



Gone With the Water: The Loss of Genetic Variability in Black and Gold Howler Monkeys (*Alouatta caraya*) Due to Dam Construction

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Specialty section:

This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 01 September 2021

Accepted: 21 January 2022

Published: 10 March 2022

Citation:

Oklander LI, Caputo M, Fernández GP, Jerusalinsky L, de Oliveira SF, Bonatto SL and Corach D (2022) Gone With the Water: The Loss of Genetic Variability in Black and Gold Howler Monkeys (*Alouatta caraya*) Due to Dam Construction. *Front. Ecol. Evol.* 10:768652. doi: 10.3389/fevo.2022.768652

Black and gold howler monkeys (*Alouatta caraya*) inhabit several eco-regions in South America with the highest population densities in riverine forests. Dam construction for electricity production represents a severe human alteration of ecosystems with consequences for primate conservation. To evaluate the possible loss of genetic diversity in *A. caraya*, we analysed and compared the genetic structure of the species across 22 study sites in Argentina (14), Paraguay (1), and Brazil (7). Four of these study sites (referred to as flooded) were sampled before dam-linked flooding which most likely caused a drastic decline or functional extinction of these populations. The genetic variability of 256 individuals was evaluated using 10 autosomal microsatellites (STRs) and 112 individuals by sequencing a fragment of 507 bp of mtDNA. DNA was extracted from tissue, blood, and faecal samples. Significantly higher values of genetic variability were observed for the flooded populations both in mtDNA and STRs. Population genetic structure showed a $K = 1, 2, \text{ or } 5$ depending on the method, separating Argentinian and Paraguayan sites from Brazilian sites and, in the case of $K = 5$, two clusters were mostly represented by flooded populations. Isolation-by-distance analyses showed that geographic distances influence gene flow. Analytical methods, such as Pairwise F_{st} 's and Nei's and regression model of Harpending and Ward, were concordant in detecting significant genetic structuring between flooded and remaining sites examined. Although some sites have very low sample sizes, these samples are of great importance since these sampling sites are currently flooded. Our results show that the study sites where dams were built had the greatest genetic diversity. As *A. caraya* is currently severely threatened by yellow fever outbreaks, the remaining populations may be more vulnerable to disease outbreaks due to impoverished genetic variability. Accordingly, it is essential to implement management actions to conserve the remaining populations. Our results

underline the importance for Environmental Impact Assessments (EIA) to include data on the genetic structure of species in the affected sites prior to their alteration or destruction. These genetic data are also remarkably important for determining where to relocate specific individuals to help avoid biodiversity loss.

Keywords: *Alouatta caraya*, howler monkeys, dam construction, genetic structure, conservation

INTRODUCTION

Hydroelectric dams have been built in primate habitat countries since the 1950s. More than 45,000 large dams (≥ 15 m tall) have been built worldwide, affecting $> 50\%$ of the largest rivers of the world, including the most biogeographically diverse ones (World Commission on Dams [WCD], 2000; Nilsson et al., 2005).

Dams store river water which, when released, powers turbines to generate electricity. Although the media commonly refer to dams as the largest source of renewable electricity in the world and are popularly considered as clean or green energy (Gibson et al., 2017), the alterations by dams of the natural flow of rivers may cause, among other disturbances, extensive modification to aquatic communities (Poff et al., 1997; Lemly et al., 2000; Prowse et al., 2002; Tockner and Stanford, 2002) and promote extensive deforestation and fragmentation of pristine forests (Finer and Jenkins, 2012). Moreover, the associated flooding of often extensive tracts of land can also destroy adjacent terrestrial ecosystems (Bauni et al., 2015) and cause anoxia which releases greenhouse gases and generates sedimentation (Rosa et al., 2004; Fearnside and Pueyo, 2012).

Additionally, many conflicts have risen from the resettlement programs associated with the inundation of indigenous and local communities. Such programs can result in adverse human health effects and substantial changes in land use patterns (Indrabudi et al., 1998; Gillet and Tobias, 2002; Richter et al., 2010; Verdum, 2012).

In recent decades, Environmental Impact Assessments (EIA) have been used to predict the potential adverse effects of dams prior to their construction (Robinson, 1992; Tullos, 2008). However, compliance with the EIA recommendations is rarely mandatory, and as a result, is not always incorporated into the final design. Hence, biodiversity impact mitigation is unlikely to occur (McAllister et al., 2001; Rodrigues, 2006). For example, populations of anuran species became extinct due to the construction of hydroelectric reservoirs in the Casca and Manso rivers in Brazil (Brandao and Araujo, 2008; Lima et al., 2015; Silva et al., 2018) despite EIA warnings.

The long-term effects of dams on biodiversity have rarely been evaluated. The World Commission on Dams (WCD) produced the most comprehensive review of dam impacts, but it was dissolved in 2001. Benchimol and Peres (2015) assessed the long-term impacts (26 years later) of a mega-hydroelectric dam on medium and large-bodied vertebrates in Central Brazilian Amazon. They found that most islands formed by the dam cannot ensure the local persistence of even a modest fraction of the original middle and large-sized vertebrate fauna.

Primates are clear candidates to be affected by dam construction, not only because of the number of islands and the areas of riverside forest lost as a result of flooding, but also with indirect impacts even before the construction itself, such as deforestation caused by clearing of the area, constructions of roads, and hunting of primates for food or capture for illegal trade (Rodrigues, 2006; Harrison-Levine et al., 2019). Primates are strongly affected by anthropogenic activities that cause loss, fragmentation, reduction, or isolation of native forest habitats (Michalski and Peres, 2005; Estrada et al., 2017), triggering secondary processes such as dispersal restrictions, resource depletion, and pathogen exposure (Oklander et al., 2010, 2017; Lecompte et al., 2017). In combination, these can reduce genetic diversity, decrease the adaptive potential of populations, increase local extinction risks, and affect long-term species survival (Frankham, 2005).

Although there are several studies of the effects of dams on primates, most have been carried out in the short term (Kinzey et al., 1988; Terborgh et al., 1997, 2001; Cosson et al., 1999; Ferrari et al., 2004). However, Benchimol and Peres (2014) found evidence of the detrimental impacts of dams on a primate community 21 years after dam construction was completed. They found that primate species richness significantly decreased according to the area and forest cover of the remaining islands in the flooded landscape, with howlers (*Alouatta macconnelli*) and brown capuchin monkeys (*Sapajus apella*) showing greater tolerance to habitat changes than other species. The rescue of fauna and their transfer to adjacent areas is a procedure used widely during the filling of the reservoirs associated with hydroelectric dams and is commonly promoted as a conservation measure. However, this procedure further destabilises adjacent areas that are already occupied by conspecifics, thereby increasing population densities (Woodford and Rossiter, 1993; Schneider, 2001; Jones et al., 2016). Many thousands of primate rescues have been conducted during and/or after flooding, but most of them have been poorly documented and very few have had subsequent follow-ups (Fournier-Chambrillon et al., 2000; Schneider, 2001). Rodrigues (2006) provides empirical evidence that the fate of the majority of individuals rescued and transported to unknown habitats is death, affirming that wildlife rescue projects are a fallacy or an unscientific measure to ameliorate the ecological impact of filling a dam in terms of public opinion.

Black and gold howler monkeys (*Alouatta caraya*, Humboldt, 1812) inhabit several ecoregions in Brazil, Argentina, Paraguay, and Bolivia (Brown and Zunino, 1994; Lynch Alfaro et al., 2015) and show a remarkable variation in density across their distribution (Zunino et al., 2001; Bicca-Marques et al., 2008;

Agostini et al., 2015; Jardim et al., 2020; Gorostiaga et al., 2021). These differences could be attributed to resource availability, interspecific competition, or the effect of epidemic diseases like yellow fever (Holzmann et al., 2010; Kowalewski et al., 2011; Lynch Alfaro et al., 2015; Oklander et al., 2019). However, the density and group size of *A. caraya* in the island system along the Paraná River under low-level human impact was found to be the highest (3.6 individuals per ha) anywhere, indicating the importance of this environment for the species (Aguilar et al., 2009; González et al., 2014). These populations also show special demographic features, with multimale groups being more common (Rumiz, 1990; Zunino et al., 2001). Demographic records show that, in continuous areas, both females and males leave their natal groups (Rumiz, 1990), but habitat fragmentation modifies the *A. caraya* dispersal pattern from bisexual to male biased (Oklander et al., 2010).

Although the International Union for Conservation of Nature (IUCN) classifies *A. caraya* as “Near Threatened” Bicca-Marques et al. (2021), Oklander et al. (2019) recommended recategorisation to “Vulnerable” based on the limited genetic variability reported in the southernmost populations, the dramatic reduction in density, and the loss of entire populations in the Atlantic forest of Brazil and Misiones Province in Argentina after two sylvatic yellow fever outbreaks (2008–2009 and 2016–2019; Holzmann et al., 2010; Almeida et al., 2012; Bicca-Marques et al., 2017; Ministério da Saúde do Brasil [MS-BR], 2018, 2019, 2021). Howlers are described as the most susceptible genus to yellow fever virus, developing clinical illness and fatal disease few days after infection (Laemmert and Kumm, 1950). As such, as sentinels for virus detection (Klitting et al., 2018), they have great importance for epidemiological surveillance, allowing the early establishment of control and prevention measures, such as targeted human vaccination campaigns in adjacent areas contributing to public health (Bicca-Marques and de Freitas, 2010; Siconelli et al., 2019).

