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Large-scale assessment of genetic diversity and population connectivity of Amazonian jaguars (*Panthera onca*) provides a baseline for their conservation and monitoring in fragmented landscapes

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ABSTRACT

Jaguar population genetics has so far not been investigated on a broad scale in the Amazon rainforest, which constitutes the largest remaining block of continuous habitat for the species. Given its size, it serves not only as a stronghold but also as a reference for jaguar conservation genetics, against which fragmented landscapes can be compared. We assessed genetic diversity and population structure of Amazonian jaguars using 11 microsatellite loci and performed comparative analyses incorporating available data from two other South American biomes (Pantanal and Atlantic Forest) in which the species has faced different amounts of habitat loss and fragmentation. Using the largest genetic data set assembled to date for jaguars ($n = 190$), we observed that all diversity indices were consistently higher for the Amazonian population, with no genetic subdivision detected in that region, indicating large-scale connectivity across > 3000 km. In contrast, we corroborate the inference of anthropic-driven genetic structure and bottlenecks for two Atlantic Forest populations. Our results indicate that the Amazon is a critically important stronghold for jaguars, comprising a highly diverse, panmictic population that allows a glimpse into the patterns of genetic connectivity that characterized this species prior to human intervention. In contrast, the Atlantic Forest populations jointly still retain considerable levels of genetic diversity, but this is currently partitioned among isolated fragments that are increasingly subjected to heavy anthropic disturbance. These results have important implications for jaguar conservation planning, highlighting the critical condition of Atlantic Forest populations and providing a genetic baseline to which they can be compared.

1. Introduction

Tropical ecosystems harbour a large proportion of global biological diversity, reaching > 50% of the world's terrestrial biodiversity (Gardner et al., 2010). Increasing human activities on those regions are exerting pressure on the biota, reducing local abundance and causing defaunation, driving thousands of species to extinction even before they are discovered (Dirzo et al., 2014). Habitat loss and fragmentation are two of the main threats to species survival, especially for large carnivores (Costa et al., 2005; Crooks, 2002), such as the jaguar (*Panthera onca*). This felid is the top predator of the Neotropics, and given its keystone role, constitutes an umbrella and flagship species for biodiversity conservation (Thornton et al., 2016). Globally, it is considered 'Near Threatened' by the IUCN (Caso et al., 2008), but it is categorized as Endangered or Vulnerable in most national red lists across its distribution (e.g. Aprile et al., 2012; ESA, 1973; Rodríguez-Mahecha et al., 2006; SEMARNAT, 2010).

In Brazil, jaguars currently occur in five out of six major biomes, and their populations are subjected to different threats on a regional basis, making them more vulnerable in some areas than others (Nijhawan, 2012; Sollmann et al., 2008). It is 'Critically Endangered' in the Atlantic Forest due to a drastic population reduction during the last three

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decades (Beisiegel et al., 2012), as this biome is severely imperilled by habitat loss and fragmentation (Tabarelli et al., 2005). In contrast, the Amazon and the Pantanal, given their extent, habitat suitability and comparatively lower levels of fragmentation, are regarded as the two main strongholds for the jaguar, both nationally and globally, although its status is ‘Vulnerable’ in both of these biomes (Cavalcanti et al., 2012; de Oliveira et al., 2012).

