Articles

GENETIC DIVERSITY OF THE ENDANGERED BLACK-HANDED SPIDER MONKEY *ATELES GEOFFROYI* (PRIMATES: ATELIDAE) IN A FRAGMENTED LANDSCAPE OF EL SALVADOR

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Abstract

Spider monkeys (genus *Ateles*) are distributed across rainforests from southern Mexico to northern Bolivia, but at risk of extinction across much of their range. Like most members of the genus, the black-handed spider monkey, or the Central American spider monkey, *Ateles geoffroyi* is particularly vulnerable to anthropogenic threats, due to its inability to persist in disturbed and/or isolated forest patches and is categorized as "Endangered" by the IUCN. Within its range in El Salvador, the smallest country in Central America, the spider monkey has now been restricted to isolated patches of semi-deciduous forests, including four fragments that lie within a disturbed agricultural landscape in Jiquilisco Bay, in Southeast El Salvador. We analyzed 73 noninvasive fecal samples from spider monkeys in this region, and we were able to identify 55 individuals. We evaluated the genetic diversity and structure of the four populations using microsatellite markers. Our results show higher observed than expected heterozygosity, but low genetic diversity compared to published data on other spider monkey populations (Ho = 0.50 – 0.57 and He = 0.39 – 0.51; allelic richness with rarefaction = 2.71 – 3.22; private alleles with rarefaction = 0.18 – 0.61). We also found significant differentiation across fragments (F*st* = 0.2, *P* < 0.001) and two genetically different groups. These findings suggest the need for conservation action to reconnect forest patches, to improve the unfavorable situation of the only non-human primate in El Salvador.

Keywords: Microsatellites, non-invasive samples, genetic structure, forest fragments, Natural Protected Areas.

Resumen

Los monos araña (género *Ateles*) están distribuidos por las selvas tropicales desde el sur de México hasta el norte de Bolivia, pero están en peligro de extinción en gran parte de su área de distribución. Como la mayoría de los miembros del género, el mono araña de manos negras, o el mono araña centroamericano, *Ateles geoffroyi* es particularmente vulnerable a las amenazas antropogénicas, debido a su incapacidad para persistir en parches de bosque perturbados y/o aislados, y está categorizado como "En peligro" por la UICN. Dentro de su área de distribución en El Salvador, el país más pequeño de Centroamérica, el mono araña se ha restringido a parches aislados de bosques semicaducifolios, incluyendo cuatro fragmentos que se encuentran dentro de un paisaje agrícola perturbado en la Bahía de Jiquilisco, en el sureste de El Salvador. Analizamos 73 muestras fecales no invasivas de monos araña en esta región, y pudimos identificar 55 individuos. Evaluamos la diversidad y estructura genética de las cuatro poblaciones utilizando marcadores de microsatélites. Nuestros resultados muestran una heterocigosidad observada superior a la esperada, pero una baja diversidad genética en comparación con los datos publicados sobre otras poblaciones de monos araña (Ho = 0,50 – 0,57 y He = 0,39 – 0,51; riqueza alélica con rarefacción = 2,71 – 3,22; alelos privados con rarefacción = 0,18 – 0,61). También encontramos una diferenciación significativa entre fragmentos (Fst = 0,2, P < 0,001) y dos grupos genéticamente diferentes. Estos hallazgos sugieren la necesidad de acciones de conservación para reconectar los parches de bosque, para mejorar la situación desfavorable del único primate no humano en El Salvador.

Palabras clave: Microsatélites, muestras no invasivas, estructura genética, fragmentos de bosque, Áreas Naturales Protegidas.

Introduction

Spider monkeys (*Ateles* spp.) are one of the most endangered primate genera in Central and South America, with most species classified as "Endangered" according to the International Union for Conservation of Nature (IUCN) (Alves et al., 2020; Cortés-Ortíz et al., 2021; Link et al., 2021; Link et al., 2020; Mittermeier et al., 2021; Ravetta et al., 2021) except for *A. hybridus* (Critically Endangered: Link et al., 2020) and *A. paniscus* (Vulnerable: Mittermeier et al., 2021). One of the major causes for population reduction is loss of forest cover resulting in loss of suitable habitats for the species (Alves et al., 2020; Cortés-Ortíz et al., 2021; Link et al., 2020; Mittermeier et al., 2019).