The genetic structure of primate populations is affected by several factors, including effective population size, dispersal distances, genetic drift, and demographic events such as bottlenecks (Di Fiore, 2009). The decrease in the population size generated by local extinctions or epizootic events implies a loss of genetic variability and fixation of allelic and/or haplotype variants due to the effect of genetic drift within small and/or isolated groups (Ayala-Burbano et al., 2017; Baden et al., 2019; Zhao et al., 2019). In this sense, it is interesting to study the genetic consequences derived from the high mortality of individuals in *A. caraya* after the flooding of several sites within its distribution.

Given the projection that there will be a rise in dam construction in primate range countries for the next 10–20 years (Tundisi et al., 2014), this study aimed to use molecular tools that use genetic markers with different mutation rates and inheritance characteristics to elucidate genetic consequences of high mortality of *A. caraya* after dam construction, analyzing 22 sites along its distribution in Argentina, Paraguay, and Brazil. Specifically, alterations in the genetic structure of the population at different time scales, with the mtDNA Control Region for ancient genetic processes and microsatellite markers (STRs) for recent population genetic processes (Oklander et al.,

2010, 2017; Kollbeck et al., 2013; Wang et al., 2019). The precise evaluation of the current genetic structure and gene flow among the populations in these sites is needed to contribute to the development of management strategies aimed at ensuring the long-term survival of this species, which is a unique ecological sentinel.

We are interested in the comparison between the locally extinct and remaining populations to analyse historical genetic variability and to identify variability in remaining populations. If populations were already isolated prior to flooding, we expect to find: (1) genetically differentiated populations, (2) that the genetic variability of some populations was irreversibly lost due to dam constructions, and (3) that remaining populations have low genetic variation perhaps making them more vulnerable to future disease outbreaks. Our findings may help in the detection of remaining *A. caraya* populations with important genetic variation to conserve and suggest management actions to preserve them.

MATERIALS AND METHODS

Study Sites Description and Sampling

Black-and-gold howler monkeys were sampled from three countries: Argentina, Paraguay, and Brazil, with a total of 256 individuals in 22 populations (Table 1 and Figure 1). Tissue or blood samples were obtained by the capture of adult individuals in the context of EIA or during flooding for dam construction in four sites (GO.BR, MT.BR, MS1.BR, and MS2.BR; Table 1 and Figures 1, 2). Consequently, we are analysing individuals that were relocated or dead.

Samples from GO.BR were collected in the EIA of Serra da Mesa Hydroelectric Power Plant, located in the Alto Tocantins Basin, Goiás state, Brazil (GO). This dam is located on the main course of the Tocantins River, and its reservoir is the largest in Brazil in terms of water volume, with 54.4 billion m³ and an area of 1,784 km². Samples from MT.BR were collected in the EIA of the Represa de Manso hydroelectric dam located on the Manso River, a tributary of the Cuiabá River, Mato Grosso state, Brazil. The reservoir has a volume of 7.3 billion m³ and an area of 427 km². During construction, many human families were displaced from the area now covered by the reservoir, and the submerged, decaying vegetation has had a negative impact on water quality and has affected edible fish stocks (Hylander et al., 2006). Samples MS1.BR and MS2.BR were collected during the flooding of the Engenheiro Sérgio Motta Hydroelectric Power Plant (also called Porto Primavera Hydroelectric Power Plant) on the Paraná River, 28 km upstream from the confluence with the Paranapanema River. This dam lies between the Municipality of Porto Primavera in the eastern part of the Mato Grosso do Sul (MS) state and southwestern-most São Paulo state. This dam has the greatest surface area in Brazil, being 10,186.20 m long with a reservoir formed by water from the Paraná River that has a flooded area of 2,250 km². The other three samples' sites in Brazil belong to the states of Paraná (PR), São Paulo (SP), and Minas Gerais (MG), in areas where no dams have been built.

TABLE 1 | Description of study sites.

Population name	N samples	Condition	Localisation		Na		Ne		Hs		Ho		AR		PA
			Latitud	Longitud	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	
GO.BR	2	Flooded	-13.833889	-48.304444	2.9	0.738	2.7	0.835	0.861	0.220	0.700	0.258	1.817	0.183	0
MT.BR	3	Flooded	-14.873611	-55.478611	3.4	1.174	3.0	1.089	0.825	0.173	0.550	0.352	1.763	0.149	0
MS1.BR	20	Flooded	-21.260833	-51.922778	7.9	3.695	5.0	3.206	0.743	0.175	0.697	0.195	1.742	0.175	2
MS2.BR	20	Flooded	-21.975000	-52.368611	7.9	3.542	5.1	2.768	0.762	0.165	0.719	0.132	1.761	0.162	3
MEAN Flooded					5.5	2.750	4.0	1.303	0.798	0.055	0.667	0.078	1.771	0.032	
PR.BR	27	Rem.BR	-22.748333	-53.258611	6.0	3.367	3.7	1.545	0.663	0.252	0.678	0.258	1.663	0.252	2
S.P.BR	3	Rem.BR	-21.240111	-48.014778	2.7	1.160	2.2	1.077	0.533	0.270	0.633	0.331	1.560	0.278	0
MG.BR	3	Rem.BR	-16.700000	-43.900000	3.4	0.966	3.0	0.914	0.775	0.286	0.533	0.322	1.727	0.271	2
MEAN Rem.BR					4.0	1.739	3.0	0.742	0.657	0.121	0.615	0.074	1.650	0.084	
Paraguay*	5	Rem.PY.AR	-27.275.156	-57.683.786	2.8	1.398	2.1	0.946	0.488	0.257	0.400	0.249	1.478	0.251	0
Isla.AR*	36	Rem.PY.AR	-27.313.561	-58.646.481	4.8	2.700	2.5	1.270	0.506	0.259	0.500	0.262	1.506	0.259	1
EBCo.AR*	40	Rem.PY.AR	-27.549.722	-58.678.889	4.5	2.953	2.2	1.124	0.448	0.253	0.465	0.255	1.448	0.253	0
PNCh.AR*	9	Rem.PY.AR	-26.793.611	-59.617.778	2.9	0.876	1.8	0.487	0.437	0.171	0.400	0.197	1.435	0.171	1
FOR.AR*	12	Rem.PY.AR	-25.984.722	-58.161.111	3.7	1.703	2.3	0.841	0.525	0.214	0.483	0.211	1.524	0.212	0
SAC. AR*	10	Rem.PY.AR	-28.306.389	-57.456.389	2.7	1.494	1.9	0.993	0.377	0.303	0.430	0.343	1.380	0.304	0
GAM.AR*	6	Rem.PY.AR	-27.466.872	-55.826.569	2.8	1.135	1.9	0.918	0.414	0.245	0.483	0.309	1.420	0.250	0
YAM.AR*	6	Rem.PY.AR	-25.574.447	-54.075.378	2.4	1.350	1.9	0.986	0.403	0.243	0.483	0.288	1.411	0.242	0
PIM.AR*	11	Rem.PY.AR	-26.500.000	-53.833.333	4.4	1.647	2.6	0.953	0.583	0.219	0.464	0.163	1.577	0.216	0
YAC.AR*	9	Rem.PY.AR	-29.445.200	-56.799.800	3.6	0.966	2.4	0.949	0.537	0.242	0.567	0.284	1.539	0.241	0
PPU.AR*	9	Rem.PY.AR	-25.920.833	-54.418.611	3.5	1.650	2.3	1.053	0.531	0.229	0.489	0.197	1.529	0.225	0
AZM.AR*	5	Rem.PY.AR	-27.984.093	-55.786.648	2.5	0.850	1.8	0.613	0.425	0.230	0.540	0.341	1.438	0.240	0
A.PM.AR*	4	Rem.PY.AR	27.910.417	-55.760.722	2.5	0.707	1.9	0.383	0.492	0.143	0.575	0.237	1.504	0.147	0
URM.AR*	4	Rem.PY.AR	-27.517.733	-55.788.489	2.2	0.789	1.7	0.568	0.404	0.255	0.450	0.284	1.411	0.255	0
SCM.AR*	12	Rem.PY.AR	-27.428.889	-55.709.722	3.8	1.619	2.3	0.689	0.546	0.210	0.500	0.242	1.545	0.210	0
MEAN Rem.PY.AR					3.3	0.834	2.1	0.285	0.474	0.063	0.482	0.052	1.476	0.060	
MEAN Rem.BR.PY.AR					3.4	1.007	2.3	0.489	0.505	0.100	0.504	0.074	1.505	0.091	

GO.BR, Goiás state; MT.BR, Mato Grosso state; MS1.BR and MS2.BR, Mato Grosso do Sul state; PR.BR, Paraná state; SP.BR, São Paulo state; MG.BR, Minas Gerais state; Paraguay; Isla.AR, Isla Brasilera in Chaco province; EBCo.AR, Estación Biológica Corrientes in Corrientes province; PNCh.AR, Parque Nacional Chaco in Chaco province; FOR.AR, Guaycolect reserve in Formosa province; SAC.AR, San Alonso in Corrientes province; GAM.AR, Garupá in Misiones province; YAM.AR, Yacutinga reserve in Misiones province; PIM.AR, Piñalito provincial park in Misiones; YAC.AR, Yapeyú in Corrientes province; PPU.AR, Provincial park Lago Uruguay in Misiones Province; AZM.AR, Azara in Misiones province; APM.AR, Apostoles in Misiones province; URM.AR, Urutaú reserve in Misiones province; SCM.AR, Santa Cecilia reserve in Misiones; Rem, Study sites that remain currently relatively undisturbed, since no major infrastructure projects or deforestation have been carried out in these sites since sampling occurred. BR, Brazil; PY, Paraguay; ARM Argentina; N samples, number of individuals sampled in each population; Na, N° of different alleles; Ne, N° of effective alleles (calculated as $1/\Sigma(\text{allele frequency})^2$); Observed Heterosigosity (Ho): number of heterozygous/N.; (Hs), Gene diversity; (AR), allelic richness (see section "Materials and Methods" for details); PA, N° of alleles unique to a single population; AR p, 0.004; Ho p, 0.001; Hsp, 0.001; Na p, 0.0006; Ne p, 0.00009. Bold values are the mean values for each condition. *Populations already analysed in Oklander et al. (2020).