The Brazilian portion of the Amazon covers nearly 3.5 million km^2, and it is assumed that jaguars occupy most of this area (de Oliveira et al., 2012). For this reason, this biome is regarded as the most important block of continuous habitat for jaguars, harbouring one of the largest populations of the species, with good perspectives for long-term persistence. Nevertheless, jaguars in this biome are threatened by illegal hunting, and the so-called “arc of deforestation” is advancing on the eastern and southern portions of the region, already representing a loss of 18% of the originally forested area (de Oliveira et al., 2012). Likewise, the Pantanal is one of the largest wetlands in the world, encompassing 140,000 km^2 (85% of which remain conserved), with jaguars occupying between 88,000 and 125,000 km^2 (Cavalcanti et al., 2012). In the Pantanal, retaliatory hunting of jaguars that prey on cattle is the main threat to the species' survival. Interestingly, ecotourism focused on jaguars in this region is currently fifty times more profitable than cattle ranching (Tortato et al., 2017), which has helped to alleviate the hunting pressure. In sharp contrast, the Atlantic Forest is a biodiversity hotspot with a high degree of endemism (Myers et al., 2000), whose primary cover has been decimated in the last four decades, declining from 1.3 million to 150,000 km^2 (Ribeiro et al., 2009). Currently, jaguars occupy < 50% of this area, persisting in small, isolated fragments in which jaguars also suffer from prey depletion and illegal hunting (Beisiegel et al., 2012; Paviolo et al., 2016).

As a large mammalian carnivore, jaguars have high mobility and, as a result, could potentially attain high levels of dispersal and gene flow across the landscape (Row et al., 2012; Tammeleht et al., 2010). However, relatively few molecular studies with jaguars have been published to date. Jaguars have shown moderate to high levels of genetic diversity (Eizirik et al., 2001; Roques et al., 2016; Ruiz-Garcia et al., 2006; Wultsch et al., 2016a), without evidence of strong population structure across their range, possibly due to a recent population expansion and high connectivity on broad spatial scales. Major geographical barriers such as the Amazon River and perhaps the Darien strait were suggested as having restricted historical gene flow among four incompletely isolated phylogeographic groups: southern South America, northern South America, Central America and Mexico-Guatemala (Eizirik et al., 2001). However, the authors of that study stressed the need for further sampling that could reveal a finer pattern of subdivision or isolation by distance on a regional level.

In-depth analyses of regional jaguar populations in Brazil initially revealed that a recently fragmented area of the inner Atlantic Forest showed evidence of drift-induced population differentiation and loss of allelic richness, driven by anthropogenic habitat loss and isolation (Haag et al., 2010). The problem is so severe that one of the sampled populations (“Porto Primavera”) was extirpated due to the flooding of a hydroelectric dam before that study was published. Valdez et al. (2015) further analysed these subpopulations in conjunction with jaguars sampled at four sites within the southern Pantanal and found that the latter region forms a single genetic cluster with higher genetic diversity than each of the Atlantic forest demes. Subsequently, Srbek-Araujo et al. (2018) analysed an isolated population from the coastal Atlantic Forest and demonstrated that it also bears signs of anthropogenic loss of diversity, at a rate that may be even higher than that of the inland fragments.

Any genetic study is sensitive to the geographic scale considered in the analysis, potential gaps in sampling, and numbers of markers and their information content (Radespiel and Bruford, 2014). Furthermore, ancient demographic processes left genetic imprints in edge-populations (vs. core-populations) that are analogous to signals detected in shrinking populations subject to contemporaneous anthropic-driven drift (Slatkin and Excoffier, 2012), potentially hindering the disentanglement of the underlying process. For instance, jaguars have shown a marked population structure altogether but a weak signal of isolation by distance across Central America, which increased when Mexican (edge-) populations were included in the analysis (Wultsch et al., 2016a, 2016b). Similarly, comparing 11 microsatellite loci typed in jaguars from Brazil and Mexico, Roques et al. (2016) found a marked genetic structure, with samples from Brazil forming three genetic clusters, corresponding to the Amazon/Cerrado, the Pantanal, and the Caatinga. Genetic differentiation was not only related to geographic distance, but also to the intensity of drift, as the isolated population from the Caatinga showed low allelic richness and reduced gene flow relative to the other areas within Brazil. This is a likely consequence of a recent (within the last 20 to 30 years) demographic reduction, which may reflect the Caatinga region contemporary habitat deterioration. Jaguars sampled in the Amazon rainforest showed high levels of genetic diversity and panmixia across considerable distances, while the genetic diversity was lower towards the limits of the species' range (Mexico, Caatinga and Pantanal). However, Roques et al. (2016) did not survey the Amazon as a whole, as their geographic sampling of this vast region was restricted to a north-south transect covering only the central portion of the biome, leaving large sampling gaps in the eastern and western Amazon. In addition, that study did not include comparisons with Atlantic Forest populations, which have been found to be severely impacted by recent fragmentation (Haag et al., 2010; Srbek-Araujo et al., 2018).

