, genetic diversity is not homogeneously distributed in the landscape. The percentage of molecular variation related to geographic structure (i.e., between islands or between archipelagos) were considerably high in *H. melanopogon*, *M. assimilis* and *M. lugubris*. It should also be noted that this last species exhibits significant values of Φst when comparing mtDNA data for the Anavilhanas and Jaú archipelagos, and that population structure analysis of this same dataset indicates the presence of more than one population for the three island specialists. Accordingly, for the nuDNA, deviations in HWE restricted to a specific geographic area may indicate differences in allele frequencies as a result of population substructure (Bradic et al., 2012).

The fact that stronger indication of population structure among archipelagos was found in the mtDNA dataset but not in the nuDNA may reflect the differences on effective population size for the two kinds of marker. Population size of island species may be low, and even lower for the haploid and maternally inherited mtDNA, making this marker faster in fixing differences among populations. It is also possible that the indication of population structure found for the mtDNA data refers to a historical isolation, with gene flow being recently re-established due to wetter conditions since the last glacial or to increased formation of island environments after the recent evolution of the lower Negro river (Latrubesse & Franzinelli, 2005).

The use of islands by generalist floodplain species also occurs in a different manner when compared to other floodplain environments. While *X. obsoletus*, *X. kienerii* and *C. icterocephalus* shared haplotypes across their ranges and did not exhibit any evidence of geographic<genetic?> structure (Aleixo, 2006; Cadena et al., 2011), *H.* melanopogon has greater nucleotide diversity, exhibiting just few haplotypes shared among individuals. Burney & Brumfield (2009) evaluated the effect of physiographic barriers on 40 bird species from the Neotropical lowlands and concluded that organisms that use different vegetation strata have distinct patterns of genetic variation, with canopy species exhibiting low genetic variation due their higher dispersal ability, while understory species were more sensitive to barriers (Burney & Brumfield, 2009). In fluvial islands this pattern cannot be expected. Birds that occupy midstory or canopy are possibly less vulnerable to water level variation, while organisms that use understory (as *H. melanopogon*) have to go through vertical or horizontal displacement in flooding periods. The pattern of genetic diversity found in *H. melanopogon* is possibly a result of several events of displacement from islands with subsequent re-colonization, due to seasonal unavailability of the islands understory. A similar pattern was observed in small rodents that occupy lower vegetation stratum of insular habitats in the Araguaia River (Rocha et al., 2014). In contrast, fluvial island specialists remain on these environments throughout the year by having adaptive features to deal with the seasonal flooding.

Biogeography of Negro river basin island birds in an Amazonian context

Although all of them occupy the same river islands, the four species studied here have distinct phylogeographic patterns (Figure 2). *Hypocnemoides melanopogon* does not show a clear geographic structure of its genetic diversity, while the three island specialist species exhibit some genetic structure related to island systems from different rivers. For these three species, the Negro basin clade diverged from populations from western Amazonia (upper and middle Solimões) during the Pleistocene, and, despite large confidence intervals, the estimated dates for this split overlap at about 500 ky before present.

The Anavilhanas archipelago probably formed due to Holocene sediment deposition (Latrubesse & Franzinelli, 2005), so that the origin of Negro River basin island specialist lineages predates the origin of this archipelago. This suggests an intense dynamic of island habitats availability in the lower Negro river region, which may have created fleeting insular environments that were occupied by ancestral lineages. This scenario is possible considering the influence of Quaternary climatic and eustatic changes on the discharge and sedimentation rates of Amazonian rivers (Iriondo & Latrubesse, 1994; Irion et al., 2009). Concerning the Negro River, rising sea levels in interglacial periods may have caused a backwater effect that promoted lentic conditions, favoring deposition of fine material and island formation (Irion et al., 2009), but also complete flooding of some island systems close to river mouths. Subsequently, glacial periods led to an increase in the energy of the drainage system, causing erosion of island fine grained sediments, and deposition of larger particles (Irion et al., 2009). It is not clear what were the real effects of these processes on insular habitats availability, but they possibly promoted the origination, evolution and extinction of islands and archipelagos, interfering on the establishment of ancestral populations in the lower Negro river region.

Accordingly, slight signals of population expansion events in the four species were dated to the Pleistocene. These events, though, seem much less pronounced than population expansions that have been reported for Amazonian upland forest species (Fernandes et al., 2012; Ribas et al., 2012). Contrary to most upland forest species, floodplain organisms seem to have smaller populations of more constant size through recent times (Aleixo, 2006). Possibly, river-created environments suffer spatial rearrangements and changes in connectivity, but not large variations in habitat availability during glacial periods (Aleixo, 2006; Irion et al., 2009).

The congruent timing of the divergence between lineages from the Negro and western Amazonian (upper and middle Solimões) islands may be related to geologic events that affected the establishment of channels and islands in central Amazonia (Almeida-Filho & Miranda 2007, Santos et al 2015). This region was probably very dynamic due to the recent connection between the formerly independent drainage systems of eastern and western Amazonia (Nogueira et al., 2013), which may have generated current patterns of genetic diversity found in several organisms. East-west variations in biological communities of flooded forests (*várzea)* have been described for trees, ants, spiders and fishes, being the confluence between the Negro and the Solimões rivers a common geographical limit for changes in composition or abundance of species (Albernaz et al., 2007; Vasconcelos, 2007; Venticinque et al., 2007; Zuanon et al., 2007). Furthermore, several várzea bird species distributions are have their limits at this same region (Cohn-Haft et al., 2007), and phylogeographic breaks in this area have been detected for *Brotogeris sanctithomae* (Canton, 2014) and for discus fishes (*Symphysodon* spp.) (Farias & Hrbek, 2008). This dynamic history of central Amazonian riverine habitats is especially evident in the recent history of the lower Negro river. A complex network of palaeochannels linked the middle Negro river with the Manacapuru River, a tributary of the Solimões (Almeida-filho & Miranda, 2007). When active, these channels may have allowed the connection between populations from the middle Negro and lower Solimões through small islands or riverbanks. Thereafter, neotectonic events promoted a mega capture of the lower Negro river (Almeida-filho & Miranda, 2007), which originated the current channel and interrupted the connectivity between these two regions. Even with the development of the Anavilhanas archipelago, Negro river basin lineages remain isolated because the final stretch of the lower Negro has no insular habitats.

Implications for Amazonian biogeography and conservation

Large Amazonian rivers are known to influence distribution and genetic diversity patterns of upland forest birds (Cracraft, 1985; Ribas et al 2012), but diversification patterns of species that occupy river-created habitats are still poorly known. Thus, understanding the history and organization of lineages from floodplain or riverine habitats may offer important information on drainage evolution and, consequently, on the origins of Amazonian biodiversity. Here we show that birds from riverine islands have a dynamic history of contact and isolation, but with small historical fluctuation of population sizes, in sharp contrast with the patterns that have been described for upland forest birds. The small genetic distances across the basin, even when there is spatial structuring of the genetic diversity, points to a recent history of the island avifauna, and possibly to a recent origination of the Amazonian flooded habitats that we know today, greatly influenced and adapted to the annual flood pulse.

Amazonian flooded habitats are currently under special pressure due to the focus on developmental projects directed to generating hydroelectric power through the construction of large dams. These dams will disrupt the natural flooding pulse and permanently flood several river islands. Species as *T. nigrocinereus* and *M. lugubris* are already considered near threatened due the possible habitat reduction in the next years (IUCN, 2015). Understanding population structure and habitat use in island specialist species is important to access and mitigate these impacts.

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SUPPORTING INFORMATION INDEX

Additional information may be found in the online version of this article:

Appendix S1 Summarized species characteristics and list of samples containing vouchers, institutions and species used in this study.

Appendix S2 Collaborators, laboratorial procedures and table of primers.

Appendix S3 Supplementary results: full table of nuclear DNA summary analyzes, pairwise F_{ST} matrices, Mantel test and Structure.

BIOSKETCH

Érik Choueri is a Masters student at the postgraduate program in Ecology, Instituto Nacional de Pesquisas da Amazônia. Has interest in Island Biogeography, with emphasis in population dynamics of birds in fluvial habitats and the evolution of these landscapes.

Author contributions: E.C. conducted the research and the writing as part of requirements to achieved his Master degree; C.R and S.B. designed the study, contributed in the discussion and writing of the manuscript; C.G. helped with laboratory procedures, data analyzes and interpretation, and manuscript revision; G. T. Provided samples and preliminary analysis. E.C. and S.B. also collected most of the samples from Anavilhanas and Jaú National Park archipelagos.

TABLES

Table 1. Number of samples per species from each locality. *Hm*: *Hypocnemoides. melanopogon*, *Ml*: *Myrmoborus lugubris*, *Ma* = *Myrmotherula assimilis* and *Tn* = *Thamnophilus nigrocinereus*. *Sample correspondent to *Thamnophilus cryptoleucus*, which is the taxon from the Solimões within the *T. nigrocinereus-T, cryptoleucus* "species complex".