Tissue or faecal material was obtained from monkeys in sites where the habitats remain intact and accessible for sampling (Table 1; Oklander et al., 2017, 2020). For some analyses, the following study sites in Brazil GO.BR, MT.BR, MS1.BR, and MS2.BR were grouped as "Flooded." The Brazilian sites PR.BR, SP.BR, MG.BR, and all sites in Paraguay and Argentina were grouped as "Remaining" (either Rem.BR or Rem.PY.AR; Table 1).

Sampling complied with Argentine, Paraguayan, and Brazilian laws and was conducted with permission from the National Resources Board (Brazil: SISBIO n. 19927-12 by ICMBio, Paraguay: Ministerio de Salud Pública, Mercosur/GMC/Res. n. 50/08, Argentina: CONICET n. 11420110100322CO and Ministry of Ecology, Misiones province, Argentina n. 9910-00086/17). We adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Primates.¹

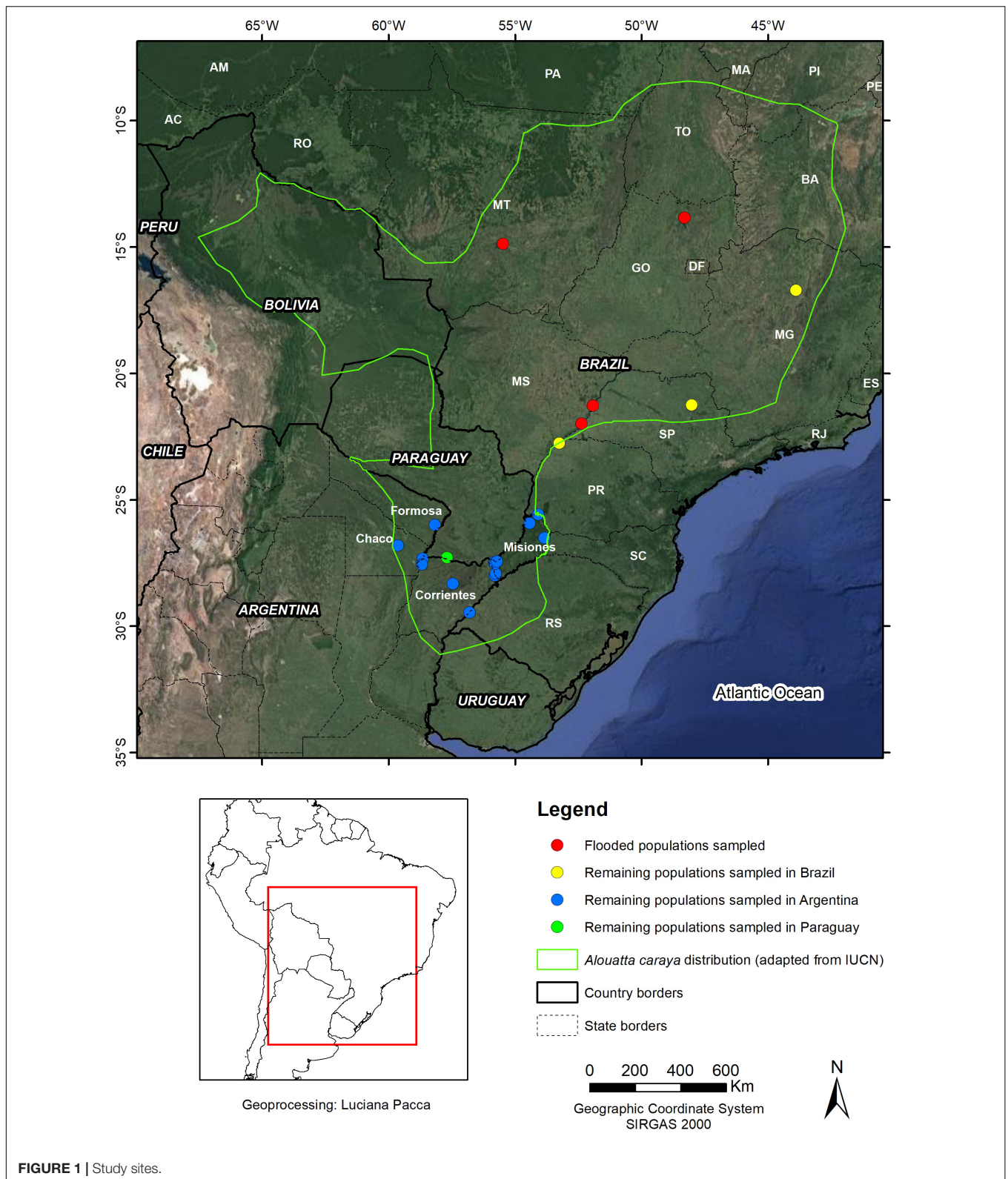
¹ <https://www.asp.org/society/resolutions/EthicalTreatmentOfNonHumanPrimate.cfm>

DNA Extraction

DNA was extracted from faecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, United States), according to the manufacturer's protocols, with slight modifications (Oklander et al., 2017). DNA was extracted from tissue and blood samples using standard SDS/Proteinase K digestion followed by phenol:chloroform organic extraction and Microcon P-100 counter-dialysis filters (Green and Sambrook, 2012).

Microsatellite or Short Tandem Repeats Amplification

Ten microsatellites or Short Tandem Repeats (STRs) characterised for *A. caraya* and previously used in population genetics studies, were amplified from each sample: AC14, AC17, AC45, TGMS1, TGMS2, D8S165, D17S804, LL1118, LL157, and AB07 (Tomer et al., 2002; Di Fiore and Fleischer, 2004; Gonçalves et al., 2004; Oklander et al., 2007; Supplementary File 1). Genotyping PCRs were performed using recommendations from



previous studies, with a final volume of 25 μ l using 5–10 ng of DNA template for tissue samples or 5 μ l of the extraction of faecal samples, 20 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂,

0.2 mM each dNTP, 1 U of GoTaq DNA polymerase (Promega, Madison, United States), 1 pmol of each forward primer bearing an M13 tail, 4 pmol of each reverse primer, and 4 pmol of M13

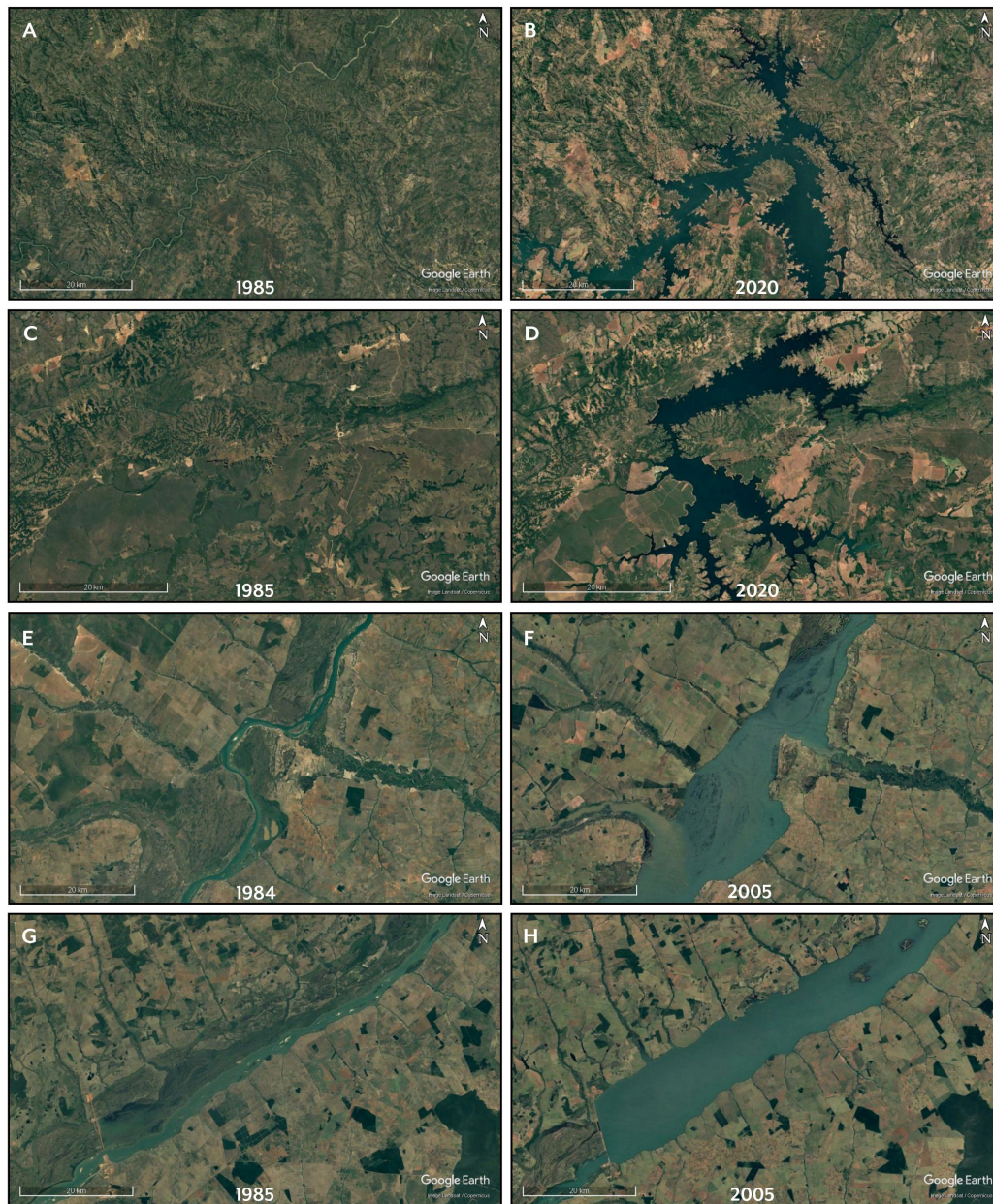


FIGURE 2 | Flooded study sites before dam construction (1985) and currently. **(A)** Tocantins River and **(B)** of Serra da Mesa Hydroelectric Power Plant in Goiás state, Brazil (site GO.BR). **(C)** Manso River and **(D)** Represa de Manso Hydroelectric Power Plant, in Mato Grosso state, Brazil (site MT.BR). **(E,G)** Parana River, **(F,H)** Engenheiro Sérgio Motta Hydroelectric Plant (Porto Primavera), in Mato Grosso do Sul state, Brazil (sites MS1.BR and MS2.BR).