The Endangered black-handed spider monkey *Ateles geoffroyi* is the only non-human primate present in El Salvador, which has a natural forest cover of only 14 % (UNSD, 2014), and the reduction of suitable habitats for *A. geoffroyi* has restricted their local home ranges (Argueta-Rivas and Rivera-Hernández, 2004). Habitat loss and fragmentation have also caused the reduction of individuals and extirpation of the species in some localities (Burt and Stirton, 1961; Morales-Hernández, 2003), and currently the species is classified as "Endangered" at the national level according to the Ministry of Environment and Natural Resources (MARN, for its Spanish acronym) of El Salvador (MARN, 2015). However, there are six different confirmed localities, which include Natural Protected Areas (NPA), that *A. geoffroyi* still inhabits: NPA Normandía (NR) (Argueta-Rivas and Rivera-Hernández, 2004), NPA Chaguantique (CH) (Morales-Hernández, 2003), El Tercio (ET) (Morales-Hernández, 2003), El Nacascolo (NA) (Rodríguez-Menjívar, 2007), Jucuarán (Pinera et al., 2020), and Olomega (Pineda et al., 2017).

The localities of NPA Normandía, NPA Chaguantique, and private areas El Tercio and Nacascolo are all part of the Jiquilisco Bay, southeast El Salvador, which is a matrix of mangroves and semi-deciduous forest patches separated by open agricultural areas, with almost no connectivity between the areas inhabited by *Ateles geoffroyi*. Human pressure on forest systems appears to negatively influence the genetic diversity of spider monkeys, as previously observed by Hagell et al. (2013) for *A. geoffroyi* at the Rivas Isthmus, Nicaragua, a human-dominated land corridor.

Therefore, in this study we assessed the genetic diversity and genetic structure of *Ateles geoffroyi* in the fragmented landscape of Jiquilisco Bay in El Salvador by using nuclear microsatellites. Considering the landscape fragmentation in the area and historical land use changes (Hecht et al., 2006; Dull, 2008; Crespin and Simonetti, 2016), we predicted that spider monkeys from the four localities would exhibit moderate levels of genetic diversity, and moderate genetic differentiation across fragments with NPA status compared to private forest patches lacking conservation policies and aggravated land use changes.

Materials and Methods

Sampling and laboratory protocols

We performed this study at Jiquilisco Bay (13.216667, – 88.533333), located in the Southeast Pacific coast of El Salvador (Fig. 1), mainly composed of semi-deciduous and mangrove forest remnants inside a cattle pasture and sugar cane plantation matrix.

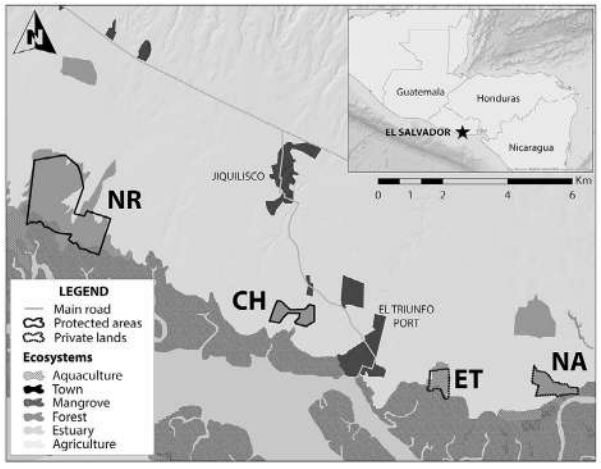

Figure 1. Land cover map of the study area in Jiquilisco Bay, showing the four sampling sites: NR Normandía, CH Chaguantique, ET El Tercio, NA Nacascolo; dark gray indicates forest, light textured gray areas indicates mangrove, light gray is agricultural land, black indicates urban infrastructure, and stone gray indicates water.

The modifications to this landscape are a consequence of historic pre-Columbian land changes due to subsistence farming, which began 4000 years ago (Dull, 2008), the forest resurgence of the civil war from 1980 to 1992 (Hecht et al., 2006), and the following post-war (2000s) trend of natural landscape loss and urban expansion across the country (Crespin and Simonetti, 2016). We sampled the four forest fragments inhabited by spider monkeys: NR, CH, ET and NA. Of these, the first two are protected areas and the last two are private lands without any conservation management strategies. The origin of the spider monkeys inhabiting NA is unclear and it has been suggested that some small groups are non-native and were introduced from a local private zoo during the 1960s – 70s (Puerto Barillas, 2010). NA is also on the property of a hotel that promotes the monkeys as a tourist attraction. These four patches have no forested connection between them, except for the mangroves in some cases (Fig. 1).