Rivers	**Reachs**	***Hm***	***Ml***	***Ma***	***Tn***	**Total**
Negro	Lower (Anavilhanas)	25	14	23	10	72
	Lower (Jaú)	26	22	8	17	73
	Middle (Mariuá)	2	-	1	-	3
Branco	Middle	2	2	1	2	7
	Lower	-	-	-	2	2
Solimões	Upper	-	-	2	-	2
	Middle	1	2	2	1*	6
	Lower	-	-	2	-	2
Madeira	Middle	-	1	1	1	3
Tapajós	Middle	-	-	-	1	1
Amazon	Upper	-	1	2	-	3
	Lower	-	-	-	1	1
Total		56	42	42	35	175

Table 2. Summary of the nuDNA data obtained for samples from the Negro river basin **(**Anavilhanas, Jaú, Mariuá and Branco islands). *n,* number of individuals; *Na*, number of alleles; *Ar*, allele richness; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *Fis*, inbreeding coefficient.

		n	*Na*	*Ar*	*Ho*	*He*	*Fis*
H. melanopogon	Anavilhanas	25	7.75	7.42	0.42	0.63	0.30
	Jaú	26	8.00	7.85	0.40	0.63	0.33
	Total	56	11.12	10.8	0.41	0.65	0.33
M. lugubris	Anavilhanas	14	4.00	3.73	0.18	0.57	0.68
	Jaú	28	7.23	6.78	0.24	0.62	0.57
	Total	42	7.62	7.27	0.24	0.64	0.62
M. assimilis	Anavilhanas	23	5.40	5.33	0.48	0.57	0.16
	Jaú	08	4.02	3.61	0.40	0.58	0.12
	Total	35	7.42	7.18	0.46	0.60	0.20
T. nigrocinereus	Anavilhanas	10	4.87	4.49	0.46	0.64	0.26
	Jaú	17	6.75	6.75	0.53	0.65	0.21
	Total	27	7.87	7.65	0.50	0.66	0.25

Table 3. Percentage of genetic variation per geographic scale for both molecular markers. Values in parenthesis refer to global dissimilarity indices F_{ST} (nuclear DNA) and Φst (mitochondrial DNA). Bold values are significative (*p-value* ≤ 0.05).

	H. melanopogon		*M. lugubris*		*M. assimilis*		*T. nigrocinereus*	
	nuDNA	mtDNA	nuDNA	mtDNA	nuDNA	mtDNA	nuDNA	mtDNA
Between archipelagos	3.12 (0.00)	5.95 (0.06)	2.85 (0.02)	24.04 **(0.24)**	7.46* **(0.07)**	21.55 **(0.22)**	5.76 (0.06)	2.68 (0.02)
Between islands	11.17 **(0.10)**	29.03 **(0.31)**	19.64 **(0.21)**	3.72 (0.05)	4.80 (0.05)	15.52 **(0.20)**	2.18 (0.02)	-2.09 (-0.21)
Within islands	85.71 **(0.10)**	65.04 **(0.35)**	77.51 **(0.22)**	72.25 **(0.28)**	87.00 **(0.12)**	62.94 **(0.37)**	92.05 (0.08)	99.42 (0.00)

Table 4. Mitochondrial DNA summary statistics and neutrality tests per different localities and total (considering Anavilhanas, Jaú, Mariuá and Branco islands). *n*, number of individuals; *Hn*, number of haplotypes; *Hd* haplotype diversity; *π*, nucleotide diversity; s.d., standard deviation in brackets; *D*, Tajima's test value; *Fs*, Fu's test value. *p<0.05, **p<0.01, ***p<0.001.

Species	Locality	*n*	*Hn*	*Hd* (±s.d.)	*π* (±s.d.)	D	Fs
Hypocnemoides melanopogon	Anavilhanas	18	16	0.988 (±0.021)	0.004 (±0.0012)	-2.36**	-7.73**
	Jaú	11	11	1.000 (±0.024)	0.007 (±0.0010)	-0.97	-6.23**
	Total	33	29	0.996 (±0.007)	0.005 (±0.0004)	-2.26**	-24.7***
Myrmoborus lugubris	Anavilhanas	12	07	0.833 (±0.100)	0.002 (±0.0003)	-1.61	-0.34
	Jaú	20	09	0.821 (±0.073)	0.001 (±0.0002)	-2.07*	-1.03
	Total	34	17	0.891 (±0.040)	0.002 (±0.0005)	-2.28**	-5.50*
Myrmotherula assimilis	Anavilhanas	17	10	0.882 (±0.059)	0.001 (±0.0002)	-1.51	-2.87
	Jaú	7	07	1.000 (±0.001)	0.003 (±0.0012)	-1.54	-2.13
	Total	26	18	0.938 (±0.033)	0.002 (±0.0004)	-2.32**	-8.45**
Thamnophilus nigrocinereus	Anavilhanas	7	07	1.000 (±0.076)	0.001 (±0.0003)	-1.36	-3.82**
	Jaú	15	08	0.867 (±0.067)	0.001 (±0.0003)	-1.54	-2.12
	Total	30	19	0.908 (±0.045)	0.001 (±0.0003)	-2.39**	-12.8***

FIGURES

Figure 1. Study area and sampled islands.

Figure 2. Haplotype networks per species. Colors refers to locality: Yellow: Anavilhanas; Red: Jaú; Green: Solimões; Pink: Branco; Blue: Mariuá. Dashed lines indicate populations inferred by the BAPS analysis.

Figure 3. Divergence times among intra specific lineages of the four studied species. Numbers close to nodes are posterior probabilities. Nodes without these values had support lower than 0.95. Blue bars are confidence intervals of divergence time estimates. Vertical bars indicate collection localities: Green: upper and middle Solimões; light green: lower Solimões; Orange: Amazon/Madeira/Tapajós; Pink: Branco River; Blue: Mariuá archipelago; Gold: lower Negro islands. Birds' images were adapted from HBW Alive (http://hbw.com). ** outgroup: *Hypocnemoides maculicauda*

Figure 4. Extended Bayesian Skyline Plots for each species. Black line indicate estimated mean. Gray background is the confidence interval.

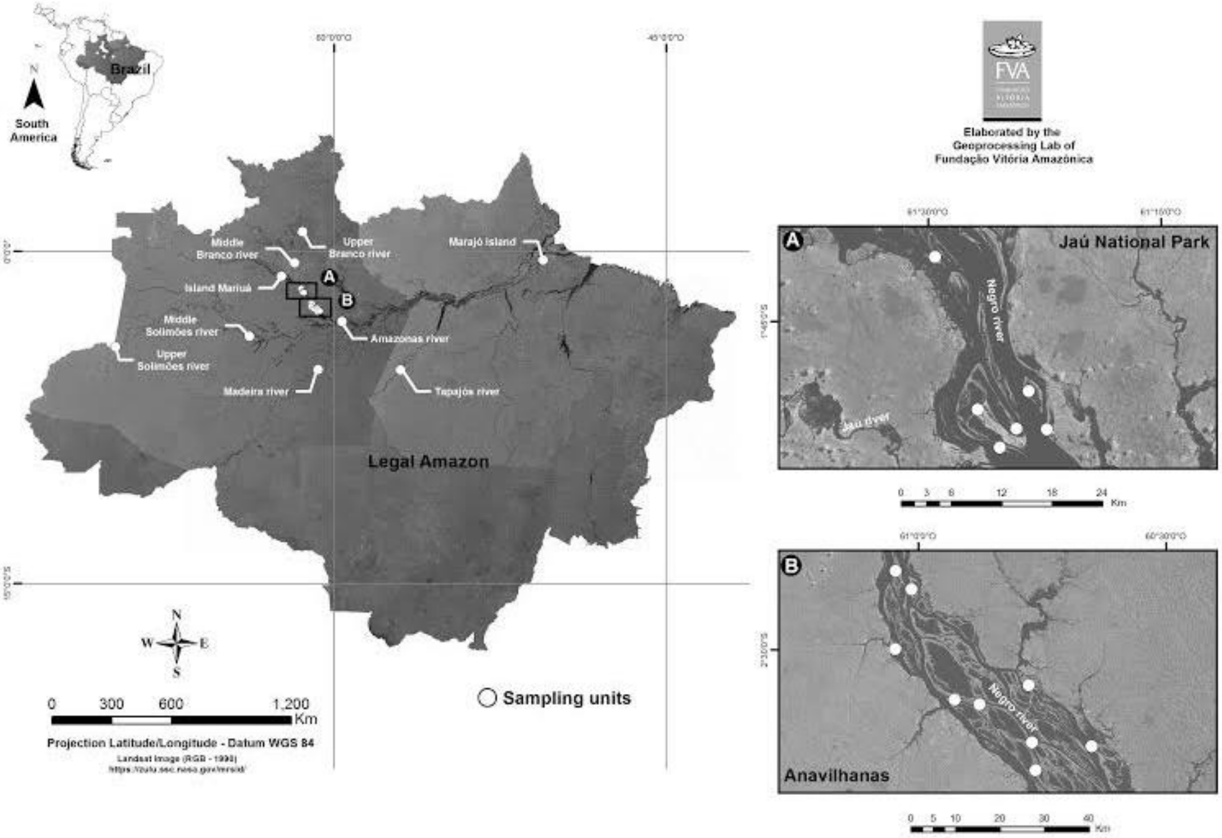

South America
Brazil
N
Elaborated by the
Geoprocessing Lab of
Fundação Vitória Amazônica
FVA
Middle Branco river
Upper Branco river
Marajó island
Island Mariuá
A
B
Middle Solimões river
Upper Solimões river
Amazonas river
Madeira river
Tapajós river
Legal Amazon
N
W
E
S
0
300
600
1,200
Km
Projection Latitude/Longitude - Datum WGS 84
Landsat Image (RGB - 1990)
https://zulu.ssc.nasa.gov/mrsid/
Sampling units
Jaú National Park
Negro river
Jaú river
0
3
6
12
18
24
Km
Anavilhanas
Negro river
0
5
10
20
30
40
Km

Figure 1.