labelled with fluorescent dyes (6-FAM, HEX or NED) on its 5' end (Schuelke, 2000; Oklander et al., 2007). Singleplex PCRs were carried out for faecal samples and multiplex of 3 or 4 STRs for tissue and blood samples (**Supplementary File 1**).

All amplifications were performed in a Gen Amp ABI 9,700 machine (Thermo Fisher, Palo Alto, United States). PCR products labelled with different fluorochromes were combined and co-injected with HD400-ROX as an internal size standard to be separated by electrophoresis on an ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific, Palo Alto, United States)

or in MegaBACE 1000 (Pharmacia, Upsala, Seden) automated sequencer (Amersham Biosciences) according to the protocols of the manufacturer. Alleles were manually scored by performing a visual inspection of electropherograms after developing the bin panel for each locus in GeneMapper ID-X v. 1.2 (Thermo Fisher, Palo Alto, United States) or software Genetic Profiler 2.2 (GE Healthcare).

For DNA extracted from faecal samples, PCR and sizing were repeated two times (in the case of a heterozygous genotype call) or four times (in case of a homozygous genotype call)

to minimise possible genotyping errors due to allelic dropout (Pritchard et al., 2000; Peakall et al., 2003). We only recorded an allele if it was observed at least two times in different amplifications from the same DNA extract. All amplification assays included negative controls. Every step of the lab work was carried out in specific laboratory spaces (DNA extraction room and PCR room), inside laminar flow hoods with negative pressure, and using aerosol-resistant filter tips to avoid between sample cross-contamination.

Short Tandem Repeat Statistical Analysis

Genetic Diversity

Genotypes were screened for null-alleles and to discriminate between errors in allele frequency estimates caused by null-alleles, allele dropout, or stutter bands using Micro-Checker v2.2.332 (Van Oosterhout et al., 2004).

Numbers of different alleles (N_a), effective and private alleles (N_e), and observed heterozygosity (H_o) were computed using GenALEX v6.5 software (Peakall and Smouse, 2012) for each locus and study site. Deviations from Hardy-Weinberg equilibrium (HWE) were assessed by employing an exact test using Arlequin v 3.5 software (Excoffier and Lischer, 2010).

Allelic richness (AR) and gene diversity (H_s) were calculated for each locus in a population using Fstat software v2.9.4 (Goudet, 2003):

$$AR = \sum \left[1 - \frac{\binom{2N - N_i}{2n}}{\binom{2N}{2n}} \right]$$

where N_i represents the number of alleles of type i between the $2N$ genes and n is sample size based on a minimum sample size of 1 diploid individual. To account for differing sample sizes, we computed a rarefied measure of allelic diversity (Allelic Richness) in Fstat based on a standard sample size of $n = 1$.

$$H_s^k = \frac{n_k}{n_k - 1} \left(1 - \sum_i p^2 k_i - H_o^k / 2n_k \right)$$

where n represents the number of alleles, p the allele frequency, and H_o the observed heterozygosity.

Based on the results of the Structure analysis (see below), and to increase the n between comparisons, we gather the study sites into three groups: 1-Flooded, 2-remaining in Brazil (Rem.BR), and 3-remaining in Paraguay and Argentina (Rem.PY.AR, **Table 1**).

For statistical comparison, AR, H_s , and H_o between these groups were compared using Fstat (Goudet, 2003), and N_a and N_e were compared with the Welch t -test (Excel).

Pairwise F_{st} 's and Nei's average number of pairwise differences between study sites (Nei, 1972) and the statistical comparison were determined with Arlequin v3.5 software and plotted with R-lequin (Excoffier and Lischer, 2010).

Structure Analysis

The genetic structure was evaluated using the non-spatial Bayesian clustering with the Structure v.2.3.4 program

(Pritchard et al., 2000). A series of 20 independent runs per K (ranging from 1 to 7) was conducted using the admixture model with correlated allele frequencies, without prior information about sampling locations, independent allele frequencies, and 1,000,000 Monte Carlo-Markov iterations after a burn-in of 50,000 replicates. Several K -estimation methods were tested, including the $Pr[X|K]$ method (Pritchard et al., 2000), the ΔK method (Evanno et al., 2005), and the parsimony method (Wang, 2019) using the KFinder software (Wang, 2019). A bar plot was constructed with the software pophelper (Francis, 2016).

Mitochondrial Amplification

We amplified a 507-bp fragment of the left domain of the mitochondrial DNA Control Region (mtDNA, CR) using primers How RA-1 (5'-CTACCATCAACACCCAAAGC-3', Ascunze et al., 2007) and RC-BugioR (5'-CCAGGTTAAGAGGGTGATAGC-3', Oklander et al., 2017). The amplified zone corresponds to the position 15,412–15,903 bp from the mitogenome published by Finstermeier et al. (2013) and deposited at Genbank: NC_021938. Amplification reactions were performed in a final volume of 25 μ l containing 25 ng of DNA extractions, 1X of GoTaq buffer (Promega, United States), 1.75 mM of MgCl₂, 0.2 mM of each dNTP, 1U GoTaq DNA polymerase (Promega, United States), 4 pmol of each primer, and 4 mg/ml of bovine serum albumin (BSA). Cycling parameters were an initial denaturation (5 min at 94°C) followed by 35–40 cycles of denaturation (1 min at 94°C), annealing (30 s at 50°C), and extension (1 min at 72°C), then a final extension step (3 min at 72°C). Sequencing was carried out using the same reverse and forward primers used for initial amplification. All samples were sequenced bidirectionally using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and visualised in an Applied Biosystems 3,500 Genetic Analyzer or the DYEnamic™ ETDye Terminator Cycle Sequencing Kit (GE Healthcare) on a MegaBACE 1,000 automated sequencer. Sequences quality was assessed with Sequencher 5.3 (LifeCodes, United States).

Mitochondrial Diversity and Structure Analysis

Sequences were edited and aligned using the CLUSTAL W algorithm (Thompson et al., 1994) in MEGA10.2.6 (Kumar et al., 2018).

Haplotype frequencies, the number of polymorphic sites (S), haplotypic (H_d), and nucleotidic (P_i) diversity were calculated in DNASP 5.0 (Librado and Rozas, 2009). Standard tests of selective neutrality and Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) with 95% confidence intervals were computed in DNASP with 1,000 simulations and a neutral infinite-sites model assuming a large constant population size (Hudson, 1990). All these parameters were performed for each study site and for the three groups (Flooded, Rem.BR, and Rem.PY.AR, **Table 2**).

A haplotype network was built using Median-Joining (Bandelt et al., 1999), and implemented in POPART (Leigh and Bryant, 2015).

TABLE 2 | Summary estimates of mitochondrial diversity.

Study sites	Number of Hap (n)	Haplotypes ID (n)	Hd (SD)	Pi	S	Tajima (D)	Fu (Fs)
GO.BR	1 (1)	Hap20 (1)					
MT.BR	2 (2)	Hap21 (1), Hap22 (1)	1	0.032	16		
MS1.BR	8 (11)	Hap1(2), Hap2 (2), Hap3(1), Hap4 (1), Hap5 (1), Hap6(1), Hap7(1), <u>Hap8</u> (2)	0.945	0.049	63	0.574	2.440
MS2.BR	8 (10)	Hap1(1), Hap3(1), Hap6(3), Hap7(1), Hap8(1), Hap9(1), Hap10 (1), Hap11 (1)	0.933	0.046	59	0.447	1.454
Total flooded	14 (24)	Hap1-Hap7 , <u>Hap8</u> , Hap9-Hap11 ; Hap20-Hap22	0.946 (0.025)	0.047	68	1.028	2.588
PR.BR	7 (17)	Hap12 (1), Hap13 (1), Hap14 (3), Hap15 (2), Hap16 (3), Hap17 (6), Hap18 (1)	0.838	0.032	46	0.759	6.321
MG.BR	1 (1)	Hap19 (1)					
Total Rem.BR	8 (18)	Hap12-Hap19	0.856 (0.059)	0.036	57	0.372	5.890
Paraguay*	1 (1)	Hap32 (1)					
Isla.AR*	6 (9)	Hap32(1), Hap36 (2), Hap37 (2), Hap38 (1), Hap39 (2), Hap40 (1)	0.911	0.012	14	1.199	0.920
EBCo.AR*	1 (11)	Hap32 (11)	0	0	0		
PNCh.AR*	3 (8)	Hap23 (2), Hap41 (4), Hap42 (2)	0.714	0.005	6	0.806	2.285
FOR.AR*	2 (8)	Hap23 (5), Hap24 (3)	0.536	0.005	5	1.764	4.413
SAC.AR*	1 (8)	Hap38 (8)	0	0	0		
GAM.AR*	5 (6)	Hap25 (1), Hap26 (1), Hap27 (2), Hap28 (1), Hap29 (1)	0.933	0.008	9	-0.113	-0.807
YAM.AR*	1 (4)	Hap30 (4)	0	0	0		
PIM.AR*	3 (7)	<u>Hap8</u> (5), Hap31 (1), Hap33 (1)	0.524	0.019	24	0.063	5.712
YAC.AR*	2 (8)	Hap34 (5), Hap35 (3)	0.536	0.006	6	1.813	5.011
Total Rem.PY.AR	21 (70)	<u>Hap8</u> , Hap23-Hap42	0.926 (0.015)	0.014	36	-0.078	-1.447
Total	42 (112)	Hap1-Hap42	0.963 (0.007)	0.033	80	0.279	-2.917

