Genomic differentiation with gene flow in a widespread Amazonian floodplain-specialist bird species

Leilton Williams Luna | Camila Cherem Ribas | Alexandre Aleixo

1Graduate Program in Zoology, Universidade Federal do Pará/Museu Paraense Emílio Goeldi, Belém, Brazil
2Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil
3Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland

Correspondence
Alexandre Aleixo, Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland. Email: alexandre.aleixo@helsinki.fi

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Abstract
Aim: Ecological, climatic and palaeogeographical processes drive biological diversification. However, the evolutionary outcomes of those mechanisms are complex and difficult to discriminate. Here, we test how alternative drivers affected connectivity along the Amazonian floodplains generating current patterns of population structure and diversity within the Striped Woodcreeper, a widespread bird species tied to forests seasonally flooded by Amazonian rivers.

Location: Amazonian floodplains.

Taxon: Xiphorhynchus obsoletus (Aves, Furnariidae).

Methods: We sequenced 2213 loci of ultraconserved elements (UCEs) and 20 exons to investigate patterns of genetic structure and connectivity across the Striped Woodcreeper distribution, using a set of spatially explicit methods. Population genetics statistics were calculated for each sampled drainage and within each identified cluster. We tested alternative evolutionary scenarios and estimated past and current demographic parameters implementing a simulation-based framework.

Results: Three genetic clusters with high admixture proportions were identified. These clusters are distributed in the western, central and eastern parts of the Amazon Basin and are not correlated with different river water types representing ecological gradients along the floodplains. Instead, migration rates indicate two putative historical barriers along the main channel of the Amazonas-Solimões River. Demographic model tests suggest a process of sequential differentiation, partitioned across the Amazon Basin, with stable population sizes and continuous gene flow.

Main conclusion: Our results support the hypothesis that late Quaternary changes in connectivity between the eastern and western Amazonian drainages were responsible for driving genetic differentiation in the Striped Woodcreeper. Genomic differentiation occurred in the presence of long-term gene flow throughout the X. obsoletus distribution, suggesting some degree of continuous historical connectivity across Amazonian floodplains. Compared to previous studies, our results suggest that although connectivity among populations of Amazonian bird species specialized in floodplain habitats varies in response to the same abiotic mechanisms, species-specific differences in habitat use may be a strong predictor of population divergence.
1 | INTRODUCTION

The current Amazonian species diversity is the result of a complex history of ecological, climatic and palaeogeographic interactions (Hoom et al., 2010; Naka & Brumfield, 2018; Oberdorff et al., 2019; Ribas et al., 2012; Silva et al., 2019). Among the different environments that compose this biome, the extensive floodplain habitats stand out for accommodating numerous endemic species. For example, roughly 15% of non-aquatic Amazonian bird species are restricted to river created habitats (Remsen & Parker, 1983). However, the processes responsible for driving biological diversification in these floodplains remain understudied (Ribas & Aleixo, 2019). Although some mechanisms have recently been proposed to explain the current patterns of genetic and species diversity (e.g., strong ecological differences between the main types of floodplains (Beheregaray et al., 2015; Cook et al., 2012), the increasing eastward expansion of the flooded ecosystems since late Pleistocene (Aleixo, 2006; Aleixo & Rossetti, 2007; Bicudo et al., 2019), and changes in the riverscape induced by climatic fluctuations during the Quaternary (Thom et al., 2020)), few studies have tested these scenarios using a framework of spatial and demographic model-based methods (but see Thom et al., 2020). Systematically testing these alternative hypotheses can help understand how current patterns of diversity and endemism have emerged along Amazonian seasonally flooded habitats.

To address this issue, we need to understand how alternative processes may affect connectivity along the floodplains, and thus generate species diversity or intraspecific population structure. Currently, Amazonian floodplain habitats are distributed along steep environmental gradients, driven by changes in river water properties along the basin (Junk et al., 2011). Sediment-rich white water rivers (i.e., várzea) with Andean sedimentary origin, and black and clear water rivers (i.e., igapó), poor in suspended sediments, and influenced by cratonic soils of the Brazilian and Guiana Shields, support very distinct floodplains in different parts of Amazonia, with várzeas distributed mainly in the west, and igapós mostly in the central and eastern parts of the basin (Junk et al., 2015). Distinct floodplain types differ by several physicochemical and ecological aspects, which determine the distribution, specialization and assembly of species in local biotic communities (Beheregaray et al., 2015; Bogotá-Gregory et al., 2020; Borges et al., 2019; Laranjeiras et al., 2019). Also, levels of habitat association with different flooded forest types can differ greatly between species and vary spatially over the distribution within species (Laranjeiras et al., 2019). These observations are the basis of the ecological gradient hypothesis, in which strong ecological differences would lead to the differentiation of populations associated with distinct water types (Wang & Bradburd, 2014), favouring population subdivision, and leading eventually to speciation. Under this scenario, divergent selection mediated by distinct ecological conditions may cause population differentiation even in the absence of physical barriers (Schluter, 2009; Wang & Bradburd, 2014).