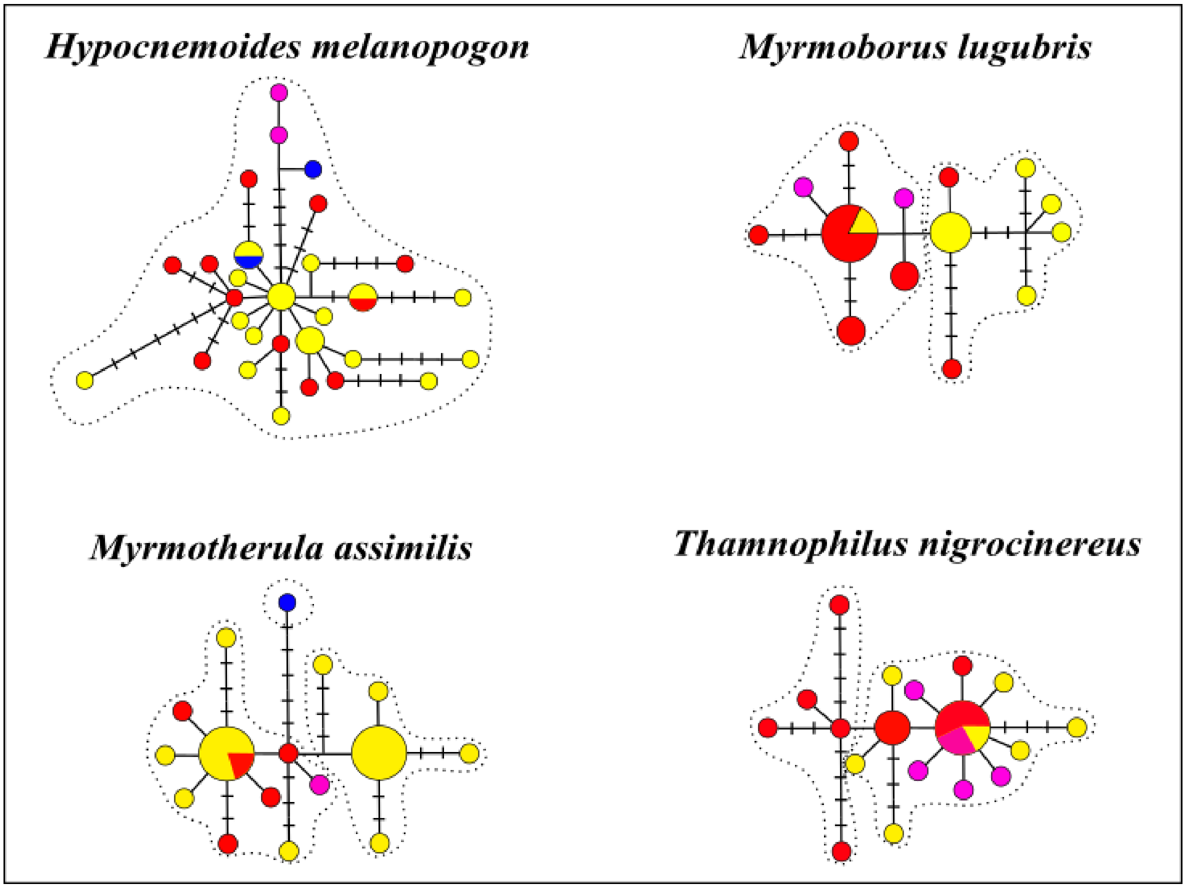
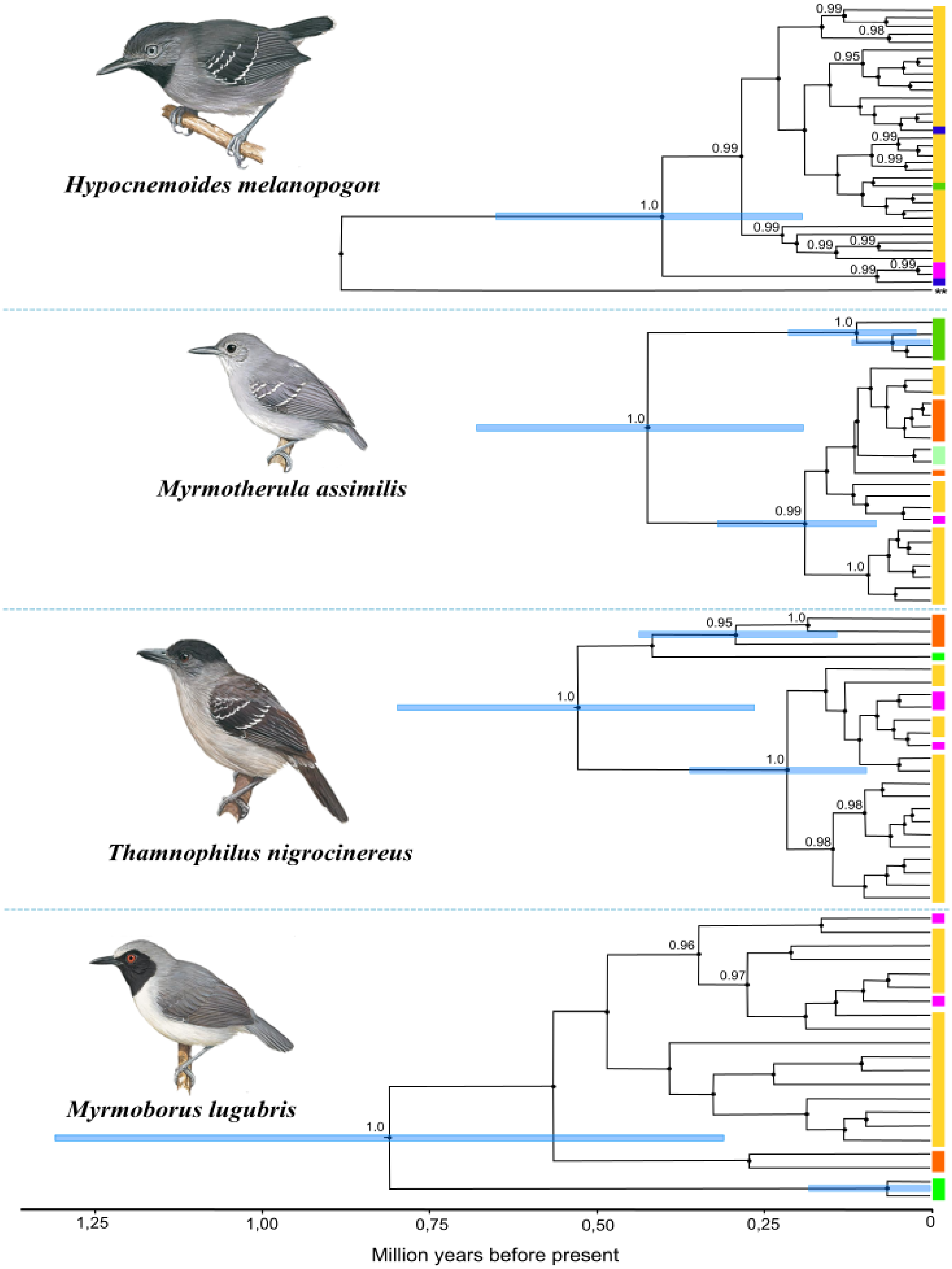

Hypocnemoides melanopogon
Myrmoborus lugubris
Myrmotherula assimilis
Thamnophilus nigrocinereus

Figure 2.


Hypocnemoides melanopogon
0.99
0.98
0.95
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0.99
1.0
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**
Myrmotherula assimilis
1.0
1.0
0.99
1.0
Thamnophilus nigrocinereus
0.95
1.0
1.0
1.0
0.98
0.98
Myrmoborus lugubris
0.96
0.97
1.0
1,25
1,00
0,75
0,50
0,25
0
Million years before present

Figure 3.