Genetic diversity estimates and neutrality tests for 16 populations of the black-and-gold howler monkeys based on 112 sequences of 491-bp mtDNA Control Region fragment sequences. Number of haplotypes (n, number of sequences); Haplotypes ID (n, number of sequences); Hd, haplotypic diversity \pm standard deviation (SD); Pi, nucleotide diversity; S: number of polymorphic (segregating) sites. Tajima's (1989) D; Fu's (1997) Fs; p-values > 0.1. In bold are shown private haplotypes by population and by group, and underlined the only haplotype (Hap8) found in more than one group. * Populations analysed in Oklander et al. (2017).

Pairwise Fst's and Nei's average number of pairwise differences between populations (Nei, 1972) were determined with Arlequin v3.5 software and plotted with R-lequin (Excoffier and Lischer, 2010). This analysis involved 112 nucleotide sequences, with all ambiguous positions removed for each sequence pair (pairwise deletion option). There were a total of 491 positions in the final dataset from the original 507 pb.

As there is evidence of hybridisation between *A. caraya* and *A. guariba* in some of the sampled sites (Aguar et al., 2007; Agostini et al., 2008), we also carried out amplifications of the same mtDNA fragment in 4 samples of *A. guariba* (2 from Argentina and 2 from Brazil) in order to obtain the evolutionary relationships for these two taxa. The optimal Neighbor-Joining phylogenetic tree obtained shows reciprocal monophyly between taxa (Supplementary File 2), ruling out that the differences found in our study are due to hybridisation with this species.

Isolation-by-Distance Analyses

We conducted Mantel tests between genetic Fst (mtDNA and STRs) and geographic distances (in Km, computed from geographical coordinates) using the *mantel* function (method = "spearman," permutations = 9,999) implemented in the "vegan" v2.4 \pm 3 package (Dixon, 2003) in R. The proportion of shared alleles by pairs of populations was calculated with PopGenReport v2.2.2 package (Adamack and Gruber, 2014) in R. If geographic distances influence gene flow, we would expect the Mantel statistic to be significant (Chiappero et al., 2016).

Regression Model Analysis

R-matrix analysis and the regression model of Harpending and Ward (1982) D2 distances derived from R-matrix analysis (Harpending et al., 1973) were employed for gaining insights into the patterns of external gene flow and genetic drift due to isolation under the island model. The broken line is the expected relationship predicted by the model according to the formula $hi = H(1-ri)$, where ri is the distance from the centroid. hi is the heterozygosity of population i , and H is heterozygosity of the total population according to Genocline v1.4 software (Peña et al., 2020). If gene flow from outside the region varies substantially from population to population, this linear relationship no longer holds. Isolated groups will be less heterozygous than the linear prediction and will therefore lie below the theoretical regression line, whereas populations receiving more gene flow from outside will be more heterozygous and lie above the line (Lakshmi et al., 2002). Consequently, the outliers above and below the theoretical regression line may give some insights into the population structure.

RESULTS

Genetic Diversity and Demographic Parameters

For the majority of the samples ($n = 243$ or 94.9%), all 10 STRs loci were amplified. For the remaining 13 samples, a minimum of nine loci were amplified. We found no evidence of significant

deviations from the Hardy-Weinberg equilibrium, with the 256 *A. caraya* individuals from 22 populations exhibiting moderate levels of microsatellite diversity (Table 1).

The highest population of genetic variability statistics were found in the four flooded study sites. The highest mean number of different alleles (NA), number of effective alleles (N_e), private alleles (PA), and observed heterozygosity (H_o) were found in the two study sites, now submerged by the Porto Primavera Hydroelectric Power Plant in Brazil (MS1.BR and MS2.BR; Table 1), and the highest mean gene diversity (H_s) and allelic richness (AR) in the other two (GO.BR, and MT.BR; Table 1 and Figures 1, 2). On the other hand, the lowest values were detected in the remaining study sites in Paraguay and Argentina (Table 1). A detailed analysis of Argentine and Paraguayan study sites is given in Oklander et al. (2017, 2020).

Mean NA, N_e , H_o , H_s , and AR showed significant differences between groups (AR $p = 0.004$, H_o $p = 0.001$, H_s $p = 0.001$, NA $p = 0.0006$, N_e $p = 0.00009$; Table 1).

The BLAST search of the 112 *A. caraya* 491-bp mtDNA fragment sequences as queries retrieved only mitochondrial sequences. Comparison showed an average identity of 94.20% (*Alouatta caraya*), 82.44% (*Alouatta guariba*, *Alouatta palliata*, and *Alouatta seniculus*), 77.26% (*Ateles* sp.), 77.66% (*Brachyteles arachnoides*), 77.30% (*Cebus olivaceus castaneus*), 77.24% (*Lagothrix* sp.), 76.82% (*Saimiri*), and 77.21% (*Sapajus* sp.). Therefore, the presence of nuclear mitochondrial DNA sequences (NUMTs) in our fragment sequences can be considered negligible.

The 112 mtDNA sequences were grouped in 42 haplotypes, of which 21 were new to this study (Genbank accession numbers: OK033252-OK033272) and the rest of haplotypes were taken from Oklander et al. (2017; MF095740- MF095754). For the complete dataset, 80 polymorphic (segregating) sites, an overall haplotypic diversity of 0.963 ± 0.007 , and an overall nucleotide diversity of $\Pi = 0.0338 \pm 0.001$ were observed. The median-joining network showed a complex pattern of relationship among observed haplotypes (Figure 3 and Supplementary File 2). The Flooded mtDNA variation, represented by the haplotypes Hap1-Hap11 (MS1.BR and MS2.BR), Hap20 (GO.BR), and Hap21-Hap22 (MT.BR) are well distributed along the network, showing a higher number of mutation steps among these 14 Control Region variants. For the rest of the remaining populations sampled in Brazil (Rem.BR) and Paraguay-Argentina (Rem.PY.AR), 8 and 21 haplotypes were obtained, respectively, with lower values of variation indices (S , H_d , and Π ; Table 2). Although the overall pattern of the network showed strong haplotype relationships, only one haplotype (Hap8) was shared between MS1 (Flooded) and PIM (Rem.PY.AR). Similarly, the general pattern of the haplotype network supports a relative demographic stability and absence of a recent population expansion, as suggested by the values obtained for the F_s of F_u and the D of Tajima (all p -values > 0.1 ; Table 2).

Mantel statistic based on Spearman's rank correlation rho showed significant results for mtDNA ($r = 0.3847$, $p = 0.0023$) and STRs ($r = 0.4603$, $p = 0.0002$) which implies an inverse proportion of effective dispersal to geographical distance.

However, for "short" distances (< 300 km) a wide range of F_{st} s is observed (from almost zero to 1; Figure 4), perhaps related to what is observed in the haplotype network. Particularly, close study sites with high genetic differentiation, up to ~ 30 difference mutations (Hap 1 and 11 from MS1.BR and MS2.BR), and low differentiation (2 mutations) between haplotypes in distant sites (Hap 11 from MS2 and Hap 19 from MG.BR; Figure 3).