In this context, the aim of this study was to survey the jaguar's genetic variability and population structure across the Amazon, and to perform comparative analyses of this data set jointly with those reported previously for Atlantic Forest (Haag et al., 2010; Srbek-Araujo et al., 2018) and southern Pantanal (Valdez et al., 2015) populations. In particular, we aimed to employ standardized molecular markers to assess the hypothesis that jaguars in the large, continuous Amazon rainforest show greater levels of genetic diversity and population size and connectivity than in the highly fragmented Atlantic Forest. We included the Pantanal biome as a control for high-quality habitat availability, as this later region currently harbours roughly the same extension as the sum of Atlantic Forest remnant fragments. This result would further corroborate our previous inference that the population structure observed in the Atlantic Forest is anthropogenic (Haag et al., 2010; Srbek-Araujo et al., 2018), and stress the importance of generating baseline data for jaguar genetics and ecology in a habitat that still retains large-scale continuity.

The specific aims of this study were as follows:

1. To contribute data on jaguar population structure and genetic diversity in the Amazon region, which currently represents its main stronghold for global conservation, but is still understudied due to its vastness and inaccessibility.
2. To compare these results with those previously published for two different biomes, the Pantanal and the Atlantic Forest, which are subjected to different intensities of anthropogenic disturbance.
3. To summarize the amounts of genetic diversity and population structure in these populations, characterizing their spatial distribution within and among biomes.
4. To provide baseline data for assessment of jaguar vulnerability to genetic erosion in its core range, as well as in other areas, which should be relevant in the context of current and projected scenarios of habitat degradation.