Alternatively, climate change during the Quaternary has been considered one of the key drivers of the enormous biological diversity of Amazonia (Silva et al., 2019; Thom et al., 2020), with precipitation varying across the region especially during the Late Pleistocene. This climatic variation, however, was stronger in eastern Amazonia, where the dry season was magnified due ultimately to orbital-forcing mechanisms, potentially leading to complex diversification and demographic scenarios, while the west kept relatively stable high precipitation patterns (Cheng et al., 2013; Wang et al., 2017). Some studies suggest that in response to increased rainfall after the last glaciation and through the Holocene, the distribution of flooded habitats expanded from western Amazonia towards the east (Irion et al., 1997). According to this scenario, we would expect that species occurring in western Amazonian floodplains expanded their distribution to the newly formed floodplains in the eastern part of the biome, generating differentiation due to consecutive founder events (Aleixo, 2006; Aleixo & Rossetti, 2007; Thom et al., 2020)—a process similar to isolation by colonization (Orsini et al., 2013).

A distinct, yet related climate-driven scenario invokes cycles of sediment supply caused by shifts in precipitation patterns across the Amazon Basin, with alternating phases of aggradation and incision of the main rivers’ channels during wet and dry periods, respectively. These sedimentation cycles would control the distribution and connectivity of seasonally flooded habitats—and of the natural populations associated to each habitat type (Pupim et al., 2019). The expectations of this model concerning population dynamics are analogous to those derived from the refugia hypothesis proposed by Haffer (1969): induced by dry-wet climatic cycles, populations would be simultaneously isolated in restricted areas (i.e., refugia or distinct river basins) during dry periods, accumulating genetic and phenotypic differences; subsequently, they would re-establish contact with other allopatrically isolated populations following habitat expansion and re-connection during wet periods. Based on this latter scenario, we hypothesize that large-scale climate shifts may have differently affected the availability and connectivity of flooded habitats throughout Amazonia. Consequently, because of distinct sedimentary regimes and the increased incision to which central Amazonia is subjected during periods of low precipitation (Thom et al., 2020), we expect those lineages inhabiting floodplains associated with river basins in western and eastern Amazonia will differ more strongly from each other than when compared to lineages occurring within each of these sectors.

Considering that the evolution of floodplain habitats may directly influence the population dynamics of species associated with these habitats, we selected the Striped Woodcreeper Xiphorhynchus | KEYWORDS

demographic model selection, geo-genomics, historical climate change, Neotropical, Pleistocene, population structure, ultraconserved elements
obsoletus (Aves: Furnariidae), a widely distributed Amazonian endemic species strictly associated with floodplain forests (Figure 1a) to test the alternative scenarios described above. Unlike most Neotropical floodplain species investigated so far under a phylogeographic perspective (Cadena et al., 2011; Thom et al., 2018, 2020), the Striped Woodcreeper can be regarded as a “generalist floodplain” species (see Choueri et al., 2017) with high population densities throughout the entire Amazon (Marantz et al., 2020). This species occurs predominantly in late-successional closed-canopy vegetation along water bodies varying in size from small creeks to the main Amazonian rivers, including both várzea (white water) and igapó (clear and black water) forests (Marantz et al., 2020). Previous genetic studies suggest a relatively old origin for the species (ca 4 Myr old; Harvey et al., 2020), with an overall lack of phylogeographic structure (Aleixo, 2006; Harvey et al., 2017), hypothesized due to high levels of gene flow among populations whose ranges were not fragmented by long-term vicariant barriers across the floodplains.

In this study, we first describe the genetic structure within the species with an unprecedented level of population and genomic sampling and then apply spatially explicit methods and demographic models to compare evolutionary expectations derived from distinct divergence scenarios. As a null hypothesis, (1) we expect a strict pattern of isolation by distance (IBD) resulting from the extensive floodplain network connectivity and lack of geographical barriers. Alternatively, (2) for the ecological gradients hypothesis, we expect discrete genetic structure boundaries that match the transitions between water types (i.e., white water vs. clear water), as well as constant gene flow that is higher within similar than between distinct environments. Finally, for the scenarios associated with historical climate change and cycles of sediment deposition, we expect: (3) that the last cycle of floodplain expansion eastward led to progressive colonization from western to eastern Amazon, generating sequential divergences in time and resulting in a genetic diversity gradient from west (higher diversity) to east (lower diversity). Alternatively, (4) if floodplain habitats have responded to cycles of aggradation and erosion during the Late Pleistocene and Holocene, geographically structured populations are expected to be partitioned across main Amazonian river basins, with evidence of genetic admixture due to recent secondary contact in areas of more intense erosion (e.g., the confluence between large rivers). Under this scenario, we expect differentiation among populations to be associated with some degree of historical isolation, followed by recent demographic expansion with secondary contact between previously isolated populations.