We collected fecal samples during two different periods (June-July 2013, and August-September 2015) by searching for and following (when possible) the monkey groups at each site. When found, monkeys often discharged their feces as a defense mechanism in response to the presence

of the researchers. The fresh scat was then promptly collected, with each scat separately placed in double plastic zipper storage bags without using tubes, alcohol or any preservative, stored in a portable cooler for transportation, and then kept in a freezer at –2 °C until analyzed at the Genetic Diversity Laboratory (LABGENCON) of the University of Costa Rica.

We performed DNA extractions using QIAmp DNA Stool Mini Kit (Qiagen) with modifications of Chaves et al. (2014). For genotyping, we first chose 12 nuclear heterologous microsatellite primer pairs: AP68 (Ellsworth and Hoelzer, 1998; Ruíz-García et al., 2007; Cortés-Ortíz et al., 2009; Hagell et al., 2013), AP74 and D8S165 (Ellsworth and Hoelzer, 1998; Ruíz-García et al., 2007; Cortés-Ortíz et al., 2009), Ceb120 (Muniz and Vigilant, 2008), D8S260, Leon2 and SB38 (Hagell et al., 2013), LL1118 (Di Fiore and Fleischer, 2004; Crespin-Guzmán, 2009; Hagell et al., 2013) LL311 (Di Fiore and Fleischer, 2004; Crespin-Guzmán, 2009), P2BH6 (Crespin, 2011), LL1110 (Grativol et al., 2001; Hagell et al., 2013), and Ceb121 (Muniz and Vigilant, 2008; Hagell et al., 2013). Primers were labeled with fluorescent dye at the Forward sequence of each pair. Prior to amplification, we used Autodimer ver. 1.0 (Vallone and Butler, 2004) to group loci into multiplexes absent of "primer-dimer" or "hairpin" amplifications. Polymerase chain reaction (PCR) volumes comprised 3 µL of DNA template (samples' concentration ranging from 30 to 200 ng/µl), 13 µL of PCR Multiplex Master Mix (Qiagen) (3 µM), 1 µL of 4 primer pairs (2 µM) and 3 µL of H2O free of RNAs, for a total volume of 20 µL. We used the touchdown PCR method (Korbie and Mattick, 2008) to reach optimal annealing temperatures: initial denaturation 95 °C (15 min), touchdown from 57 °C, decreasing 1 °C each cycle (45 sec) until reaching 52 °C. Extension step was denaturation 95 °C (45 sec), annealing 52 °C (45 sec), extension 72 °C (45 sec) for 34 cycles, and final extension 72 °C (7 min). All products were analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems) with 600 LIZ dye Size Standard (Applied Biosystems) and were manually scored using GeneMarker v1.91 demo (SoftGenetics LLC).

We followed precautions in Hagell et al. (2013) to avoid genotypic errors concerning non-invasive samples. For the positive control, we used a blood DNA sample template of captive *Ateles geoffroyi* from a local wildlife sanctuary, previously analyzed by Crespín-Guzmán (2009), while the negative control was PCR mix without DNA template. We repeated all PCR reactions three to five times and confirmed the final genotype call only when at least two repetitions were consistent, similar to Frantz et al. (2003). We validated all alleles using the R package MsatAllele 2.0.1 (Alberto, 2009). To check for null alleles, we used Microchecker 2.2.3 (Van Oosterhout et al., 2004) and, to check for allelic dropout, false alleles, the probability of identical genotypes (prob. > 90 %), and whether all loci were informative, we used GIMLET 1.3.3 (Valière, 2002). Finally, we removed all samples that were missing more than two alleles.

Data analysis

We performed Fisher's exact test to identify deviations from Hardy-Weinberg Equilibrium (HWE) in GENEPOP 4.0.11 (Raymond and Rousset,1995) using 100 batches and 10,000 iterations per batch. We tested for linkage disequilibrium across all 12 loci using the log-likelihood *G*-test in GENEPOP 4.0.11 (Raymond and Rousset, 1995). For both HWE and likelihood disequilibrium analyses, we adjusted the *alpha* value ($\alpha = 0.05$) with a Bonferroni correction (Rice, 1989).