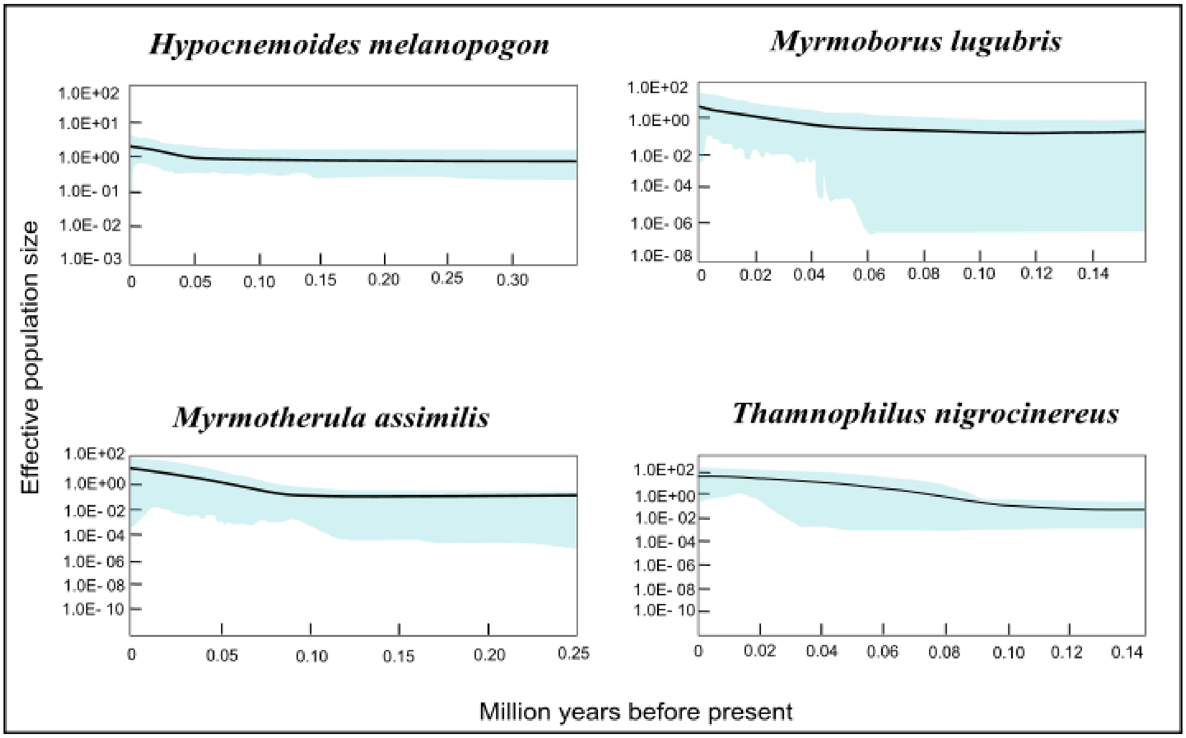
Hypocnemoides melanopogon
Myrmoborus lugubris
Myrmotherula assimilis
Thamnophilus nigrocinereus
Effective population size
Million years before present
1.0E+02
1.0E+01
1.0E+00
1.0E- 01
1.0E- 02
1.0E- 03
0
0.05
0.10
0.15
0.20
0.25
0.30
1.0E- 04
1.0E- 06
1.0E- 08
1.0E- 10
0.02
0.04
0.06
0.08
0.12
0.14

Figure 4.

SUPPORTING INFORMATION

Appendix S1

Table S1. Morphological and ecological characteristics of studied species as described by Zimmer & Isler (2003, [1]) and personal observations ([2]).

Species	**Average size (cm)[1]**	**Average weight (g)[1]**	**Ratio wing length/weight[2]**	**Vegetation Stratum[2]**
Hypocnemoides melanopogon	12	14	4.90	Understory (0 – 2 m)
Myrmoborus lugubris	13	21	2.97	Understory (0 – 3 m)
Myrmotherula assimilis	09	09	5.60	Understory/ mid-story
Thamnophilus nigrocinereus	16	30	2.58	Understory/ mid-story

Table S2. Samples used including Locality, Species identification, Voucher institution, voucher number and geographical coordinates.

Locality	Specie	Institution	Voucher #	LAT	LONG
NA	*Gymnocichla nudiceps*	LSUMZ	2228	NA	NA
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16761	-2.49767	-61.0462
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16763	-2.49767	-61.0462
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16770	-2.60948	-60.8759
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16774	-2.60948	-60.8759
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16781	-2.60948	-60.8759
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16974	-2.60948	-60.8759
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16975	-2.60948	-60.8759
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16978	-2.60019	-60.9261
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16996	-2.37669	-61.0136
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16994	-2.37669	-61.0136
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16990	-2.33978	-61.0455
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16992	-2.33978	-61.0455
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-16993	-2.33978	-61.0455
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17003	-2.57064	-60.7767
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17012	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17013	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17018	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17021	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17022	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17023	-2.68614	-60.7698
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17027	-2.69386	-60.6498
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17028	-2.69386	-60.6498
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17040	-2.69386	-60.6498
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17041	-2.74236	-60.7625
Anavilhanas	*Hypocnemoides melanopogon*	INPA	A-17048	-2.74236	-60.7625
Jaú	*Hypocnemoides melanopogon*	INPA	A-10595	-1.84383	-61.3801
Jaú	*Hypocnemoides melanopogon*	INPA	A-10596	-1.84383	-61.3801
Jaú	*Hypocnemoides melanopogon*	INPA	A-10509	-1.87567	-61.3671
Jaú	*Hypocnemoides melanopogon*	INPA	A-10575	-1.87567	-61.3671
Jaú	*Hypocnemoides melanopogon*	INPA	A-10577	-1.87567	-61.3671
Jaú	*Hypocnemoides melanopogon*	INPA	A-10594	-1.87567	-61.3671
Jaú	*Hypocnemoides melanopogon*	INPA	A-10601	-1.87567	-61.3671
Jaú	*Hypocnemoides melanopogon*	INPA	A-4511	-1.88512	-61.4228
Jaú	*Hypocnemoides melanopogon*	INPA	A-4512	-1.88512	-61.4228
Jaú	*Hypocnemoides melanopogon*	INPA	A-4513	-1.88512	-61.4228
Jaú	*Hypocnemoides melanopogon*	INPA	A-4514	-1.88512	-61.4228
Jaú	*Hypocnemoides melanopogon*	INPA	A-15775	-1.855	-61.4383

Jaú	*Hypocnemoides melanopogon*	INPA	A-15778	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15779	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15780	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15789	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15790	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15791	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15792	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15793	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15794	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15795	-1.855	-61.4383
Jaú	*Hypocnemoides melanopogon*	INPA	A-15822	-1.68083	-61.4925
Jaú	*Hypocnemoides melanopogon*	INPA	A-15826	-1.68083	-61.4925
Jaú	*Hypocnemoides melanopogon*	INPA	A-15831	-1.68083	-61.4925
Jaú	*Hypocnemoides melanopogon*	INPA	A-15832	-1.68083	-61.4925
Rio Branco	*Hypocnemoides melanopogon*	INPA	A-8378	-0.53	-61.7992
Rio Branco	*Hypocnemoides melanopogon*	INPA	A-8380	-0.53	-61.7992
Mariuá	*Hypocnemoides melanopogon*	INPA	A-2273	-1.13333	-62.3833
Mariuá	*Hypocnemoides melanopogon*	INPA	A-2274	-1.13333	-62.3833
Solimões	*Hypocnemoides melanopogon*	INPA	A-052	-3.90278	-62.8222
Mato Grosso (Midwest Brazil)	*Hypocnemoides maculicauda*	ZMCU	S1301	-9.6	-55.9
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16773	-2.60948	-60.8759
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16799	-2.60019	-60.9261
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16792	-2.60019	-60.9261
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16793	-2.60019	-60.9261
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16808	-2.33978	-61.0455
Anavilhanas	*Myrmoborus lugubris*	INPA	A-16809	-2.33978	-61.0455
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17001	-2.57064	-60.7767
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17002	-2.57064	-60.7767
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17010	-2.68614	-60.7698
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17011	-2.68614	-60.7698
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17017	-2.68614	-60.7698
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17035	-2.69386	-60.6498
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17042	-2.74236	-60.7625
Anavilhanas	*Myrmoborus lugubris*	INPA	A-17043	-2.74236	-60.7625
Jaú	*Myrmoborus lugubris*	INPA	A- 3419	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 3566	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 10504	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 10505	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 10592	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15798	-1.84383	-61.3801