FIGURE 1 (a) Geographic distribution of the Striped Woodcreeper (Xiphorhynchus obsoletus) genetic samples sequenced in this study along with different water colour types (white, clear and black) of the Amazonian rivers. Pie charts represent the proportion of admixture between the three genetic clusters (K = 3) identified in the conStruct analysis for each sampled locality, and (b) the delimited proportion of ancestry across populations associated with white water Andean sourced rivers and clear and black water rivers sourced in the cratonic Brazilian and Guiana shields.
2 | MATERIALS AND METHODS

2.1 | Species studied and sampling design

We sequenced 59 Striped Woodcreeper tissue samples from the following institutions: American Museum of Natural History (AMNH), Louisiana State University Museum of Natural Sciences (LSUMNS), Instituto Nacional de Pesquisa da Amazônia (INPA) and Museu Paraense Emílio Goeldi (MPEG). The Striped Woodcreeper is widely distributed throughout Amazonia, occurring along all major tributaries but also penetrating deep into some interfluvas through minor tributaries and even small creeks (Marantz et al., 2020). Because our primary goal was to test alternative hypotheses involving environmental and historical aspects of floodplains that could drive intraspecific divergence in this species, we focused sampling on populations directly associated with major rivers in the Amazon drainage basin. The sampling design covered a large portion of the species’ distribution, including most large tributaries, and covering all water types (Figure 1a). The number of specimens sampled at each locality ranged from one to three (Table S1).

2.2 | Genetic data processing

We obtained genomic data using a probe set for 2321 ultraconserved elements (UCEs) and a set of 96 exons used in avian evolutionary studies (Faircloth et al., 2012; Harvey et al., 2017; Thom et al., 2018, 2020). UCE’s flanking regions and exon’s third codon positions have been shown to contain variability with a good statistical resolution for inferences about phylogeographic patterns and population history (Amaral et al., 2018; Harvey et al., 2016; Smith et al., 2014; Thom et al., 2018). DNA from all samples was extracted using the QIAGEN DNeasy tissue and blood kit following the manufacturer’s protocol. Subsequent laboratory procedures were performed by RAPID Genomics. The raw reads files obtained from the 150 bp paired-ends sequencing in Illumina Hiseq 2500 had their qualities evaluated in FastQC 0.11.5 (Andrews, 2014). Then, the raw reads were cleaned using Illumiprocessor for individuals, with the removal of the adapters and low-quality reads (Faircloth et al., 2012). We assembled the clean reads samples using Trinity 2.4 (Grabherr et al., 2014), followed by mapping the contigs with the UCEs and exons reference probes set, as well as obtaining summary statistics for all loci using PHYLUCE 1.4 (Faircloth et al., 2012). All sequences per locus were aligned in MAFFT (Kotoh & Standley, 2013). Finally, to avoid including markers with different inheritance patterns, we identified UCEs and exons linked to the Z-allosomal chromosome, using the local BLAST search based on the zebra-finch Z-chromosome (Ensembl taeGut3.2.4).

For SNPs calling, we constructed a matrix including all individuals without filtering missing-data and trimming, where the longest sequences were selected as references. The reference sequences were aligned with the matrix of reads for each sample using the BWA (Li & Durbin, 2009). The aligned reads with the references were mapped using a hard-masking low-quality criterion (< 30), maintaining sites with the minimum read depth of > 8, using the Unified Genotyper and Variant Annotator of GATK (McKenna et al., 2010). The final matrix was assembled by randomly calling one SNP per locus from a database of biallelic SNPs without missing-data, using a minor allele frequency (MAF) of 0.01.

2.3 | Population genetic structure

To determine the number of populations (K) and verify whether the genetic structure in X. obsoletus is determined by continuous (IBD) or discrete (geographical or ecological barriers) processes, we used a model-based clustering method implemented in the R package conStruct (Bradburd et al., 2018). This statistical framework assumes that the degree of relatedness declines as a function of geographical distance, thus using spatially explicit information to simultaneously model allelic and geographic covariation in patterns of continuous and discrete population structure. Also, this method allows the comparison of spatial and non-spatial models, through the application of cross-validation, which infers the predictive accuracy of a given model relative to the best probabilistic model for the partitioned data (0.9 for training and 0.1 for model testing [Bradburd et al., 2018]). We explore one to nine K—which means a set of populations geographically distributed throughout the distribution of the species (for more details see Bradburd et al., 2018). For each number of K, we performed 10 replicates with 10,000 Markov chain Monte Carlo (MCMC) iterations and compared each replicated model using cross-validation. As a final selection criterion for the best fit value of K, we evaluated the covariance contribution of each layer in the model, with a threshold of > 0.02.