We used GenAlEx 6.502 (Peakall and Smouse, 2012) and HP-RARE ver 1.0. to determine allelic richness and private alleles with rarefaction from each site regardless of different sample sizes, as measures of genetic diversity (Kalinowski, 2004), and calculated F-estimators in Arlequin 3.5. To evaluate genetic clusters, we carried out a Discriminant Analysis of Principal Components (DAPC) using the R package Adegenet (Jombart and Collins, 2015), choosing 10 principal components and $K = 2$ according to the Bayesian information criterion (BIC) (Jombart and Collins, 2015). We also performed a genetic structure analysis in STRUCTURE 2.3.4 (Pritchard et al., 2000). Simulations were run using an admixed model with correlated allelic frequencies, not including previous information. Run parameters were $1 < K < 7$ with a burn-in of 100,000 and 100,000 Markov chain Monte Carlo (MCMC) replications after burn-in with 10 iterations for each K. To determine the optimal number of K, we implemented ΔK (Evanno et al., 2005) in STRUCTURE HARVESTER (Earl and VonHoldt, 2012). To analyze the results from replicated STRUCTURE runs, we used CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007), and plotted the results in DISTRUCT 1.1 (Rosenberg, 2004).

We tested the hypothesis of genetic bottleneck by performing the sign test and Wilcoxon's sign-rank test both under the Stepwise mutation model (SMM) and the Two-phase model (TPM), with a 95 % of one step mutation and 5 % of multiple step mutation in BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996; Piry et al., 1999).

Results

In total, from 73 samples, we obtained microsatellite-based genotypes of 55 unique individuals from the four sampling sites. Seven out of 18 samples had identical genotyping, possibly being individuals sampled multiple times, and 11 had missing data for three or more loci. The overall genotyping error was low for all loci with allelic dropout and false alleles percentage of 0.038 and 0.013 respectively. We decided to remove

from further analysis three microsatellites (AP68, LL311 and LL1118) with positive PCR percentage below 0.78. We did not find HWE deviations ($P \leq 0.001$), and only three pairs of loci showed likelihood disequilibrium: AP74/D8S260 ($P < 0.005$), AP74/Leon2 ($P < 0.005$), and Ceb121/LL1110 ($P < 0.005$). Expected heterozygosity was moderate among all loci, according to Hagell et al. (2013), except for Ceb120, Leon2, and P2BH6 (Table 1).

Genetic diversity across the four study sites was low (He = 0.385 - 0.507) (Table 2). All sites exhibited low allelic richness, but NA showed the highest number of private alleles compared to the other three sites.

The F*ST* values for all 55 individuals in Jiquilisco Bay were high and significant (0.20, $P < 0.005$), which indicates a degree of differentiation among fragments. There were indications of strong genetic differentiation between NA *vs*. CH, NR, and ET since *D* and G*st* estimator (Table 3) were higher than 0.150 for all sites compared to NA. In addition, ET showed some degree of genetic differentiation with CH and NR, but the value was not high enough to be considered as strong differentiation (< 0.150). Based on the estimator results, CH and NR's spider monkeys are genetically more homogeneous ($D = 0.026$, G*st* = 0.018) than individuals from the other study sites.

Table 1. Genetic diversity in nine nuclear microsatellites loci of spider monkeys (*Ateles geoffroyi*) across all four fragments in Jiquilisco Bay, El Salvador.

Locus	N	Allelic range	*A*	*ne*	Ho	He	EHW(*P*)	F
AP74	55	134-155	4	2.447	0.552	0.559	0.050	−0.027
Ceb120	53	187-195	3	1.536	0.349	0.301	0.302	−0.103
Ceb121	53	182-210	6	2.236	0.666	0.552	0.842	−0.209
D8S260	53	212-234	7	3.096	0.772	0.662	0.012	−0.178
Leon2	54	184-200	5	1.506	0.365	0.309	1.000	−0.157
LL1110	51	213-221	5	2.000	0.612	0.461	0.230	−0.289
SB38	55	145-159	4	2.077	0.640	0.492	0.374	−0.283
P2BH6	44	105-155	8	1.726	0.231	0.355	0.013	0.397
D8S165	55	126-138	4	2.106	0.619	0.524	0.484	−0.172

N, individual genotypes; A, allelic richness; ne, effective number of alleles; Ho/He, observed/expected heterozygosity; EHW(P), probability exact test of Hardy-Weinberg equilibrium; F, Fixation index.