Jaú	*Myrmoborus lugubris*	INPA	A- 15803	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15804	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15805	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15806	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15807	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15808	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15809	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15810	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15812	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 15815	-1.84383	-61.3801
Jaú	*Myrmoborus lugubris*	INPA	A- 3095	-1.87567	-61.3671
Jaú	*Myrmoborus lugubris*	INPA	A- 3100	-1.87567	-61.3671
Jaú	*Myrmoborus lugubris*	INPA	A- 3103	-1.87567	-61.3671
Jaú	*Myrmoborus lugubris*	INPA	A- 10508	-1.87567	-61.3671
Jaú	*Myrmoborus lugubris*	INPA	A- 10589	-1.87567	-61.3671
Jaú	*Myrmoborus lugubris*	INPA	A- 10591	-1.87567	-61.3671
Rio Branco	*Myrmoborus lugubris*	INPA	A-8376	-0.53	-61.7992
Rio Branco	*Myrmoborus lugubris*	INPA	A-8379	-0.53	-61.7992
Solimões	*Myrmoborus lugubris*	INPA	A-960	-3.78106	-64.0254
Solimões	*Myrmoborus lugubris*	INPA	A-971	-3.84583	-63.8301
Madeira	*Myrmoborus lugubris*	LGEMA	ETA-438	NA	NA
Amazonas	*Myrmoborus lugubris*	LGEMA	ETA-518	NA	NA
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16758	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16759	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16760	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16765	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16766	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16967	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16968	-2.49767	-61.0462
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16970	-2.60948	-60.8759
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16976	-2.60948	-60.8759
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16982	-2.37669	-61.0136
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16985	-2.37669	-61.0136
Anavilhanas	*Myrmotherula assimilis*	INPA	A-16995	-2.37669	-61.0136
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17000	-2.33978	-61.0455
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17005	-2.57064	-60.7767
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17009	-2.68614	-60.7698
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17015	-2.68614	-60.7698
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17019	-2.68614	-60.7698
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17026	-2.69386	-60.6498
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17029	-2.69386	-60.6498
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17036	-2.69386	-60.6498
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17037	-2.69386	-60.6498

Anavilhanas	*Myrmotherula assimilis*	INPA	A-17051	-2.74236	-60.7625
Anavilhanas	*Myrmotherula assimilis*	INPA	A-17052	-2.74236	-60.7625
Jaú	*Myrmotherula assimilis*	INPA	A-15802	-1.84383	-61.3801
Jaú	*Myrmotherula assimilis*	INPA	A-15811	-1.84383	-61.3801
Jaú	*Myrmotherula assimilis*	INPA	A-15813	-1.84383	-61.3801
Jaú	*Myrmotherula assimilis*	INPA	A-15788	-1.855	-61.4383
Jaú	*Myrmotherula assimilis*	INPA	A-15796	-1.855	-61.4383
Jaú	*Myrmotherula assimilis*	INPA	A-15797	-1.855	-61.4383
Jaú	*Myrmotherula assimilis*	INPA	A-15823	-1.68083	-61.4925
Jaú	*Myrmotherula assimilis*	INPA	A-15834	-1.68083	-61.4925
Mariuá	*Myrmotherula assimilis*	INPA	A-2270	-1.13333	-62.3833
Rio Branco	*Myrmotherula assimilis*	INPA	A-8346	-0.53	-61.7992
Solimões	*Myrmotherula assimilis*	INPA	A-937	-3.78106	-64.0254
Solimões	*Myrmotherula assimilis*	LGEMA	ETA-212	-3.37944	-64.6411
Solimões	*Myrmotherula assimilis*	LGEMA	ETA-046	-4.32436	-69.8862
Solimões	*Myrmotherula assimilis*	LGEMA	ETA-051	-4.32436	-69.8862
Solimões	*Myrmotherula assimilis*	LGEMA	ETA-336	-3.28775	-60.215
Solimões	*Myrmotherula assimilis*	LGEMA	ETA-338	-3.28775	-60.215
Madeira	*Myrmotherula assimilis*	LGEMA	ETA-399	-4.37547	-60.9542
Amazonas	*Myrmotherula assimilis*	LGEMA	ETA-490	-3.15878	-58.3703
Amazonas	*Myrmotherula assimilis*	LGEMA	ETA-549	-2.57986	-56.6792
NA	*Thamnomanes caesius*	LSUMZ	B-9482	NA	NA
NA	*Thamnophilus punctatus*	USNM	B-4172	NA	NA
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16771	-2.60948	-60.8759
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16785	-2.60948	-60.8759
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16786	-2.60948	-60.8759
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16798	-2.60019	-60.9261
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16804	-2.37669	-61.0136
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16817	-2.69386	-60.6498
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-16980	-2.60019	-60.9261
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-17038	-2.69386	-60.6498
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-17046	-2.74236	-60.7625
Anavilhanas	*Thamnophilus nigrocinereus*	INPA	A-17049	-2.74236	-60.7625
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 4153	-1.87394	-61.3954
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 3093	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 3104	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 3565	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 10583	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15799	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15800	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15801	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15814	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15816	-1.84383	-61.3801

Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15817	-1.84383	-61.3801
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 3099	-1.87567	-61.3671
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 3101	-1.87567	-61.3671
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 10585	-1.87567	-61.3671
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 4721	-1.88512	-61.4228
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 4722	-1.88512	-61.4228
Jaú	*Thamnophilus nigrocinereus*	INPA	A- 15786	-1.855	-61.4383
Branco	*Thamnophilus nigrocinereus*	INPA	A-5621	0.8827	-61.4338
Branco	*Thamnophilus nigrocinereus*	INPA	A-5622	0.8827	-61.4338
Branco	*Thamnophilus nigrocinereus*	INPA	A-8360	-0.53	-61.7992
Branco	*Thamnophilus nigrocinereus*	INPA	A-8394	-1.29528	-61.8625
Solimões	*Thamnophilus cryptoleucus*	INPA	A-943	-3.78106	-64.0254
Madeira	*Thamnophilus nigrocinereus*	INPA	A-295	-5.34932	-60.7337
Tapajós	*Thamnophilus nigrocinereus*	INPA	A-12366	-5.35292	-56.9646
Amazonas	*Thamnophilus nigrocinereus*	MPEG	MAYA-003	-0.38684	-50.5541

Appendix S2
Laboratory procedures

To amplify mitochondrial regions, approximately 50 ng of DNA were used in Polymerase Chain Reaction (PCR). The PCR mix consisted of PCR Green GoTaq® Flexi Buffer (5x), Magnesium Chloride (25.0 mM), dNTP (2 mM), specific primers at 10 mM and 5U of Taq Polymerase (Invitrogen) with the following profile: initial denaturation of 4 min at 94°C; 30 cycles of 60s at 94°C to denature, 60s at specific primer's annealing temperature (Table S2) and 2 min at 72°C. A final extension step of 10 min at 72°C finish the cycles. PCR product was purified using PEG 8000 (Polyethylene glycol, NaCl and Milli-q water). Purified products were sequenced with Big Dye® Terminator Kit XTerminator v3.1 cyclosequencing (Applied Biosystems), following the manufacturer's protocol on a ABI 3130 Genetic Analyzer (Applied Biosystems).

Microsatellite variability was assessed using the following protocol: 4 min at 94°C, followed by 30 cycles at 94°C for 30 s, 45 s at specific annealing temperature (Table S3) and 45 at 72°C, and 8cycles at 94°C for 30 s, 45 s at 53°C and 72°C for 45 seconds, finishing at 72°C for 10 min. Genotyping was performed on a ABI 3130 Genetic Analyzer (Applied Biosystems) using GeneScan™ 500 ROX Size Standard (Applied Biosystems), according to the manufacturer's protocol.

Table S3: Mitochondrial primers for *cytb* and *ND2* (Sorenson et al., 1999). Annealing temperatures (Tm) were determined for each specie, being *Hm*: *H. melanopogon*, *Ml: M. lugubris*, *Ma*: *M. assimilis* and *Tn: T. nigrocinereus*.

Primer	Sequence (5' - 3')	Tm (°C)			
		Hm	*Ml*	*Ma*	*Tn*
Cytb					
L14990	5'-AATATTTCAGCCTGATGGAA-3'	52	50	56	50
H16065	5'-AACTGCAGTCATCTCCGGTTTACAAGA-3'				
ND2					
L5204	5'-TAACTAAGCTATCGGGCCCAT-3'	60	56	56	56
H6313	5'-ACTCTTRTTTAAGGCTTTGAAGGC-3'				

Table S4: Microsatellite primers and annealing temperature (Tm) for each species (Ágreda et al., 2006; Barnett et al., 2007; Feldheim et al., 2010). *Hm*: *Hypocnemoides melanopogon*, *Ml*: *Myrmoborus lugubris*, *Ma*: *Myrmotherula assimilis* e *Tn*: *Thamnophilus nigrocinereus*.