We expect that, if genetic diversity is strictly determined by the continuous IBD process, the result will strongly support the spatial model with a value of K = 1. Alternatively, if discrete processes predominate (i.e., geographic or ecological isolation) we expect to observe a value of K > 1. Furthermore, if the genetic structure within X. obsoletus has a spatial relationship with watercolour along the Amazon basin, population limits should coincide with transitions between different water colour types (i.e., ecological differences).

2.4 | Genetic diversity

We used the R package diveRsity v1.9.90 (Keenan et al., 2013) to estimate genetic diversity parameters, such as observed, expected and unbiased heterozygosity, for each sub-basin sampled and within each genetic cluster identified in the population genetic structure analyses. For each distinct genetic cluster, we estimate the genetic diversity of the set of individuals used in historical demographic simulations (for more details, see Demographic history).
2.5 | Effective migration surface

We investigated the spatial pattern of genetic variability and population connectivity, implementing the Estimated Effective Migration Surface method (EEMS; Petkova et al., 2015). The EEMS is based on isolation by resistance concept (McRae, 2006), where the variation in migration rates is measured between adjacent locations (demes) in a stepping-stone model, providing as an outcome a visual resource to recognize potential barriers or corridors for gene flow across the landscape (Petkova et al., 2015). This approach evaluates the decay of genetic similarity from geographically isolated genetic data, helping to highlight areas that are potentially deviating from a null expectation of IBD, without the need to include environmental variables or topographic information. To capture the spatial heterogeneity of the genetic structure and to ensure that the deme size (i.e., grid area) did not influence the estimation of the migration surface, we selected three distinct values (200, 400, and 600). These deme size values should better capture variation in migration rates across the Striped Woodcreeper distribution, considering the broad geographic sampling of our study. We applied three independent runs for each deme size with MCMC length of $2 \times 10^6$ and burn-in of $2 \times 10^6$. The convergences of the chains were evaluated, and the results of the runs combined using the reemplot R package (Petkova et al., 2015), generating map graphs of effective migration surface ($m$) and effective genetic diversity ($q$).

2.6 | Demographic history

To test among alternative demographic scenarios that may have driven the observed pattern of population structure in *X. obsoletus*, we used a model-based approach implemented in FASTSIMCOAL v2.5.2 (FSC—Excoffier et al., 2013). This robust simulation-based framework enables inference of historical demographic parameters (divergence time, gene flow, past and present population size) from the likelihood of observing joint site frequency spectrum (JSFS) obtained from a dataset of SNPs calculated in dadi v1.7 (Gutenkunst et al., 2009).

We used the results inferred by conStruct (see Results) to compare the probabilities of four different demographic models, based on expectations of scenarios of spatial and temporal variation of the Amazonia climate during the Late Pleistocene (Pupim et al., 2019; Thom et al., 2020). We conducted a hierarchical model selection, first comparing models with simultaneous or sequential divergence, where Model (1) assumes simultaneous divergences across the landscape, with constant gene flow and stable effective population sizes, and Model (2), which assumes sequential divergences with constant gene flow and stable effective population sizes. We then selected the best-fit model and tested exponential population growth after the beginning of the separation, described here as Model (3): sequential divergence times, constant gene flow and exponential population growth. Alternatively, we simulated eastward colonization events in Model (4), which assumes sequential divergences in time, following a bottleneck with subsequent population expansion and asymmetric gene flow with a higher migration rate from source to sink (western to eastern) populations.

To avoid uncertainty of inputting highly admixed individuals in the demographic parameter estimates, we excluded from the analysis nine samples with an undetermined assignment to a specific genetic cluster (using a conservative threshold of 60% individual membership in each cluster, see Table S2 for population assignments). Highly admixed individuals are an issue if the goal is to infer the formation of putative barriers in the history of differentiation, since they could be the result of recent secondary contact between previously isolated populations (Massatti & Knowles, 2020; Momigliano et al., 2021). The run with the highest composite-likelihood for each model was evaluated using the relative weight of the Akaike information criterion (AIC—Akaike, 1973), to select the best-fit model. For the parameter estimates and model comparison, 50 runs were performed for each model with 40 conditional maximization algorithm cycles (ECM—Excoffier et al., 2013) and 100,000 simulations for likelihood maximization. The best-fit model parameter confidence intervals were calculated from 50 parametric bootstrap replications through new JSFS simulations from the run with the best composite likelihood value.