Table 2. Genetic diversity across nine nuclear microsatellite loci of spider monkey in sampling sites from Jiquilisco Bay, El Salvador.

Site	N	*A*	Ar	Apr	*ne*	Ho	He	EHW (P)
NR	12	31	3.220	0.440	2.099	0.514	0.482	0.042
CH	23	33	3.120	0.290	2.150	0.554	0.499	0.004
ET	10	25	2.710	0.180	1.921	0.502	0.385	0.626
NA	10	30	3.140	0.610	2.153	0.566	0.507	0.575

N, individual genotypes; A, allelic richness; Ar, Allelic richness with rarefaction; Apr, private alleles with rarefaction; ne, effective number of alleles, Ho/He, observed/expected heterozygosity; EHW (P), probability exact test of Hardy-Weinberg equilibrium.

Table 3. Pairwise analysis of genetic differentiation of *D* (Jost, 2008) and G*st* (Nei, 1977) of spider monkeys across four sampling sites from Jiquilisco Bay, El Salvador.

Site 1	Site 2	*D*.est	*P*-value	G*st*.est	*P*-value
CH	NR	0.026	0.052	0.017	0.018
CH	ET	0.093	0.001	0.051	0.001
CH	NA	0.531	0.001	0.193	0.001
NR	ET	0.111	0.001	0.041	0.001
NR	NA	0.503	0.001	0.194	0.001
ET	NA	0.509	0.001	0.239	0.001

The DAPC showed two genetically different groups (Fig. 2), one with nine individuals from NA, and the other group with all individuals from NR, CH, ET, plus one spider monkey from NA. Although ET belongs to group one, it did not overlap with CH and NR's individuals. The Mantel test between the genetic distances (Nei) and the geographic distance (Euclidean) was not significant ($P > 0.05$) and showed a negative value of – 0.004. This suggests that isolation-by-distance (IBD) does not explain the genetic differentiation between the *A. geoffroyi* populations in Jiquilisco Bay.

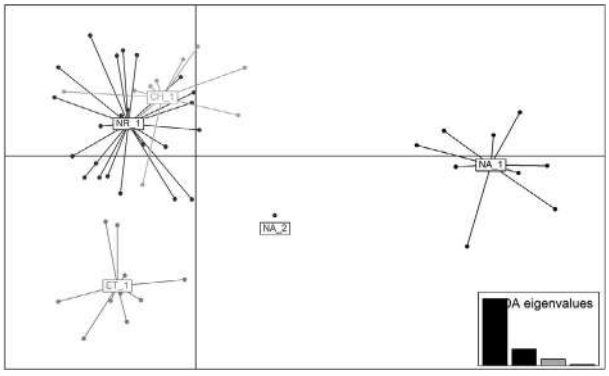

Figure 2. Scatter plot of Discriminant Analysis of Principal Components (DAPC) for spider monkeys from Jiquilisco Bay, El Salvador. Each dot represents a genetically identified individual.

The STRUCTURE analysis shows two genetically different groups (K = 2) as the most probable number of clusters according to the individual membership coefficient (Q). K1 group corresponded to individuals from NR, CH, and ET. Whereas, K2 groups all NA individuals, except for one sample with a higher proportion of the K1 group (Fig. 3).

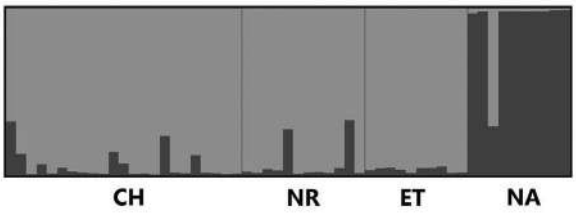

Figure 3. Bayesian clustering plot of STRUCTURE analysis for spider monkeys from Jiquilisco Bay, El Salvador, estimated for K = 2, evidencing two genetic clusters: K1 for CH, NR, ET and K2 for NA.