2. Methods

2.1. Sampling protocol

We obtained samples of biological material from 73 Amazonian

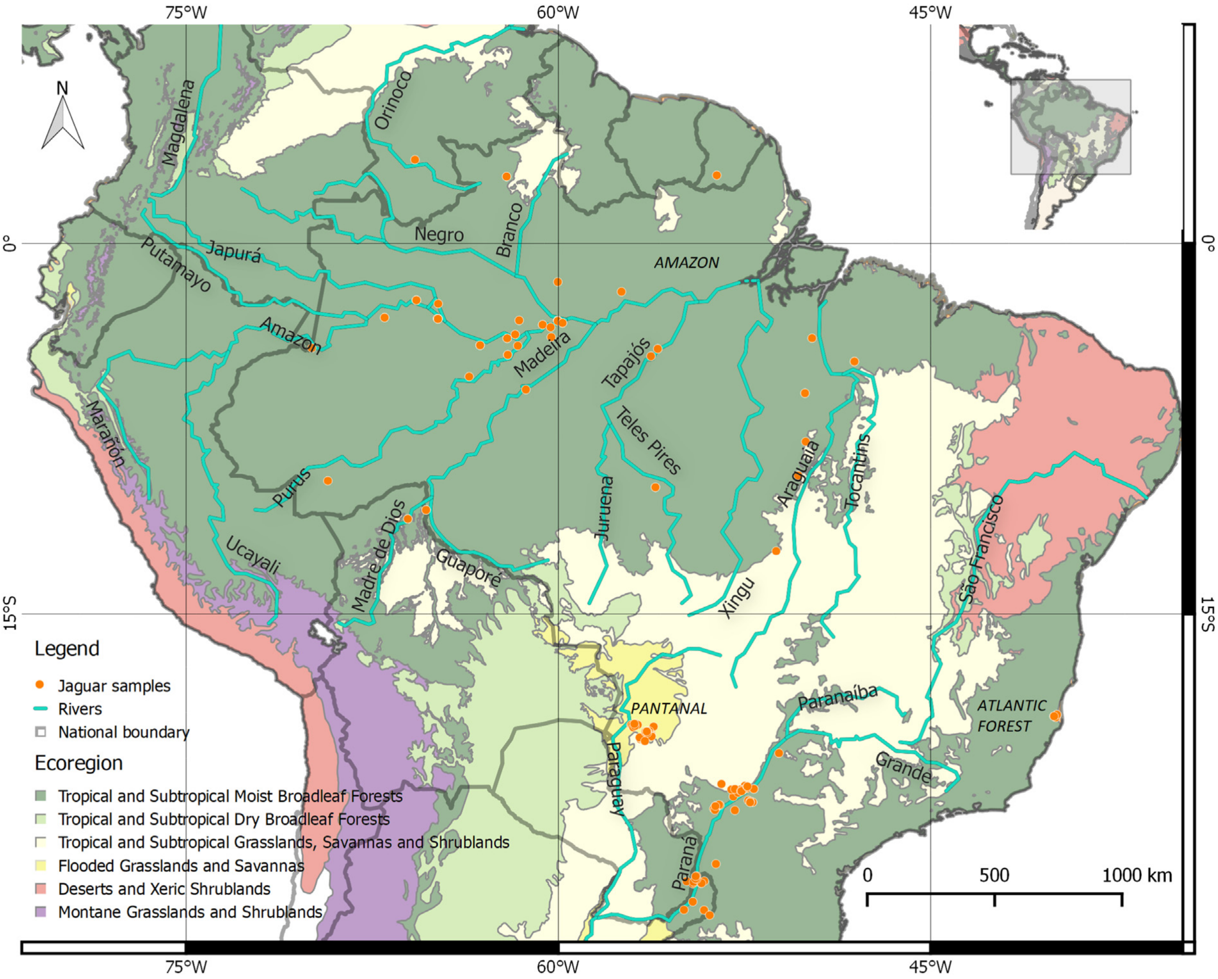

Fig. 1. Study area in South America. Points represent the sampling locations for genotyped jaguars. The Amazon basin was surveyed in three sub-regions: upper Amazon ($n = 46$), northeastern Amazon ($n = 18$), and southeastern Amazon ($n = 9$), with an estimated sampled area, calculated with convex hulls polygons, roughly encompassing 1.2 million, 400,000 and 95,000 km^2, for each region, respectively. As for the Pantanal ($n = 52$), the estimated total area covered was *ca.* 5000 km^2, while for the Atlantic Forest ($n = 59$ for inner and $n = 11$ for coastal areas) it was approximately 110,000 km^2.

jaguars, including blood samples from animals captured for field ecology studies or kept in captivity, and pelt/hair samples from material confiscated by local environmental authorities or collected during wildlife surveys within indigenous and other riverine human communities (Supporting information Table S1). Field-captured animals were covered by capture permit 11095-8, issued by SISBIO/ICMBio, Brazil. The overall Amazonian sample included three sub-regions: upper Amazon ($n = 46$), northeastern Amazon ($n = 18$) and southeastern Amazon ($n = 9$). The vast extent of the sampled area, calculated by convex hulls polygons in QGIS v.2.8, roughly encompassed 1.2 million, 400,000 and 95,000 km^2 in each of these sub-regions, respectively (Fig. 1). Blood samples were preserved with EDTA, followed by mixing with an equal volume of the buffer TES (100 µM Tris, 100 µM EDTA, 2% SDS). Pelts, tissues, and hairs were preserved in 96% ethanol. Faecal samples were stored in sterile vials containing silica gel at a ratio of 4 g silica: 1 g stool (Wasser et al., 1997). All samples were stored at −20 °C prior to DNA extraction.