The wide distribution of UCEs across the genome and the variation along their flanking regions have made these markers useful for investigations of recent evolutionary histories (Amaral et al., 2018; Faircloth et al., 2012; Smith et al., 2014). However, due to their unique characteristics, mutation rates for UCEs are still debated in the literature. To account for this uncertainty, we employed two alternative mutation rates previously applied to UCE data (Bocalini et al., 2021; Thom et al., 2020; Winker et al., 2018), as follows. First, a mutation rate of $6.75 \times 10^{-9}$ substitution/site/generation estimated for UCEs of two closely related passerine species based on comparisons across the Psittaciformes-Passeriformes phylogeny using whole-genomes and fossil-dated divergence ages (Winker et al., 2018); second, an average genome-wide mutation rate of $2.5 \times 10^{-9}$ substitution/site/generation reported for other well-studied passerine lineages (Nadachowska-Brzyska et al., 2015). To calculate the relative times of demographic events, we employed a generation length of 3.25 years, an estimated average value for the Striped Woodcreeper derived from models that incorporate age of first reproduction as a parameter (Bird et al., 2020).

3 | RESULTS

3.1 | Genetic summary information

The processing of the genetic data generated a matrix of 2213 loci (UCEs = 2193 loci, exons = 20 loci, total nucleotides = 101,060,580 bp), with the average contigs size of 1427 bp (min 293 bp, max. 4274 bp) from a set of 2321 UCEs and 96 exons (allowing 20% missing data). A total of 24,924 informative sites were obtained with a mean of 11.26 variable sites per locus (min 0, max...
We called a total of 4372 biallelic SNPs and after selecting a single SNP for each autosomal locus without missing data, we obtained a final matrix of 1529 loci.

### 3.2 Spatial population structure and connectivity

Cross-validation analyses in conStruct indicated that the spatial model had greater predictive accuracy when compared to the purely discrete (non-spatial) model (Figure S1), suggesting that the incorporation of geographic information is important to describe the number of genetic clusters in the Striped Woodcreeper. Also, the cross-validation supported a genetic structure of $K = 3$ (Figure 1a) as the most likely number of ancestral populations in *X. obsoletus* (see Table S3 and Figure S1). Additional $K$ values showed lower predictive accuracy and little contribution to the total covariance for each cluster (less than 0.02, Table S3 and Figure S2). The three genetic clusters include populations distributed respectively in the western (Solimões, Japurá, Branco, Negro—white, clear and black waters), central (Amazonas and Tapajós—white and clear waters), and eastern (Xingu and Araguaia—clear waters) Amazon Basin, with no spatial relationship with the current geographic distribution of the different water colour types across the basin (Figure 1b). These longitudinally structured clusters also present a high proportion of admixture, mainly in the Madeira River basin and at the confluence of the Xingu and Amazonas rivers.

The EEMS results were consistent with the structure inferred by conStruct, revealing two major landscape barriers that potentially inhibit migration (darker shades of orange, Figure 2a), suggesting a strong deviation from IBD. These below-average migration rate values coincide with the main channel of the Amazonas-Solimões River, especially in the following regions: (1) central Amazon Basin, including the lower Solimões, lower Madeira, lower Negro and upper Amazonas rivers confluences; and (2) at the confluence between the lower Xingu and Jari rivers and the mouth of the Amazon River (Figure 2a). On the other hand, gene flow corridors (i.e., areas with above-average migration rates) were observed mostly within the entire length or stretches of river basins of the following Amazonian tributaries: Negro/Branco, Madeira, Tapajós and Tocantins (Figure 2a). In addition, the spatial analysis of the genetic diversity across the Striped Woodcreeper’s distribution highlights the central Amazonia and the lowermost Xingu River, which act as barriers to gene flow, as having high levels of variability (i.e., a greater proportion of heterozygotes), suggesting that these areas are contact zones between genetically divergent populations (Figure 2b). Contrastingly, populations from most river basins of the Brazilian and Guiana Shields (Negro, Branco, Tapajós, and Xingu) presented genetic diversity values below average, except for the Tocantins River.

### 3.3 Diversity statistics

Heterozygosity values ($H_o$, $H_e$, $\mu H_e$) were higher for populations occurring along main trunk rivers such as the Solimões and Amazonas, with consistently lower values for other Amazonian rivers sampled (Table 1). Among the genetic clusters identified in the population genetic structure analysis, the western cluster showed slightly higher diversity values than the central and eastern clusters (Table 1).