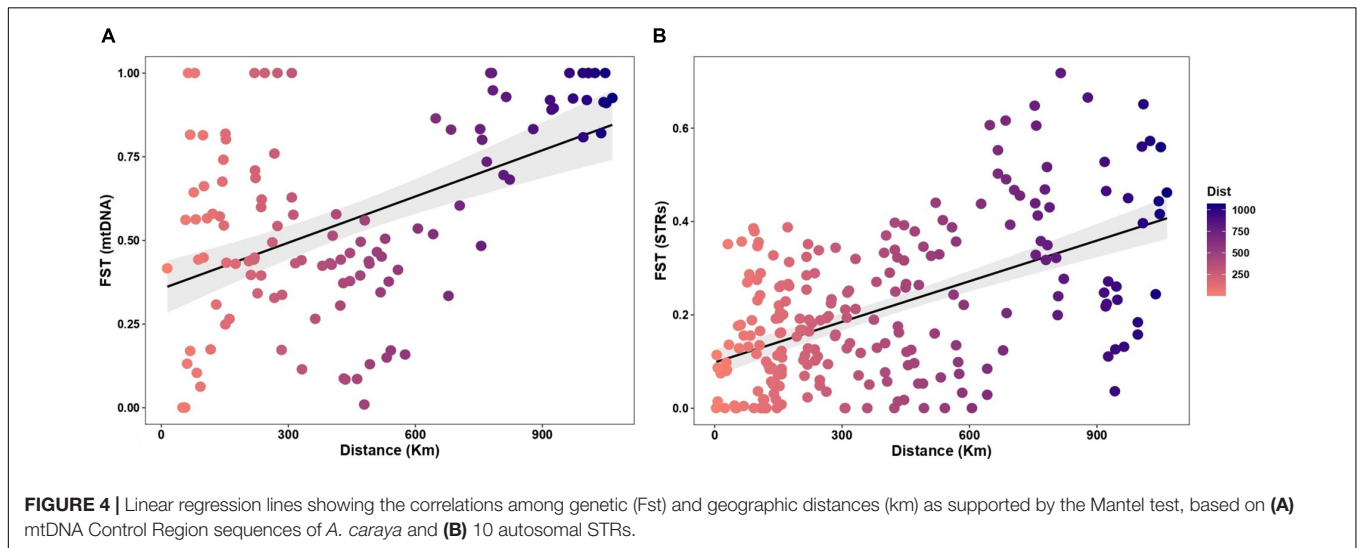
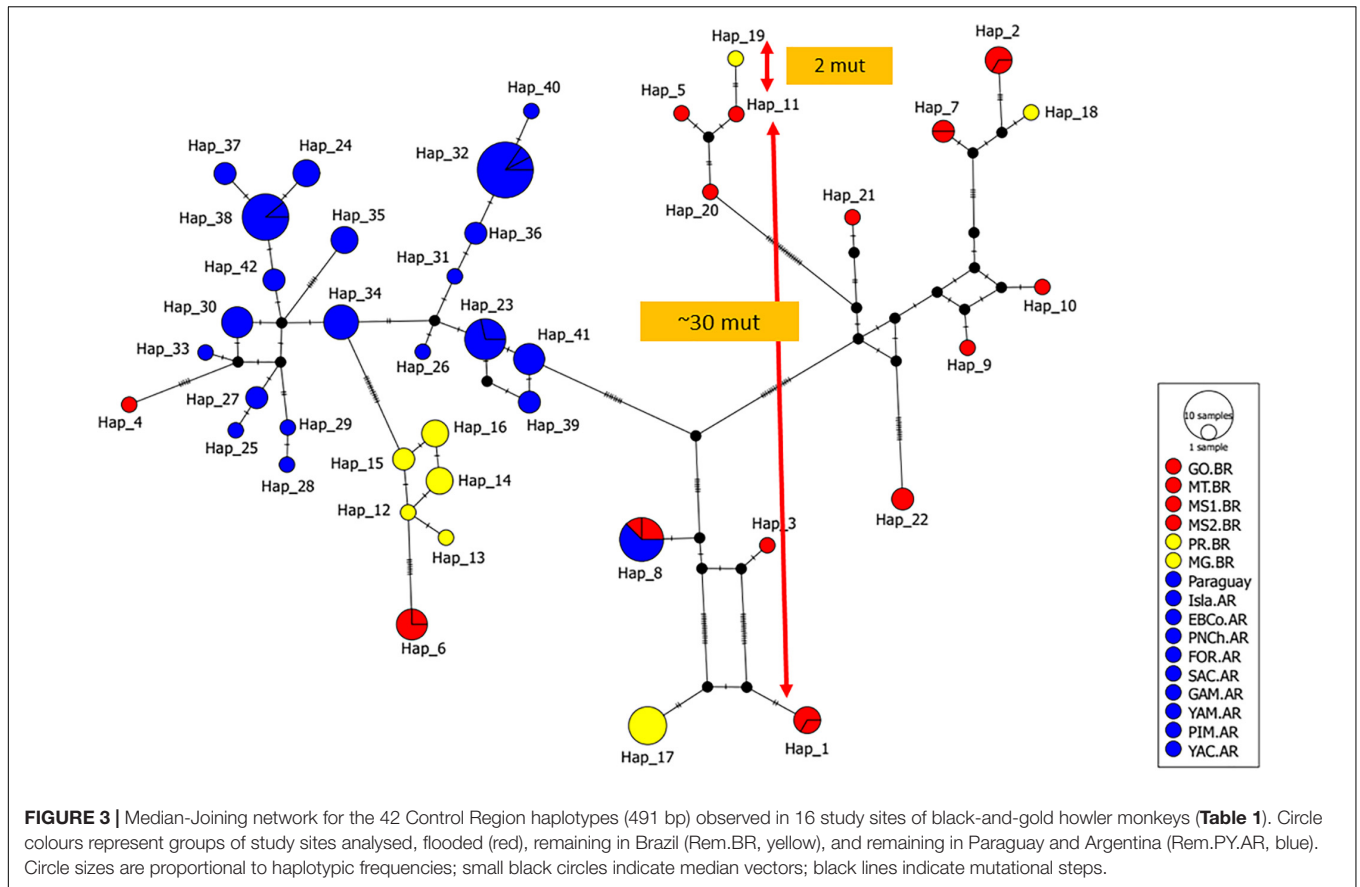
Genetic Structure

The STRUCTURE results identified a genetically different number of clusters (K) depending on the method. The parsimony method only detected one cluster grouping in the 22 study sites (Figure 5; $K = 1$). The Evanno method results showed $K = 2$ with a clear separation between the remaining study sites of Argentina and Paraguay (Rem.PY.AR, represented as green in Figure 5; $K = 2$) from all other studied sites—all of which were in Brazil (represented as blue in Figure 5; $K = 2$). Finally, the $Pr[X|K]$ method results detected five genetically differentiated clusters ($K = 5$) as best explaining the nuclear genetic variation observed in *A. caraya*. Two of these clusters are mostly represented by the four-flooded sites for dam construction coloured as yellow and blue in Figure 5, $K = 5$ (Flooded: GO.BR, MT.BR, MS1.BR, and MS2.BR), although the yellow cluster is still represented in the remaining MG:BR study site. The third cluster is represented in orange in Figure 5, $K = 5$, and is mostly represented in the remaining study sites in Brazil (PR.BR and SP.BR). Finally, the remaining Argentine and Paraguayan study sites were grouped in two clusters (green and grey) with this method (Figure 5; $K = 5$). These study sites, grouped as Rem.PY.AR, were previously studied in Oklander et al. (2017, 2020) and remain currently relatively undisturbed since no major infrastructure projects or deforestation have been carried out in these sites since sampling occurred.