Primer	Repeat motif	Size (pb)	Sequence (5’-3’)	Tm (°C)			
				Hm	*Ml*	*Ma*	*Tn*
Mex 033	(TATC)13	220-252	GGTGGGATGAACCTTATCTAAAA GGAGCAAATCTGGGACAAAT	56	56	56	56
Mex 178	(TGGA)14	229-253	GGCCACTACAGCAGATTTCA AATTAAACTCATGGCCGAGGT	55	57	55	55
My Ex041	(GGAT)16	233-261	TGGCACCTCACTTGTAGGATGTAG CAGAGTTCCCTAGCCTGGATTTAC	49	53	57	57
My Ex052	(GT)2TT(GT)9TT(GT)8	213-283	GAGGCCAGTCAGTATGCTTTTCTG CTGGCACCAAGTTTCACTAACACA	52	56	57	59
Thamno MicA11	(CW)43	274-306	AAGAAAATTGTGGAGGCATGTCTGC ACCTTAGGGACTGGAGAGGCAC	60	60	60	60
Thamno MicD07	(CA)14	181-191	GCCATGCTGGCTGCATCCTTGTG CAGAGAGTTCGGTTCTGAAGCGGTG	57	57	57	57
Thamno MicD09	(GAGT)11	190-230	ACAGTTAATGTGACTCCCCTAG CACAGGGAACTGTCAACAAGC	60	60	60	60
Thamno MicG03	(GGAT)10 (AGAY)3 (GGAT)11	302-370	TGAAAGTGTTGTCTAAATGCTACC ATCTCTCAATCTCCCCTCAGAC	53	58	58	58

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Appendix S3

Table S5. Nuclear DNA summary statistics per loci. *n,* number of individuals; *Na*, number of alleles; *Ar*, allele richness; *Ho*, heterozygosity observed; *He*, heterozygosity expected; *Fis*, inbreeding coefficient. Asterisks represents significates deviations in HWE after Bonferroni correction. “Total” include samples of Anavilhanas, Jaú, Branco and Mariuá.

Species	Locality (*n*)	Locus	*Na*	*Ar*	*Ho*	*He*	*Fis*
H. melanopogon	Anavilhanas (*n*=25)	Mex033	3	2.80	0.12	0.12	-0.02
		ThamnoMicA11	5	4.92	0.16	0.36	0.55*
		Mex178	9	9.00	0.75	0.80	0.06
		ThamnoMicD07	4	3.79	0.36	0.46	0.23
		MyEx041	13	12.15	0.56	0.90	0.38*
		ThamnoMicD9	9	8.66	0.50	0.85	0.41*
		MyEx052	9	8.33	0.21	0.71	0.70*
		ThamnoMicG03	10	9.55	0.76	0.87	0.13
		Mean	7.75	7.4	0.4275	0.63	0.30
	Jaú (*n*=13)	Mex033	3	2.92	0.08	0.07	-0.01
		ThamnoMicA11	3	2.92	0.03	0.24	0.84*
		Mex178	8	8.00	0.70	0.84	0.16
		ThamnoMicD07	6	5.76	0.73	0.60	-0.21
		MyEx041	12	11.68	0.76	0.87	0.12
		ThamnoMicD9	6	6.00	0.40	0.63	0.37
		MyEx052	17	16.67	0.26	0.94	0.71*
		ThamnoMicG03	9	8.91	0.26	0.85	0.69*
		Mean	8	7.85	0.40	0.63	0.33
	Total (*n*=55)	Mex033	3	2.99	0.09	0.09	-0.02
		ThamnoMicA11	6	5.96	0.09	0.32	0.73*
		Mex178	11	11.0	0.69	0.82	0.17
		ThamnoMicD07	6	5.81	0.54	0.53	0.00
		MyEx041	18	17.25	0.68	0.90	0.25*
		ThamnoMicD9	10	9.96	0.45	0.79	0.44*
		MyEx052	24	23.04	0.23	0.91	0.75*
		ThamnoMicG03	11	10.68	0.53	0.87	0.37*
		Mean	11.12	10.83	0.41	0.65	0.33

Table S5. Continuation

Species	Locality (*n*)	Locus	*Na*	*Ar*	*Ho*	*He*	*Fis*
M. lugubris	Anavilhanas (*n=14)*	ThamnoMicA11	3	2.88	0.14	0.56	0.75*
		Mex178	6	6.00	0.11	0.83	0.87*
		ThamnoMicD7	4	3.52	0.50	0.61	0.19
		ThamnoMicD9	4	3.61	0.09	0.33	0.73*
		MyEx052	3	2.64	0.07	0.53	0.87*
		Mean	4	3.73	0.182	0.572	0.682
	Jaú (*n*=22)	ThamnoMicA11	4	3.90	0.36	0.43	0.23*
		Mex178	9	8.89	0.16	0.85	0.67*
		ThamnoMicD07	10	8.70	0.54	0.63	0.28*
		ThamnoMicD9	6	6.00	0.13	0.68	0.76*
		MyEx052	7	6.44	0.04	0.52	0.93*
		Mean	7.2	6.786	0.246	0.622	0.574
	Total (*n*=38)	ThamnoMicA11	4	3.92	0.31	0.54	0.44*
		Mex178	10	10.00	0.20	0.85	0.73*
		ThamnoMicD07	10	8.90	0.52	0.66	0.26*
		ThamnoMicD9	7	7.00	0.14	0.61	0.77*
		MyEx052	7	6.54	0.05	0.56	0.91*
		Mean	7.6	7.272	0.244	0.644	0.622

Table S5. Continuation

Specie	Locality (*n*)	Locus	*Na*	*Ar*	*Ho*	*He*	*Fis*
M. assimilis	Anavilhanas (*n*=23)	Mex033	3	2.96	0.17	0.20	0.14
		MyEx041	3	2.99	0.40	0.54	0.27
		ThamnoMicD9	5	5.00	0.47	0.59	0.20
		MyEx052	10	10.00	0.47	0.78	0.40*
		ThamnoMicG03	6	5.72	0.90	0.77	-0.18
		Mean	5.4	5.334	0.482	0.576	0.166
	Jaú (*n*=8)	Mex033	2	1.87	0.25	0.23	-0.76
		MyEx041	3	2.86	0.28	0.48	0.42
		ThamnoMicD9	4	4.00	0.60	0.64	0.07
		MyEx052	6	5.21	0.37	0.82	0.56
		ThamnoMicG03	5	4.12	0.50	0.73	0.33
		Mean	4	3.612	0.4	0.58	0.124
	Total (*n*=33)	Mex033	4	3.71	0.21	0.22	0.03
		MyEx041	4	3.99	0.35	0.57	0.39*
		ThamnoMicD9	9	9.00	0.50	0.65	0.23*
		MyEx052	13	12.46	0.44	0.82	0.43*
		ThamnoMicG03	7	6.78	0.81	0.77	-0.06
		Mean	7.4	7.188	0.462	0.606	0.204

Table S5. Continuation

Species	Locality (*n*)	Locus	*Na*	*Ar*	*Ho*	*He*	*Fis*
T. nigrocinereus	Anavilhanas (*n*=10)	Mex033	3	2.79	0.40	0.35	-0.14
		ThamnoMicA11	7	6.36	0.30	0.82	0.64
		Mex178	3	2.80	0.10	0.51	0.81
		ThamnoMicD07	4	3.93	0.50	0.64	0.23
		MyEx041	4	4.00	0.62	0.72	0.14
		ThamnoMicD9	5	4.56	0.80	0.67	-0.19
		MyEx052	8	6.93	0.40	0.70	0.44
		ThamnoMicG03	5	4.60	0.60	0.72	0.18
		Mean	4.87	4.49	0.46	0.64	0.26
	Jaú (*n*=17)	Mex033	5	5.00	0.23	0.36	0.36
		ThamnoMicA11	11	11.00	0.47	0.81	0.43*
		Mex178	3	3.00	0.05	0.51	0.88*
		ThamnoMicD07	7	7.00	0.58	0.66	0.11
		MyEx041	7	7.00	0.58	0.66	0.12
		ThamnoMicD9	5	5.00	1.00	0.72	-0.40
		MyEx052	9	9.00	0.41	0.73	0.45*
		ThamnoMicG03	7	7.00	0.94	0.75	-0.24
		Mean	6.75	6.75	0.53	0.65	0.21
	Total (*n*=28)	Mex033	6	5.70	0.28	0.34	0.17
		ThamnoMicA11	13	12.83	0.39	0.89	0.56*
		Mex178	4	3.92	0.07	0.49	0.85*
		ThamnoMicD07	7	6.91	0.53	0.63	0.15
		MyEx041	8	8.00	0.61	0.70	0.13
		ThamnoMicD9	6	5.84	0.92	0.72	-0.29
		MyEx052	11	10.16	0.39	0.73	0.47*
		ThamnoMicG03	8	7.91	0.82	0.80	-0.01
		Mean	7.87	7.65	0.50	0.66	0.25

Table S6: Pairwise matrices of dissimilarity between archipelagos per species. *F_{ST}* values (nuclear DNA) below the diagonal, *Φst* (mitochondrial DNA) above. Bold values are significant after Bonferroni's correction. Values with asterisks should not be considered due small sample size. Negative values tend to zero.