### 3.4 Historical demographic inference

Comparisons between the different divergence models tested, revealed Model 2 as the most likely (Table 2; Figure 3), supporting a scenario of sequential divergence across the landscape, with stable
population sizes and continuous gene flow. Other demographic models tested had no significant contribution to the AIC weight (Table 2). The parameter estimates of the best fit model based on the composite likelihood for two alternative mutation rates (Table 3) suggest a large ancestral population size of 892,407–1,065,406 diploid individuals, with the first divergence \( T_1 \) between the western genetic cluster and the central and eastern groups starting between 162,348 and 738,919 years ago. The second and most recent divergence \( T_2 \), between the central and eastern populations, took place around 37,247–204,002 years ago. The population size parameters were higher in the western population \( (N_{e1} = 419,088–516,303 \text{ diploid individuals}) \) when compared to values of central \( (N_{e2} = 288,758–302,205 \text{ diploid individuals}) \) and eastern population size \( (N_{e3} = 224,249–275,801 \text{ diploid individuals}) \). Even with the exclusion of individuals with large proportions of admixture among clusters (<60%), migration rates per generation were relatively high, ranging from 3.464–4.721 \( (M_{12}) \) to 5.055–5.339 \( (M_{23}) \). Among the three populations, the central genetic cluster had the highest rate of gene flow to adjacent populations, probably acting as a source of migrants.

4 | DISCUSSION

By combining spatially explicit methods and demographic model selection, we resolved with an unprecedented detail the spatio-temporal context of the intraspecific genetic structure within the floodplain specialist Striped Woodcreeper (Xiphorhynchus obsoletus) and assessed expectations from distinct scenarios for the evolution of Amazonian floodplains. Despite the species’ current continuous distribution across Amazonia, our findings support a scenario of spatially and temporally variable connectivity among populations. Both mutation rates used to estimate divergence times suggest that differentiation among populations was centred in the Late-Pleistocene and is best explained by partial isolation, probably due to changes in the connectivity of floodplain habitats due to large-scale climate shifts across the Amazon Basin (Pupim et al., 2019; Thom et al., 2020). Below, we summarize the main genetic evidence supporting this hypothesis.

Our extensive geographical and genomic sampling, with the explicit inclusion of spatial information, brought a new resolution to the intraspecific genetic structure of the Striped Woodcreeper,
detecting three genetic clusters with high admixture proportions (Figure 1). Despite previous results suggesting little geographical structure and low rates of diversification across Amazonian floodplains compared to upland forests (Aleixo, 2006; Cadena et al., 2011; Harvey et al., 2017), our findings add to a growing body of evidence supporting a pattern of recent population differentiation, with constant (albeit reduced) gene flow or secondary contact (Choueri et al., 2017; Thom et al., 2018, 2020), revealing a much more complex scenario for the evolution of Amazonian seasonally flooded habitats.

By explicitly testing alternative hypotheses to explain the population subdivisions within *X. obsoletus*, we were able to provide some important insights about the history of connectivity among Amazonian floodplain habitats. For example, although geographical distance is a key factor influencing genetic structure in the Striped Woodcreeper (Aleixo, 2006; Harvey et al., 2017), the null hypothesis

| Parameters | Demographic units | Prior distribution | Estimate based on $\mu = 6.75 \times 10^{-10}$ (CI 95%) | Estimate based on $\mu = 2.75 \times 10^{-9}$ (CI 95%) |
|------------|-------------------|-------------------|---------------------------------|---------------------------------|
| Ne1        | Current Western population size | $10^4$–$5 \times 10^5$ | 419,088 (390,140–572,552) | 516,303 (456,207–488,014) |
| Ne2        | Current Central population size | $10^4$–$5 \times 10^5$ | 288,758 (174,006–291,332) | 302,205 (258,402–314,004) |
| Ne3        | Current Eastern population size | $10^4$–$5 \times 10^5$ | 224,249 (85,550–292,677) | 275,801 (95,687–244,854) |
| Na123      | Ancestral size before first split time | $5 \times 10^5$–$5 \times 10^6$ | 892,407 (740,678–1,001,253) | 1,065,406 (726,220–1,582,863) |
| Na23       | Ancestral size before second split time | $10^5$–$10^6$ | 599,407 (415,635–565,333) | 878,052 (397,339–725,880) |
| T1         | First split time (years) | $10^4$–$10^5$ | 738,919 (493,962–857,200) | 162,348 (104,223–198,061) |
| T2         | Second split time (years) | $10^4$–$10^5$ | 204,002 (73,604–298,203) | 37,247 (18,105–78,465) |
| M12        | Migration from Western to Central | 0.1–4 | 3.464 (3.001–8.307) | 4.721 (2.829–6.238) |
| M21        | Migration from Central to Western | 0.1–4 | 5.339 (4.015–6.868) | 5.055 (2.670–8.517) |
| M23        | Migration from Central to Eastern | 0.1–4 | 4.595 (3.909–5.731) | 3.801 (1.555–3.009) |
| M32        | Migration from Eastern to Central | 0.1–4 | 3.635 (3.635–4.008) | 1.824 (0.668–2.463) |

CI, confidence interval.
that genetic variation is strictly determined by IBD could be rejected. These findings contradict the notion of a long-term extensive connectivity of flooded forest environments across Amazonia supporting unrestricted dispersion between adjacent populations (Aleixo, 2006; Cadena et al., 2011), even for a generalist flooded habitat species such as the Striped Woodcreeper. However, when visualizing the spatial pattern of migration rates, we note that drastic reductions in gene flow occur mainly along the main channel of the Amazonas / Solimões River, while higher values are observed within the sub-basins (Branco, Negro, Madeira, Tapajós, and Tocantins), suggesting populations in IBD equilibrium at least at a regional scale and within distinct river basins (Figure 2a).