2.2. Data collection and dataset construction

We performed DNA extractions from Amazonian samples using the commercial kits Puregene DNA Purification Kit (GENTRA), ChargeSwitch Forensic DNA Purification Kit (INVITROGEN), or QIAmp DNA Stool Mini Kit (QIAGEN), following the manufacturers' instructions. In the case of faecal samples, species-level identification was confirmed using the approach reported by Haag et al. (2009). We used all DNA extracts to genotype 13 microsatellite loci, one with a dinucleotide repeat (FCA742), two with trinucleotide repeats (F146 and F98), and ten with tetranucleotide repeats (FCA741, FCA740, FCA723, FCA453, FCA441, FCA391, F124, F85, F53, and F42). We scored microsatellite alleles using a MegaBACE 1000 automated sequencer and the ET-ROX 550 size standard, and then analysed them with the accompanying GENETIC PROFILER software v.2.2, as described by Haag et al. (2010).

To allow comparisons on a broader scale, we jointly analysed these Amazonian data with genotypic matrices generated by Haag et al. (2010) and Srbek-Araujo et al. (2018) for the Atlantic Forest ($n = 59$, and $n = 11$, respectively), as well as Valdez et al. (2015) for the Pantanal ($n = 52$). These studies used the same loci, and their data are available on the Dryad digital repository (https://doi.org/10.5061/dryad.1884/1; https://doi.org/10.5061/dryad.371c6). Genotyping for all these previous studies and for the present one was performed with the same protocols and equipment, including replicated control samples to allow identical binning of microsatellite alleles.

2.3. Genotyping quality control

All datasets were screened for genotyping errors and missing data using the StrataG package v.2.0.2 (Archer et al., 2016). First, we identified samples with missing loci using a threshold equal to 0.69, i.e.

only individuals genotyped for at least nine out of 13 (69%) loci were included in the analysis. We then assessed the percentage of missing samples per locus, using a cut-off value of 0.20. We removed loci below this threshold from the analysis. For the novel Amazon dataset, we also checked for duplicate genotypes, using an identity threshold of 1.0. We assessed departures from Hardy-Weinberg Equilibrium (HWE) proportions, using the exact test of Guo and Thompson (1992) for heterozygote deficit, as well as linkage disequilibrium between loci in GENEPOP v.1.0.5 (Rousset, 2008). For both tests, we estimated *P*-values by the Markov chain method with 10,000 dememorization steps, 200 batches and 5000 iterations per batch. For some downstream analysis (i.e. effective population size estimation, see below), we previously tested for the presence of closely related individuals (parent-offspring, and full-siblings) using the software ML-RELATE v.1 (Kalinowski et al., 2006).

2.4. Genetic diversity and population structure

We calculated standard diversity and differentiation indices with Adegenet v.2.1.1 (Jombart, 2008) and diveRsity v.1.9.90 (Keenan et al., 2013) packages in R, and GenAIEx v.6.503 in EXCEL (Peakall and Smouse, 2012). We also calculated allelic richness by rarefaction using HP-RARE v.1 (Kalinowski, 2005). We assessed population structure with *F*-statistics computed in GenAlEx, including pairwise standardized measures (G_{st}), which are better suited for hypervariable markers such as microsatellite loci than F_{st} indices (Hedrick, 2005), using 1000 permutations to estimate *P*-values.