Genetic Differences Between Flooded and Remaining Sites

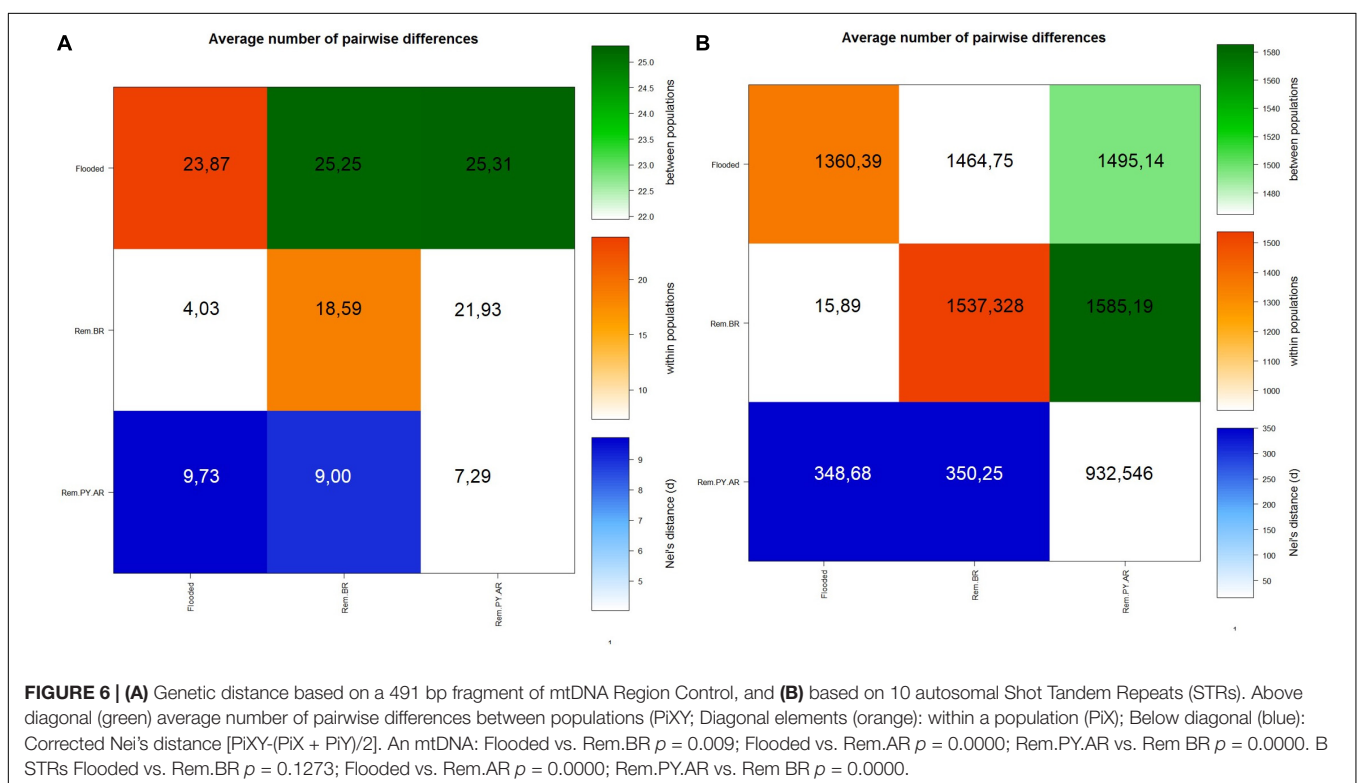
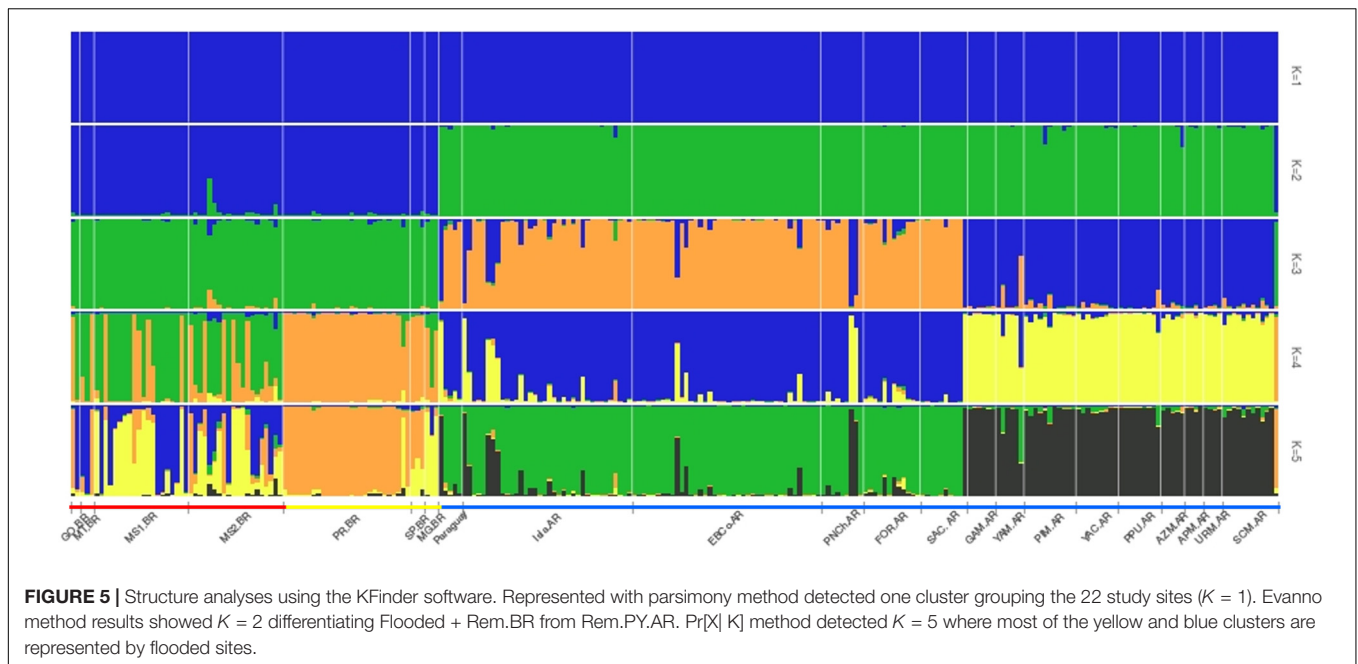
Pairwise F_{st} 's and N_e 's were used to further investigate the genetic differences between flooded (Flooded) and remaining sites (Rem.BR and Rem.PY.AR), with both markers revealing different gene flow situations in time (Figure 6). The mtDNA analysis showed that the flooded study sites showed the highest genetic diversity estimates and shared one haplotype with one Argentine site (Table 2 and Figure 3). In addition, the highest average pairwise between-population differences occur between the Flooded group and the two remaining groups (Flooded vs. Rem.BR $p = 0.009$; Flooded vs. Rem.AR $p = 0.0000$; Rem.PY.AR vs. Rem BR $p = 0.0000$; Figure 6A), suggesting that they historically exchanged migrants with other populations. The remaining populations of Brazil showed an intermediate variability (Table 1) and differentiated mtDNA haplotypes from both the flooded populations and the remnants of Argentina, suggesting isolation processes between all the sites (Table 2 and Figure 3).

On the other hand, STR analysis showed that the major mean within-population pairwise difference occurs in the Rem.BR group, and the major average pairwise differences



between populations are from both Flooded and Rem.BR groups compared to the Rem.PY.AR group. Meanwhile, Nei's distance shows greater values for both molecular markers between the two Brazilian groups (Flooded and Rem.BR) compared to the Argentine and Paraguayan sites (Flooded vs. Rem.BR $p = 0.1273$; Flooded vs. Rem.AR $p = 0.0000$; Rem.PY.AR vs. Rem.BR $p = 0.0000$, Figure 6B).

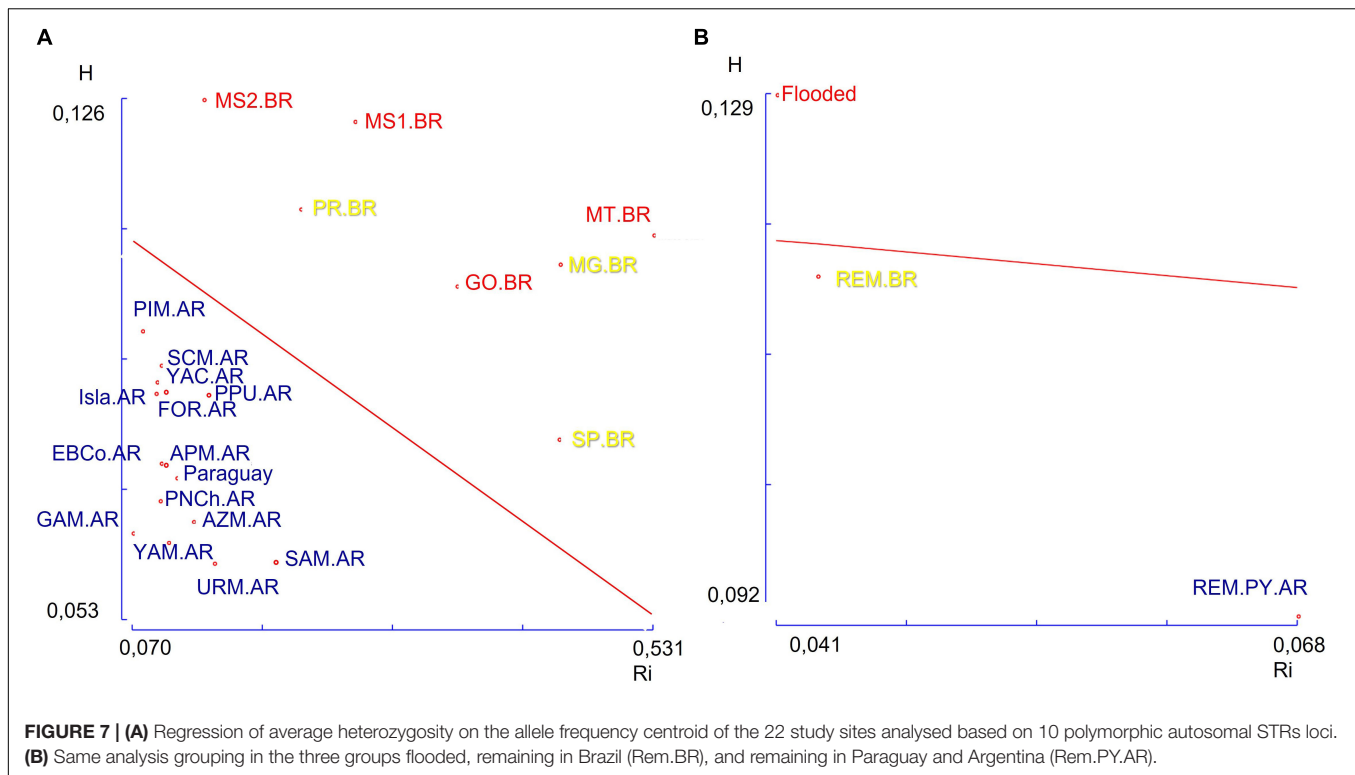
Tajima's Neutrality Test comparison of the mtDNA from three groups (Table 2) again showed significantly higher parameter values for the Flooded group compared to those in the remaining groups. According to R-matrix analysis and the regression model of Harpending and Ward (1982), the Brazilian study sites received more gene flow as they have greater heterozygosity and lie above the theoretical regression line.



In contrast, Argentine and Paraguayan sites are more isolated and less heterozygous, hence they lie below the theoretical regression line (Figure 7A). When we perform the same analysis grouping in the three groups already mentioned, we see that only the Flooded group lies above the theoretical regression line (Figure 7B).

DISCUSSION

The populations of primates are declining with an unprecedented proportion of species at elevated risk for extinction (66%) due to numerous anthropogenic activities (Estrada et al., 2017). While many primates inhabit flooded environments to which



they have adapted in evolutionary times, modifications in the habitat resulting from dam construction forces primates to make significant changes to their ecology virtually overnight (Harrison-Levine et al., 2019).

Alouatta caraya is considered a resilient species, presenting phenotypic plasticity and ability to survive in fragmented environments (Zunino et al., 2007; Bicca-Marques, 2013), even incorporating unusual resources in its diet such as eggs (Bicca-Marques et al., 2009). However, the immense loss of native forest and yellow fever outbreaks during the last decades has exceeded its resistance capacity, as is evident with its status of Vulnerable in Argentina, Endangered in Misiones, and several Brazilian states (Margarido and Braga, 2004; Oklander et al., 2019; Rio Grande do Sul, Decreto Estadual n° 51.797 de 8/9/2014).