The spatial pattern of population genetic structure provided little support for the ecological gradient hypothesis. Our predictions for this scenario posited that adaptations due to strong ecological differences (i.e., between rivers with different water colours) would tend to reduce gene flow, irrespective of geographical distance (Wang & Bradburd, 2014). However, the population limits found for the Striped Woodcreeper do not match the transition areas between the major types of flooded environments associated with distinct water colours in Amazonia (Figure 1b). In addition, the spatial structure pattern visualized through EEMS (Figure 2a) does not show continuous gene flow (i.e., above-average migration rate) between rivers sharing the same water colour type (e.g., white water rivers such as the Amazonas/Solimões and Madeira and even along the entire trunk of the Amazonas/Solimões), reinforcing the conclusion that the population differentiation assessed here cannot be explained by ecological differences between habitats flooded by distinct water types, unlike reported for some fishes (Beheregaray et al., 2015; Cook et al., 2012).

Contrary with the hypothesis of eastward expansion, genetic diversity did not show considerable reductions from western to eastern rivers of the Amazon Basin (Table 1; Figure 2b), which would be an expected outcome of a colonization model that predicts consecutive bottleneck events originating in western Amazonia (Model 4—Table 2). Instead, the variation in local genetic diversity is mainly attributable to localities with a high proportion of admixture between distinct populations, especially in central Amazonia, where major white, black and clear water tributaries conflate with the Amazonas/Solimões River (Figure 1a; Figure 2b).

The demographic model selection supports a scenario of recent and sequential differentiation with gene flow between demographically stable X. obsoletus populations (Model 2, Figure 3c; Table 2). However, the model posterior estimates of current and historical effective population sizes for each Striped Woodcreeper population indicate lower and non-overlapping values for both central and eastern populations when compared to the western population (Table 3). This is illustrated well by the "Ancestral size before second split time" parameter, which originated the central and eastern populations, and which is significantly smaller than the "Ancestral size before first split time" estimate, related to the ancestral effective population size of the entire species. These estimates of varying effective population sizes can be reconciled with rather demographically

stable populations through time across the range of X. obsoletus by verifying the lesser extent of floodplains present in ranges of the central and eastern populations (Miguez-Macho & Fan, 2012). The area covered by flooded wetlands in western Amazonia is greater than that in the central and eastern parts of the basin (Hess et al., 2015; Miguez-Macho & Fan, 2012), and this may have led to historically smaller effective populations sizes of the Striped Woodcreeper inhabiting these areas.

The time estimates obtained for the sequential population splits in the Striped Woodcreeper using two distinct mutation rates converge to the late Quaternary (Table 3), a period when changes in precipitation are documented, especially in eastern Amazonia (Cheng et al., 2013; Wang et al., 2017). These cyclical variations in precipitation patterns have been related to the dynamics of floodplain construction and erosion, affecting connectivity among habitats that are subjected to seasonal flooding (Pupim et al., 2019). Especially during the Last Glacial Maximum (LGM), precipitation levels in the central and eastern parts of Amazonia were nearly half (i.e., 58%) current amounts (Wang et al., 2017), resulting in significant changes in the landscape affecting the extent and distribution of floodplain forests (Pupim et al., 2019). In this case, our results best fit the hypothesis of differentiation among X. obsoletus populations being related to historical changes in the connectivity of distinct Amazonian riverine basins and flooded environments driven by climate change during the Quaternary (Pupim et al., 2019; Thom et al., 2020).