In addition, we used Bayesian clustering in STRUCTURE v.2.3.4 (Pritchard et al., 2000) parallelized with StrAuto v1.0 (Chhatre and Emerson, 2017) to reduce running time. The optimal value of *k* was defined using the Puechmaille method (Puechmaille, 2016) calculated on the STRUCTURE SELECTOR webserver (Li and Liu, 2018), based on 20 replicates per *k*, with 2^6 burn-in steps and 2^6 additional Markov Chain Monte Carlo (MCMC) sampled generations per run. Many studies have based the choice of optimal *k* using the Evanno approach (Janes et al., 2017). However, it has been shown (Gilbert et al., 2012) that this works well only for datasets that harbour at least two genetic clusters; therefore, it does not perform well when the population shows no structure (i.e. $k = 1$). Moreover, the Puechmaille method has shown a better performance than Evanno's technique in cases of uneven sampling (Puechmaille, 2016), as is the case in the present study. The genetic clusters for the best value of *k* were visualized in geographic space through the interpolation of the admixture coefficients onto a South America raster map, using the R script provided by Jay et al. (2012), as a companion to the spatially explicit Bayesian clustering approach TESS v.2.3 (Chen et al., 2007). For this, the 20 replicate runs of STRUCTURE generated with the optimal *k* value were merged with CLUMMP v.1.1.2 (Jakobsson and Rosenberg, 2007) using the greedy algorithm and 10,000 repeat configurations, in order to generate a single admixture matrix (*Q*-matrix) as input for TESS. Finally, we also ran STRUCTURE with the LOCPRIOR option, using the putative population origin of each sample as a prior (Supporting information Appendix S1). Isolation by distance (IBD) patterns were assessed within and among biomes using individual-based pairwise Mantel tests (Mantel, 1967), comparing genotypic (proportion of shared alleles) and geographic matrices with the distance-based module and the correlogram module in GenAIEx.

2.5. Effective population size and contemporary bottlenecks

We estimated the contemporary effective population size (N_e) for each of the inferred populations using the programs SPEED-Ne v.2.3 (Hamilton et al., 2018), NeESTIMATOR v.2.1 (Do et al., 2014), and LdNe v.1.31 (Waples and Do, 2008), incorporating two values for the minor allele frequency (MAF, 0 and 0.01), and discarding seven closely related individuals detected by the relatedness analysis (Supporting information Appendix S1). Finally, we searched for signals of drastic contemporary population reductions with the software BOTTLENECK v.1.2.02 (Piry et al., 1999).

3. Results

3.1. Dataset features

For the joint data set, using the 0.69 threshold of genotyped loci, we discarded three individuals that did not meet this criterion. After checking for exact duplicate genotypes, one additional individual was removed from the Amazon dataset (likely deriving from tube mislabelling during sample collection or processing), as well as another one showing an excess of homozygous genotypes. Two out the 13 loci showed > 20% of missing genotypes: F124 ($n = 48$; 24.7% missing) and FCA741 ($n = 41.5$; 21.4% missing), and we removed them from further analyses, for a final dataset of 190 individuals reliably genotyped at 11 loci. Before estimating effective population size, we removed seven individuals from the Amazon dataset that potentially could downwardly bias the estimates, which were part of two parent-offspring pairs, three full-sibling pairs, and one full-sibling triplet (Supporting information Appendix S1).

The Amazon population showed no significant deviations from HWE ($P > 0.05$), except for the loci FCA740, FCA391 and F98, which presented a heterozygote deficit. For the Atlantic Forest dataset, two loci (FCA723, FCA441) showed signs of a heterozygote deficit. The linkage disequilibrium test did not detect any significant non-random associations between pairwise locus comparisons. Since there was no consistent trend of the same loci showing departures from equilibrium, and to maximize information content, we kept the full dataset for all the analyses described below.

3.2. Genetic diversity

Overall, Amazon jaguars showed considerably high levels of genetic variability across most of the loci (Table 1), with most of the estimates being higher than those of the Atlantic Forest and the Pantanal (Table 2). Confidence intervals for the estimates of allelic richness (*Ar*) per locus did not overlap among the three biomes, indicating significantly higher diversity in the Amazon than in the Atlantic Forest, which was significantly more diverse than the Pantanal. Expected heterozygosity followed the same pattern, but observed heterozygosity showed the opposite trend, with lower values in the Amazon (Table 2). Total and private alleles ranged from 10.2 and 2.7 for the Amazon, to 6.5 and 0.40 for the Pantanal (Table 2).