Yellow fever outbreaks between 2016 and 2020 in Brazil have been considered the most significant ones of the last 70 years, with more than 15,000 primate epizootics (Ministério da Saúde do Brasil [MS-BR], 2018, 2019, 2021). In 2016, a yellow fever outbreak started in Minas Gerais state and spread to São Paulo, Espírito Santo, and Rio de Janeiro states in 2017 and 2018 before finally reaching Santa Catarina and Paraná states in 2019 (Silva et al., 2020). Notably, these were all states where *A. caraya* is endemic. However, our analysis was carried out with samples taken before this outbreak. Therefore, our results are independent of them.

Genetic Diversity Gone With the Water

Genetic diversity is important in ensuring species survival as populations that have high levels of genetic diversity are

better able to face environmental change than those that have low levels (Hoban et al., 2020). The sites of *A. caraya* included in the current study cover a large part of the distribution of the species (Figure 1). Our results showed: (1) genetically differentiated populations between the Brazilian and the southernmost populations of the species, (2) that a large part of the genetic variability and unique genetic lineages of the Brazilian populations were probably lost due to dam constructions, and (3) that remaining studied populations have low genetic variation, perhaps making them more vulnerable to future threats as fragmentation and disease outbreaks.

As our sampling does not cover all the occurrence sites or the entire species range of *Alouatta caraya*, we cannot assert that the genetic variability lost in the flooded sites is not represented in any other remaining population. However, the densities and group sizes recorded for the species on the upper and middle Parana River (Aguiar et al., 2009) were larger than those reported elsewhere in Brazil (Moura, 2007; Silva and Codenotti, 2007). Therefore, the processes of dam construction described in these areas undoubtedly affected at least the frequency of these variants as a whole for this species. On the other hand, as we have detected that genetic variability is related to geographic distance, the unsampled populations at the extreme west of the distribution are also highly likely to be differentiated from those sampled in this study.

At the mtDNA level, of the 42 haplotypes described in the 22 study sites, 14 belong to the flooded sites, of which only one is shared with one of the remaining sampled sites. In other words, these 13 haplotypes found only in the now submerged areas

are absent from any other studied population and have possibly disappeared from the genetic variability of the species.

At the autosomal level, the study sites of *A. caraya* included in the current study had a mean microsatellite diversity of 0.531 ± 0.095 . This is slightly lower than most congeneric species studied with some of the same markers employed here [*Alouatta pigra*: 0.588 (Van Belle et al., 2012), *Alouatta belzebul*: 0.640 (Gonçalves et al., 2004), *Alouatta palliata*: 0.584 (Milton et al., 2009)]. Flooded study sites were the most diverse, as shown by the genetic indexes and the presence of exclusive alleles and haplotypes (Tables 1, 2). If we now consider only the variability of the remaining sites (0.504 ± 0.074), it is likely that, on average, they are genetically impoverished compared to other howler species.

The results from the population structure can be hierarchical as evidenced in the different *k*-value estimation methods that detect one, two, or five clusters depending on the model used. The Pr[X|K] method results detect five genetically differentiated population clusters ($k = 5$), of which two are practically gone with the water, with only one poorly represented in the remaining MG:BR study site.

To preserve what is left of the gene pool of *A. caraya*, we recommend that the remaining four genetic clusters must be given high conservation priority. Our evidence supports significant genetic differentiation between populations, indicating contemporary restricted gene flow between *A. caraya* geographic distribution. These results have direct implications for the conservation of howlers and should be considered by policymakers in future management plans, as well as during dam construction in the habitat of these vulnerable Neotropical primates.

Implications for the Conservation of *Alouatta caraya*

During the flooding to create the lake for the Engenheiro Sérgio Motta Hydroelectric Plant (sites MS1.BR and MS2.BR in the current study), 4,000 *A. caraya* individuals were rescued along a 180 km stretch of the Paraná River, with their subsequent release in nearby forest areas (Inglêz, 2006). However, as can be seen in Figure 2, there are almost no remnants of forest on the sides of the flooded area.

The impact of dams on primate populations is not exclusively due to the loss of the flooded forest area. Before and during construction, numerous anthropogenic modifications are also generated in the surrounding areas, such as clearings, arrival of workers, resettlement of local people, and construction of roads and infrastructure, housing, and agricultural needs for these people, all of which diminishes the quality of adjacent forests (Tan and Yao, 2006; Moore et al., 2010; Harrison-Levine et al., 2019). After construction is complete, animals that have remained in the area of the reservoir either drown, remain in treetops that rot and die shortly after, or may try to disperse by swimming. During the flooding of a dam in the Brazilian Amazonia, caiman and jaguars were observed capturing howler monkeys while swimming toward the banks, while eagles captured the animals that remained on emergent trees (Harrison-Levine et al., 2019).

Both “rescued” animals and those arriving in non-flooded areas by their own means are exposed to an unfamiliar environment, difficulties finding resources, such as food, water, and a social group, and a higher risk of stress, disease, predation, and/or human hunting (World Commission on Dams [WCD], 2000; Martin, 2004). There are reports of primates found malnourished, injured, infected with parasites, and stressed during post-flood rescue operations at dams in Thailand (Nakhasathien, 1989), French Guiana (de Thoisy et al., 2001), and Brazil (Harrison-Levine et al., 2019). De Sá (2004) observed an increase in the population density of *Plecturocebus*, *Pithecia*, and *Samiri* in the adjacent habitat just after the release, but an abrupt decrease in the same in subsequent years. Another example, also in Brazil, reports a telemetry follow-up that found that only 7% translocated *Cebuella pygmaea* survived 3 months after release (Dias et al., 2015).

Historically, our work has aimed to discern the genetic diversity of this species to provide guidelines for the future management of populations (Oklander et al., 2017, 2020, 2021). For all the reasons mentioned above, we assume that the populations analysed in the flooded areas are functionally extinct (Säterberg et al., 2013). Therefore, in the current study, we unfortunately present results of “fossil” genetics. Since the largest number of dams in the future is scheduled in South America (Fearnside, 2006; Zarfl et al., 2015), an awareness of both their long-term detrimental impacts on biodiversity and developing strategies to mitigate such effects should be considered to be of utmost importance, especially in the case of *A. caraya*, a species demonstrated to be of public health importance. We believe this work provides strong evidence for the need for attention when planning and implementing future hydroelectric dams.

It has already been reported that pre-construction EIA studies fail to: (1) estimate the indirect impact on surrounding areas (Tan and Yao, 2006; Moore et al., 2010), (2) include intra-specific measures of biodiversity, such as genetic variation of species (Simões et al., 2014), and (3) implement management plans to minimise biodiversity losses (Schneider, 2001; Alho, 2011). The occurrence of a severe population declines of the already “Critically Endangered” (CR) Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) (from 130 to 40 individuals in 13 years, Hoang, 2010) was predicted by an EIA conducted prior to dam construction in Vietnam (Scott Wilson Asia Pacific Ltd [SWAPL], 2000). Despite this, the dam was still built, and few of the recommended mitigation measures were implemented. In the current study, we show an important consequence of the construction of dams for the species *A. caraya* as the extensive loss of genetic variation, leaving surviving populations greatly genetically impoverished.

To prevent this type of irreversible biodiversity loss from continuing, it should be mandatory for hydropower infrastructure to include all items mentioned in EIA in the final construction. Construction of future hydroelectric plants should explicitly consider appropriate release sites for animal rescues with sufficient resources, minimal anthropogenic threats, and relatively low population densities of conspecifics. In addition, affected primate populations should be part of long-term

monitoring programs, including impacts on behavior, health status, and genetics (McAllister et al., 2001).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because some of the samples used in this study belong to previous research such as Environmental Impact Assessments and were in the collection of the Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. Other samples are feces, the collection of which does not represent any danger or prejudice in the primate populations studied.

AUTHOR CONTRIBUTIONS

LO, DC, and SB formulated the research idea. LO, LJ, and SO conducted fieldwork. LO, MC, and GF performed laboratory analyses. MC, GF, and DC performed statistical analyses. LO, MC, GF, LJ, and DC prepared the figures and tables. LO, MC, GF, and DC wrote the manuscript. All authors reviewed and approved the final submission.

FUNDING

This work was supported by the CONICET grants to LO and the DNA Fingerprinting Service (SHDG), School of

Pharmacy and Biochemistry, University of Buenos Aires, Argentina, and by CNPq, CAPES, and FAPERGS grants from Brazil to SB. LO, MC, and DC were members of the Carrera de Investigador (CONICET-Argentina). GF was funded by the Universidad Nacional del Noroeste de la Provincia de Buenos Aires (UNNOBA-Argentina). LJ was funded by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Ministry of Environment, Brazil.

ACKNOWLEDGMENTS

We greatly appreciate the collaboration with the Ministerio de Ecología y Recursos Sustentables de la Provincia de Misiones, Instituto Misionero de Biodiversidad – IMiBio, Programa Macacos Urbanos (Universidade Federal do Rio Grande do Sul, Brazil), and Programa de Revitalização da Bacia Hidrográfica do Rio São Francisco (Ministério do Meio Ambiente, Brazil). We thank Maria de Nazaré Klautau-Guimarães for collaborating with samples. We are grateful to Luciana G. Pacca (ICMBio/CPB) and Vinicius A. Roberto (ICMBio/CPB), for the map in **Figure 1**, and Keoma C. Rodrigues (ICMBio/CPB) for producing **Figure 2**. LO wants to especially thank Atenea, Ivy, and Julian Baigorria for their time and patience. We are grateful to Adrian Barnett, Sam Shanee, and Karen DeMatteo for providing comments on the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.768652/full#supplementary-material>

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