Hypocnemoides melanopogon

Archipelagos	Mariuá	Jaú	Anavilhanas	Branco
Mariuá	-	0.052*	0.203*	0.600*
Jaú	-0.001	-	0.061	0.267*
Anavilhanas	-0.007	0.000	-	0.367 *
Branco	-0.171*	-0.026*	-0.054*	-

Myrmoborus lugubris

Archipelagos	Jaú	Anavilhanas	Branco
Jaú	-	**0.291**	-0.002*
Anavilhanas	-0.001	-	0.215*
Branco	0.043*	**0.11***	-

Myrmotherula assimilis

Archipelagos	Mariuá	Branco	Jaú	Anavilhanas
Mariuá	-	0,100 *	0,069*	0,130*
Branco	-0,010*	-	0,031*	0,031*
Jau	0,030*	0,050*	-	0,126
Anavilhanas	0,090	-0,080*	0,030	-

Table S6: Continuation

Thamnophilus nigrocinereus			
Archipelago	Jaú	Anavilhanas	Branco
Jaú	-	0.084	-0.460
Anavilhanas	-0.020	-	-0.683
Branco	0.057*	0.006	-

Figure S1: Phylogenetic trees obtained in the MrBayes analysis based on mtDNa sequences (cytb and ND2). Values above nodes are posterior probabilities. Colored bars refer to sampling locality, being: Gold: lower Negro archipelagos; Pink: Branco River; Blue: Mariuá archipelago; Green: Solimões; Light green: lower Solimões; Orange: Amazon, Madeira or Tapajós rivers.

Figure S2: Mantel's test plots. *p-values* refer to statistical significance, while *r* is linear regression adjustment.

Figure S3: Plots of maximum likelihood (LnP(*k*)) for different numbers of clusters (*k*), followed by correspondent barplot (except for *T. nigrocinereus*, as *k*=1). Numbers below barplots represent geographic area, being: 1: Solimões; 2: Jaú; 3: Anavilhanas; 4: Branco; 5: Mariuá.

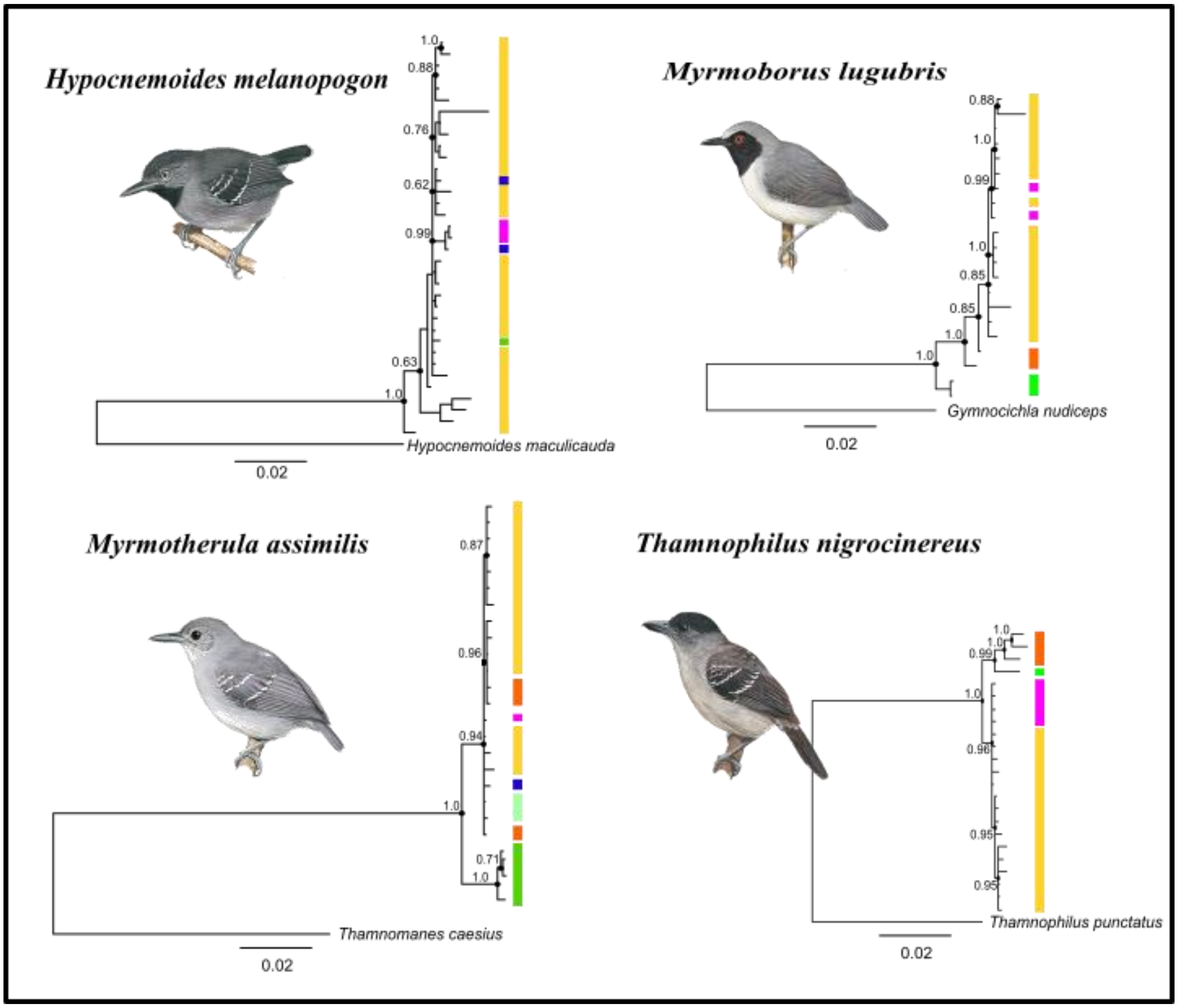
Hypocnemoides melanopogon
1.0
0.88
0.76
0.62
0.99
0.63
1.0
Hypocnemoides maculicauda
0.02
Myrmoborus lugubris
0.88
1.0
0.99
1.0
0.85
0.85
1.0
1.0
Gymnocichla nudiceps
0.02
Myrmotherula assimilis
0.87
0.96
0.94
1.0
0.71
1.0
Thamnomanes caesius
0.02
Thamnophilus nigrocinereus
1.0
1.0
0.99
1.0
0.96
0.95
0.95
Thamnophilus punctatus
0.02

Figure S1.

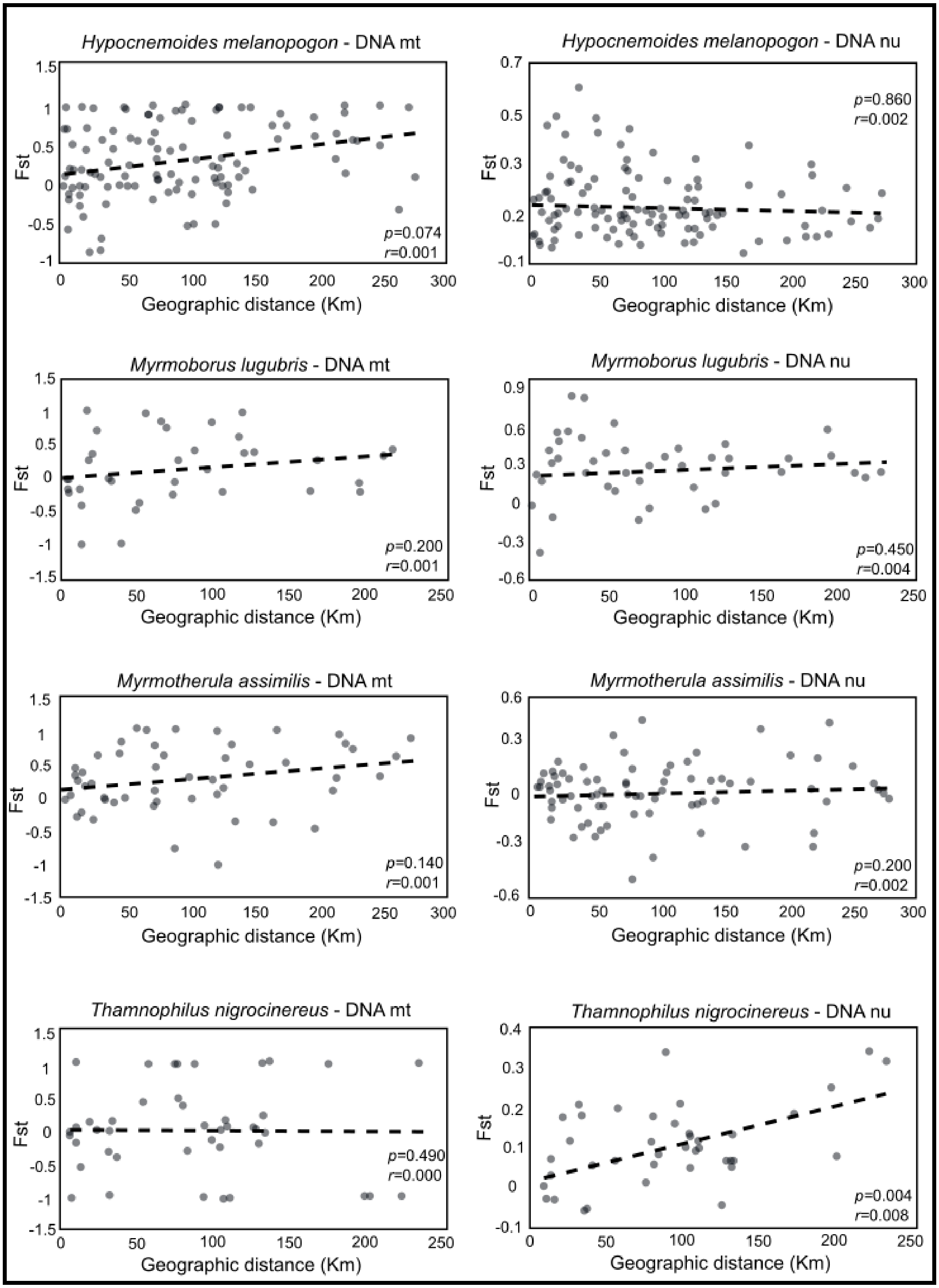
Hypocnemoides melanopogon - DNA mt
Fst
Geographic distance (Km)
p=0.074
r=0.001
Hypocnemoides melanopogon - DNA nu
Fst
Geographic distance (Km)
p=0.860
r=0.002
Myrmoborus lugubris - DNA mt
Fst
Geographic distance (Km)
p=0.200
r=0.001
Myrmoborus lugubris - DNA nu
Fst
Geographic distance (Km)
p=0.450
r=0.004
Myrmotherula assimilis - DNA mt
Fst
Geographic distance (Km)
p=0.140
r=0.001
Myrmotherula assimilis - DNA nu
Fst
Geographic distance (Km)
p=0.200
r=0.002
Thamnophilus nigrocinereus - DNA mt
Fst
Geographic distance (Km)
p=0.490
r=0.000
Thamnophilus nigrocinereus - DNA nu
Fst
Geographic distance (Km)
p=0.004
r=0.008