3.3. Population structure

F-statistics among major populations were quite low, with F_{st} values ranging from 0.037 to 0.052, although their confidence intervals did not overlap zero, indicating modest but significant differentiation among biomes (Table 3). G_{st} values were higher, and followed the same trend, indicating that the highest levels of differentiation were observed between the Atlantic Forest and the Pantanal, and the lowest ones between the Pantanal and the Amazon.

Differences in allelic frequencies determined with STRUCTURE led to the identification of four major population clusters, one corresponding to the Amazon, the second one representing the Pantanal, and the third and fourth ones dividing the Atlantic Forest into two spatial domains (Fig. 2). One of them grouped the Green Corridor (the southern block of the Upper Parana Atlantic Forest [UPAF]) with the coastal Vale population, on opposite sides of the surveyed region, while the other group assembled individuals from a central area, comprising the small fragments of the northern block on the UPAF (Porto Primavera, Ivinhema and Morro do Diabo).

Extensive admixture was observed among the three biomes, and the Amazon cluster included a few individuals with a large proportion of

Table 1
Genetic diversity at 11 loci microsatellite in three populations of jaguars in South America. N number of individuals genotyped, A number of alleles, A_r allelic richness, N_p private alleles, H_e expected heterozygosity, H_o observed heterozygosity.

	Amazon (n = 71) (This study)						Atlantic Forest (n = 68) (Haag et al., 2010; Srbek-Araujo et al., 2018)						Pantanal (n = 51) (Valdez et al., 2015)					
Locus	N	A	A_r	N_p	H_e	H_o	N	A	A_r	N_p	H_e	H_o	N	A	A_r	N_p	H_e	H_o
FCA742	67	26	23.5	8.6	0.93	0.78	65	14	14.3	0.5	0.88	0.83	50	13	12.8	0.1	0.86	0.90
FCA723	66	9	7.8	2.1	0.63	0.52	66	7	6.7	1.0	0.67	0.48	48	7	6.9	1.9	0.63	0.67
FCA740	68	6	5.7	0.7	0.77	0.72	64	5	4.7	0	0.71	0.72	50	5	5.0	0	0.69	0.58
FCA441	69	8	7.9	1.9	0.80	0.75	68	6	6.0	0	0.74	0.59	48	7	6.9	1.0	0.62	0.52
FCA391	66	8	7.9	0	0.85	0.79	66	8	7.9	0.1	0.76	0.80	51	6	6.0	0	0.76	0.86
F98	71	5	4.6	1.6	0.80	0.69	67	4	4.0	1.0	0.55	0.60	50	3	3.0	0	0.66	0.66
F53	64	16	14.6	4.4	0.86	0.70	66	12	11.4	1.1	0.85	0.86	48	6	6.0	0	0.77	0.79
F146	62	8	7.2	2.7	0.42	0.35	68	5	4.7	0	0.59	0.54	48	3	3.0	0	0.30	0.23
F85	65	13	12.3	2.8	0.78	0.63	62	12	11.8	2.4	0.80	0.77	48	9	8.9	1.0	0.78	0.82
F42	65	13	12.4	2.5	0.88	0.72	58	9	8.9	0	0.77	0.69	48	7	7.9	0	0.84	0.85
FCA453	62	9	8.5	2.7	0.73	0.65	62	6	5.7	0.7	0.71	0.63	46	6	6.0	0	0.76	0.80

ancestry coming from the Pantanal and the Atlantic Forest (Fig. 2a). In the next hierarchical level of structure, neither the Amazon nor the Pantanal showed further subdivision (k = 1 each), whereas the Atlantic forest showed a marked structure into five genetic clusters (Supporting information Appendix S1).

3.4. Isolation by distance

We did not find significant patterns of isolation by distance (IBD) within and among the biomes (Fig. 3). However, Mantel tests of the proportion of shared alleles vs. geographic distance indicated a slight inverse relationship for all the biomes except the Amazon. This pattern was clearer for the Atlantic Forest (Spearman $R = -0.475$; Fig. 3c), followed by the three biomes assessed jointly ($R = -0.222$; Fig. 3d) and the Pantanal by itself ($R = -0.178$, Fig. 3b). The R-value for the Amazon was nearly null ($R = 0.034$, Fig. 3a), although the spatial correlogram indicated that this small signal of IBD derives from a negative correlation between genetic similarity and geographic distance observed up to a distance of 400 km (Fig. 4). Within this range, the negative correlation is significantly different from the null expectation (of no correlation) up to a distance of 150 km between sampling points.