The Bottleneck analysis (sign test and Wilcoxon's sign-rank test for SMM and TPM) showed four of nine loci that had an excess of heterozygosity, but all *P*-values were higher than 0.05. Therefore, we were not able to confirm the existence of a genetic bottleneck in the spider monkey populations of Jiquilisco Bay.

Discussion

Genetic diversity

Results show that *A. geoffroyi* in El Salvador has very low microsatellite genetic diversity (He = 0.385 – 0.507) in comparison to previous studies of other *Ateles spp.* wild populations. In a similar fragmented landscape composition in the Rivas Isthmus, Nicaragua, Hagell et al. (2013) found the loss of genetic diversity in *A. geoffroyi* (He = 0.63 – 0.74) possibly related to accelerated human pressures on that forest systems but got lower Ho than He, reflecting a likely threat from inbreeding in said population. Ruíz-García et al. (2006) registered a wide range of microsatellite genetic diversity for *A. geoffroyi vellerosus* (He = 0.57 ± 0.36) from Petén, Guatemala, the second lowest diversity found (after *A. hybridus*) when compared with other *Ateles* taxa (e.g., *A. belzebuth belzebuth, A. fusciceps robustus, A. f. fusciceps, A. paniscus paniscus, A. chamek*). In contrast, DiFiore (2009) found higher genetic diversity (He = 0.72 – 0.78) for *A. belzebuth* in a relatively intact forest of Yasuní National Park, Ecuador. These differences in genetic diversity could be related to the disturbance level in each landscape.

We obtained low values of expected heterozygosity per locus. However, Crespín-Guzmán (2009) reported high heterozygosity levels for three loci analyzed in captive spider monkeys at three different wildlife sanctuaries from El Salvador (Mean He = 0.72). The higher genetic diversity of captive spider monkeys in El Salvador could be explained by the origin of the individuals, as some came from different populations or subspecies and ended up in captivity after being seized from the illegal pet trade (Morales-Hernández, 2003).

Few studies have used microsatellite loci in wild populations of *Ateles geoffroyi*, reporting variable allelic ranges for the same loci. In our analysis, we called AP68 monomorphic with only one allele at 172, as did Cortés-Ortíz et al. (2009) and Hagell et al. (2013) for the same locus. The 172 allele could be already fixed in the populations of these three studies. We registered a wide allelic range and allelic richness (Ar) for P2BH6 (105 – 155, Ar: 8). This locus was first analyzed in *A. geoffroyi* by Crespín-Guzmán (2009) with a similar range (104 – 124, Ar: 9). We successfully obtained polymorphic amplification of Ceb120 in *A. geoffroyi*, with a different allelic range than in other *Ateles* species like *A. belzebuth* (195 – 211) and *A. fusciceps* with a monomorphic allele at 185 (Muniz and Vigilant, 2008).

We obtained both low He and Ar for our sample. Unlike heterozygosity, allelic richness is more sensitive to the presence of unusual alleles and, in a bottleneck event, allelic richness is reduced more rapidly than heterozygosity (Kalinowski, 2004). Considering this, as well as the excess of heterozygosity in our results, we inferred a bottleneck event (Kalinowski, 2004; Nei et al., 1975). Nevertheless,

the bottleneck analysis was negative for both mutation models. NA was the only site showing a "mode-shift" allelic distribution, which could indicate that the site is not at genetic-drift equilibrium.

Genetic population structure and differentiation

The F*st* value for all individuals was high (0.20), revealing high genetic differentiation within the studied population (Allendorf and Luikart, 2009). Spider monkeys from NR and CH did not show genetic differentiation among them, according to G*st* and *D* values, which was also reflected in the STRUCTURE analysis. In the clustering plot, NR, CH, and ET formed one genetically different group, but for ET, G*st* and *D* indicated low differentiation. Considering that monkeys from NR, CH and ET are native (Morales-Hernández, 2003), it could be inferred that the connectivity between ET to the other sites was the first to decrease in relation to the connectivity between CH and NR, possibly due to the construction of Puerto el Triunfo port in 1829 (FISDL, 2012) that divided the landscape between CH and ET. For all sample sites *D* was higher than G*st*, suggesting the population had a high genetic diversity previous to the differentiation (Leng and Zhang, 2011).