Figure S2.

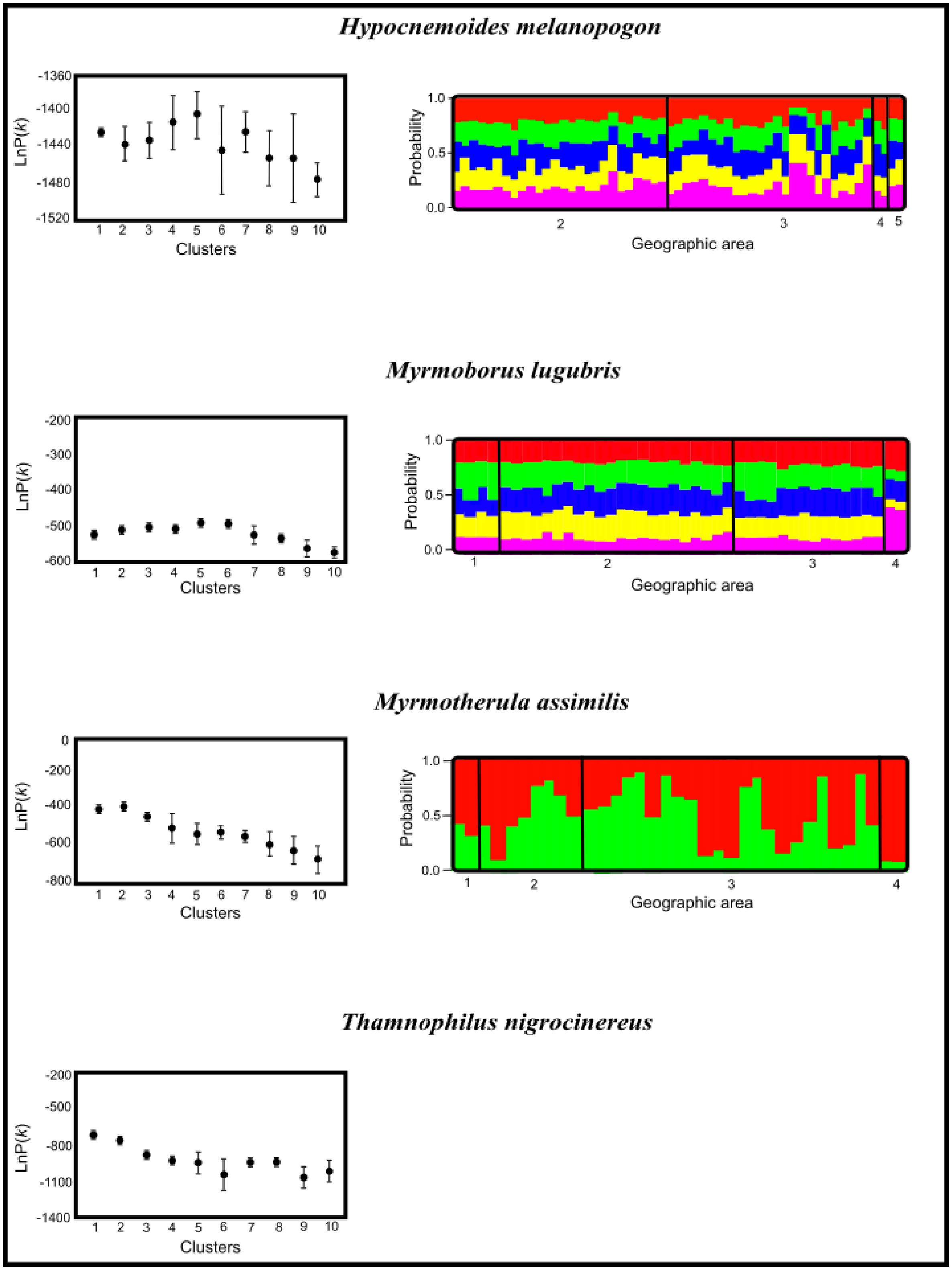
Hypocnemoides melanopogon
LnP(k)
-1360
-1400
-1440
-1480
-1520
1 2 3 4 5 6 7 8 9 10
Clusters
Probability
1.0
0.5
0.0
2 3 4 5
Geographic area
Myrmoborus lugubris
LnP(k)
-200
-300
-400
-500
-600
1 2 3 4 5 6 7 8 9 10
Clusters
Probability
1.0
0.5
0.0
1 2 3 4
Geographic area
Myrmotherula assimilis
LnP(k)
0
-200
-400
-600
-800
1 2 3 4 5 6 7 8 9 10
Clusters
Probability
1.0
0.5
0.0
1 2 3 4
Geographic area
Thamnophilus nigrocinereus
LnP(k)
-200
-500
-800
-1100
-1400
1 2 3 4 5 6 7 8 9 10
Clusters

Figure S3.

CONCLUSÕES

Todas as espécies estudadas não exibiram estruturações populacionais claras nas ilhas do baixo curso do Rio Negro. Apesar disso, espécies que utilizam ambientes insulares de forma preferencial apresentaram linhagens diferentes estabelecidas entre os rios Negro e Solimões. Estes resultados são contrastantes com os obtidos em outros estudos de aves de áreas alagadas, nos quais estes ambientes foram propostos como “corredores ao fluxo gênico”. Cabe salientar que a ausência de estruturação nestes estudos possivelmente relaciona-se às características ecológicas dos organismos avaliados, sendo estes generalistas de áreas alagadas.

Corroborando, os resultados obtidos para *H. melanopogon*, espécie que também apresenta pouca especialização em micro-habitats, indicaram ocorrência de fluxo gênico mesmo em ampla escala geográfica. Para este organismo, o padrão de alta diversidade genética somada com a ausência de estruturação em fina escala pode ser uma consequência ao pulso sazonal de inundação afetando as ilhas. A indisponibilidade dos estratos vegetacionais inferiores durante o período da cheia possivelmente propicia que indivíduos deixem os ambientes insulares. Com a seca, animais de linhagens distintas voltam as ilhas para ocupar o sub-bosque disponível.

As divergências das espécies especialista de ilhas, datadas do Pleistoceno, possivelmente refletem à dinâmica das conexões entre drenagens do oeste e leste amazônico. Linhagens distintas de peixes, estruturações em populações de aves e comunidades biológicas diferenciadas ocorrendo no eixo Solimões/Amazonas evidenciam a importância da dinâmica histórica destes cursos na organização dos organismos. Neste caso, eventos neotectônicos e eustáticos foram os agentes responsáveis por reger o estabelecimento da drenagem atual e das ilhas fluviais, interferindo nas linhagens atualmente estabelecidas.

Ainda neste contexto, as flutuações paleoclimáticas características deste período não afetaram diretamente o tamanho histórico das populações das espécies estudadas. Tal resposta vai de acordo com a obtida para outros organismos de planície alagada, mas contrasta com o padrão congruente de expansão populacional encontrado em espécies de Terra Firme. Possivelmente as variações de umidade e pluviosidade não proporcionaram drásticas alterações na disponibilidade dos habitats de várzea e igapó, permitindo que o tamanho populacional das espécies estabelecidas fosse relativamente constante.