Historically, these regions highlighted by the EEMS in central Amazonia and the lower portion of the Amazonas River (Figure 2a), experienced an intense process of sedimentary activity during the Plio-Pleistocene, partially driven by precipitation changes (Peizhen et al., 2001; Pupim et al., 2019). In central Amazonia, shifts from sediment aggradation to incision stages in the main river channels resulted in cyclical erosion of floodplain habitats, associated with narrowing of the floodplains (Nogueira et al., 2013; Pupim et al., 2019; Rossetti et al., 2015). These sedimentary processes disrupted large tracts of riverine habitats, creating for instance up to 150 km of floodplain discontinuity between the mouths of the Negro and Madeira rivers in central Amazonia (Irión et al., 2010), which may have reduced connectivity among X. obsoletus populations. This same region in central Amazonia of historically restricted gene flow for X. obsoletus populations, was shown to exert the same effect on populations of three additional floodplain bird species (Thom et al., 2020). The additional region of restricted gene flow within X. obsoletus, in the lower Amazonas River, correspond to the easternmost limits of the Amazon Basin, as the Tocantins River basin is currently isolated, independently arriving to the Amazon delta. The fish species composition (Dagostas & Pinna, 2017) and the avifauna (T. O. Laranjeiras personal communication) associated with flooded environments has been shown to be distinct between the Amazonas and Tocantins basins (Figure 1a). This is reflected in the X. obsoletus population structure as well, although, interestingly, in more recent times than the split estimated between western and central Amazonian populations (see T1 in Table 3). Other widespread floodplain-associated Amazonian lineages exhibiting significant
genetic differentiation in the Tocantins drainage are the river dolphins of the genus Inia (Hrbek et al., 2014) and the Arapaima fishes (Nogueira et al., 2020). Details on the dynamics of past connections between the Tocantins and the Amazon’s floodplains have yet to be clarified, but the Tocantins floodplain is known to have had a very recent dynamic history and high loads of Quaternary sediments (Jesus et al., 2020), which have impacted the extent and distribution riverine-created habitats.

Models predicting historically stable population sizes were statistically favoured (Table 2), contrary with expectations of bottleneck effects with subsequent population growth associated with cyclical reductions/expansions in the extent of floodplains and the formation of putative refugia (Thom et al., 2020). Instead, populations were isolated by an incomplete barrier (e.g., fragmentation and reduction in area and connectivity of the floodplain forest matrix across Amazonian drainages), which reduced but did not prevent gene flow. This distinct pattern relative to the “floodplain refugia” described in Thom et al. (2020) may be associated with the fact that while the species studied by Thom et al. (2020) and most of those by Choueri et al. (2017) are tied to river islands, X. obsoletus is more generalist, using many kinds of riverine habitats, including those away from the channels of major Amazonian rivers. Similarly, another generalist floodplain species—the Black-chinned Antbird Hypocnemoides melanocephala, showed no population structuring across parts of central and western Amazonia, in sharp contrast with the other three sympatric antbird species tightly associated with river islands (Choueri et al., 2017). This reinforces the notion that habitat specificities are strong predictors of population divergence in Amazonian bird species (Burney & Brumfield, 2009; Harvey et al., 2017). Especially in flooded habitat specialist birds, restrictions on habitat use (e.g., river islands, river-edges) reflect in greater genetic structure across the landscape when compared to more generalist species (Choueri et al., 2017; Thom et al., 2018, 2020). In this case, although X. obsoletus is a typical species of floodplain forests such as várzea, igapó, and river islands, it may also penetrate upland forest close to wooded swamps and other water bodies (Marantz et al., 2020). This plasticity in the use of alternative habitats may have ensured the long-term maintenance of gene flow across parts of the range of the Striped Woodcreeper even in the absence of extensively flooded environments during periods of lower sediment input or higher erosion cycles (Bicudo et al., 2019; Pupim et al., 2019; Thom et al., 2020).

In summary, by investigating alternative divergence mechanisms using population genomic analysis, we were able to support the hypothesis that spatial and temporal variations in connectivity between the eastern and western drainages of the Amazon Basin during the late Quaternary drove intraspecific genetic differentiation in the Striped Woodcreeper, a widespread floodplain generalist species. Besides, we demonstrated that these processes occurred in the presence of long-term gene flow throughout the X. obsoletus distribution, which indicates a certain degree of continuous connection among flooded forest patches even during drier climatic periods, particularly in eastern Amazonia. When contrasting our results with those obtained by other studies on different floodplain lineages, we found that although divergences seem to be associated with the same abiotic mechanisms (historical cycles of sediment deposition and erosion along river channels), biotic differences in habitat use across species may explain the variation in gene flow across putative barriers (e.g., extensive floodplain areas replaced by terra firme forest), leading to distinct degrees of genetic differentiation. However, to test this hypothesis, comparative population genomic investigations including many species with a different propensity to dispersion (associated with habitat availability and use) coupled with distinct times of lineage persistence in the landscape, will be necessary.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
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ORCID
Leilton Williams Luna https://orcid.org/0000-0001-7902-6294
Camila Cherem Ribas https://orcid.org/0000-0002-9088-4828
Alexandre Aleixo https://orcid.org/0000-0002-7816-9725

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BIOSKETCH
The authors share wide interests in research related to how species-specific traits interact with geographic and ecological barriers during evolutionary diversification processes across time and space, especially for Neotropical birds.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

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