Mantel's test shows no correlation between genetic and geographic distances; thus, we rejected the isolation by distance hypothesis, contrary to Hagell et al. (2013) in a similar landscape. Genetic drift was not at equilibrium for our data, implying there could be another reason for the genetic differentiation, like the presence of geographical barriers, environmental, landscape characteristics (Storfer, 1999), or historical land use changes. For El Salvador, these changes were accentuated in the late 20th century and early 21st, at the peak of the country's agricultural industry.

Apr values from NA varied from the rest in our data, and we found similar results in the differentiation values, where NA has a higher genetic distance vs. the other three locations. It is possible that these monkeys have a different origin than the others. During the decades of the 1960s and 70s, the owners of NA had a private zoo on this site. After the agrarian reform process in El Salvador, most of the animals were hunted down, but a group of monkeys survived according to historical records (Puerto Barillas, 2010). Another possible explanation is that government entities and wildlife nonprofit organizations released confiscated individuals from illegal trafficking at this site, though no confirmation could be found of the same through records in the public domain.

Conservation implications of spider monkey in El Salvador

Spider monkeys' rapid movement patterns and subgroup fission and fusion make this genus difficult to survey (Fedigan and Baxter, 1984). From 2003 to 2014, the highest number of individuals reported per site were 51 for CH, 45 for ET, 21 for NR, and 22 for NA (Morales-Hernández, 2003; Rodríguez-Menjívar, 2007; Girón et al., 2014). In Jiquilisco Bay, poaching spider monkeys for bushmeat was a regular practice until the late 1980s, as well as killing females to capture their infants for the illegal pet trade (Rodríguez-Menjívar, 2007). The low density and low genetic diversity of Jiquilisco Bay monkeys could make them susceptible to diseases and more studies of stress dynamics and parasite vulnerability (Behie and Pavelka, 2013) are necessary to gauge their vulnerability, especially to zoonotic diseases.

Inbreeding is another imminent risk for El Salvador's spider monkeys, due to the lack of connectivity and low number of individuals in some forest patches. Local reproduction between consanguineous monkeys is a concern for their conservation (Hagell et al., 2013), since it has been linked to congenital malformations (Charpentier et al., 2007) and child mortality in other primate species (Rails and Ballou, 1982). However, we observed spider monkeys of all ages during the field phase of our study, while Hagell et al. (2013) observed infants but not juveniles during their field phase in Nicaragua.

The subspecies of *Ateles geoffroyi* in El Salvador are also not well defined. The latest phylogenetic study showed that El Salvador's spider monkey samples grouped in the clade with the samples from Nicaragua, Costa Rica (A.*g. frontatus*), and Panama (*A.g. azuerensis and A.g. ornatus*) (Morales-Jimenez et al., 2015). Overall, there is a lack of phylogenetic studies including wild spider monkeys' samples from Guatemala, Honduras, and El Salvador (pers. comm., L. Cortés-Ortíz). Thus, it is necessary to define which subspecies is/are present in El Salvador, especially if the haplotype present is unique compared to the other populations of *A. geoffroyi* in the region (Morales-Jimenez et al., 2015). This knowledge would be useful to propose better conservation and management actions, especially for confiscated spider monkeys of unknown origin.

The two genetically different groups found represent two different conservation units, and there should be appropriate conservation measures for both. In keeping with the structure and differentiation results, the establishment of biological corridors among NR and CH should be a priority, considering they belong to the same genetic group and could contribute to increase gene flow between patches. Even though it is unclear how these spider monkeys use the matrix and its different land cover types on Jiquilisco Bay, it is possible they may use food resources and travel within secondary forest (Arroyo-Rodríguez et al., 2017). Depending on the proximity between fragments, a minimum area size where *Ateles* spp. inhabit can be associated with canopy density and tree diameter values related to less logged forest fragments (Marsh et al., 2016). *A. geoffroyi* can feed and travel in secondary vegetation (i.e., < 30-year forest

succession and a canopy shorter than 20 m), live fences, riparian corridors, tree crops like *Mangifera indica* and *Theobroma cacao*, and also isolated trees in the landscape matrix (Arroyo-Rodríguez et al., 2017), which could facilitate the efficiency of restoration processes of the fragmented area, considering the ecological importance that the species has as seed disperser (Chaves et al., 2011).

The low genetic variability could have negative consequences for the species in the country. Multidisciplinary management actions are necessary to ensure the survival of spider monkeys in El Salvador.

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