3.5. Effective population size and bottlenecks

Estimates of contemporary effective population size based on linkage disequilibrium were lowest for the Atlantic Forest and highest for the Amazon, ranging from 20 to 887 individuals, respectively (Table 4). Using these figures and assuming that N_e represents on average ca. one-tenth of the census size (N_c) for a given population (Frankham, 1995), we estimate that N_c point estimates range from 1152 to 8877 individuals in the Amazon; 499 to 812 in the southern Pantanal, and 169 to 262 in the Atlantic Forest (Table 4). We did not detect signals of recent bottlenecks for the Amazon and Pantanal populations. However, when we performed the analysis on the four separate clusters of the Atlantic Forest, the Morro do Diabo and Ivinhema demes appeared bottlenecked.

Table 3
Fixation indices reflecting jaguar population differentiation in three South American biomes. Values above the diagonal are F_{st} *Nei*, with G_{st} *Hed* in parentheses; values below the diagonal are 95% confidence intervals for F_{st}.

Population	Amazon	Pantanal	Atlantic Forest
Amazon	–	0.037 (0.124)	0.041 (0.149)
Pantanal	0.025–0.055	–	0.052 (0.164)
Atlantic Forest	0.032–0.064	0.045–0.097	–

4. Discussion

4.1. General patterns

Genetic diversity studies constitute a pillar in the field of conservation biology, although their practical application has often not been fully achieved so far (de la Torre et al., 2018; Hoban et al., 2013; Rivers et al., 2014). As a contribution to fill this gap, we analysed the most broadly distributed set of genetic samples for Amazonian jaguars surveyed to date, and directly compared it with two other biomes, potentially serving as a baseline for the assessment of jaguar population genetics across the species' range. As a result, we highlight the following features. The Amazonian jaguar population showed (1) moderate to high levels of microsatellite diversity, for example as assessed by allelic richness; (2) large-scale connectivity with signals of panmixia across thousands of kilometres, both south, north and across the Amazon River; (3) relatively large effective population size (but see caveats below), with no signals of recent bottlenecks. The Pantanal population displayed (4) lower genetic diversity but a relatively large effective population size derived from only a small surveyed portion of the available habitat in that area (see discussion below). In contrast, for the Atlantic Forest population we corroborated previous findings identifying (5) intermediate levels of diversity, with a marked structure

Table 2
Summary of population genetic parameters for Amazon, Atlantic Forest, and Pantanal jaguar populations, based on 11 autosomal microsatellite loci. Number of genotyped individuals (N), mean number of observed alleles per loci (N_a), mean number of effective alleles per locus (N_f), mean number of private alleles per loci (N_p), allelic richness (A_r), rarefied allelic richness (A_f), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{is}), confidence interval 95% (*CI*), standard error (*SE*).

Population	N	Na	Nf	Np	Ar	(*CI*)	Af[a]	H_o (*SE*)	H_e (*SE*)	F_{is}	(*CI*)
Amazon	71	11	5.64	3.27	9.38	(8.55–10.18)	10.22	0.674 (0.041)	0.759 (0.043)	0.11	(0.062–0.142)
Atlantic Forest	68	8	4.26	0.55	7.75	(7.27–8.18)	7.82	0.684 (0.038)	0.730 (0.030)	0.06	(0.002–0.103)
Pantanal	51	6.5	3.98	0.36	6.36	(5.91–6.73)	6.58	0.698 (0.060)	0.698 (0.047)	0.02	(−0.044–0.054)

[a] Allelic richness rarefied to 92 gene copies (N = 46).

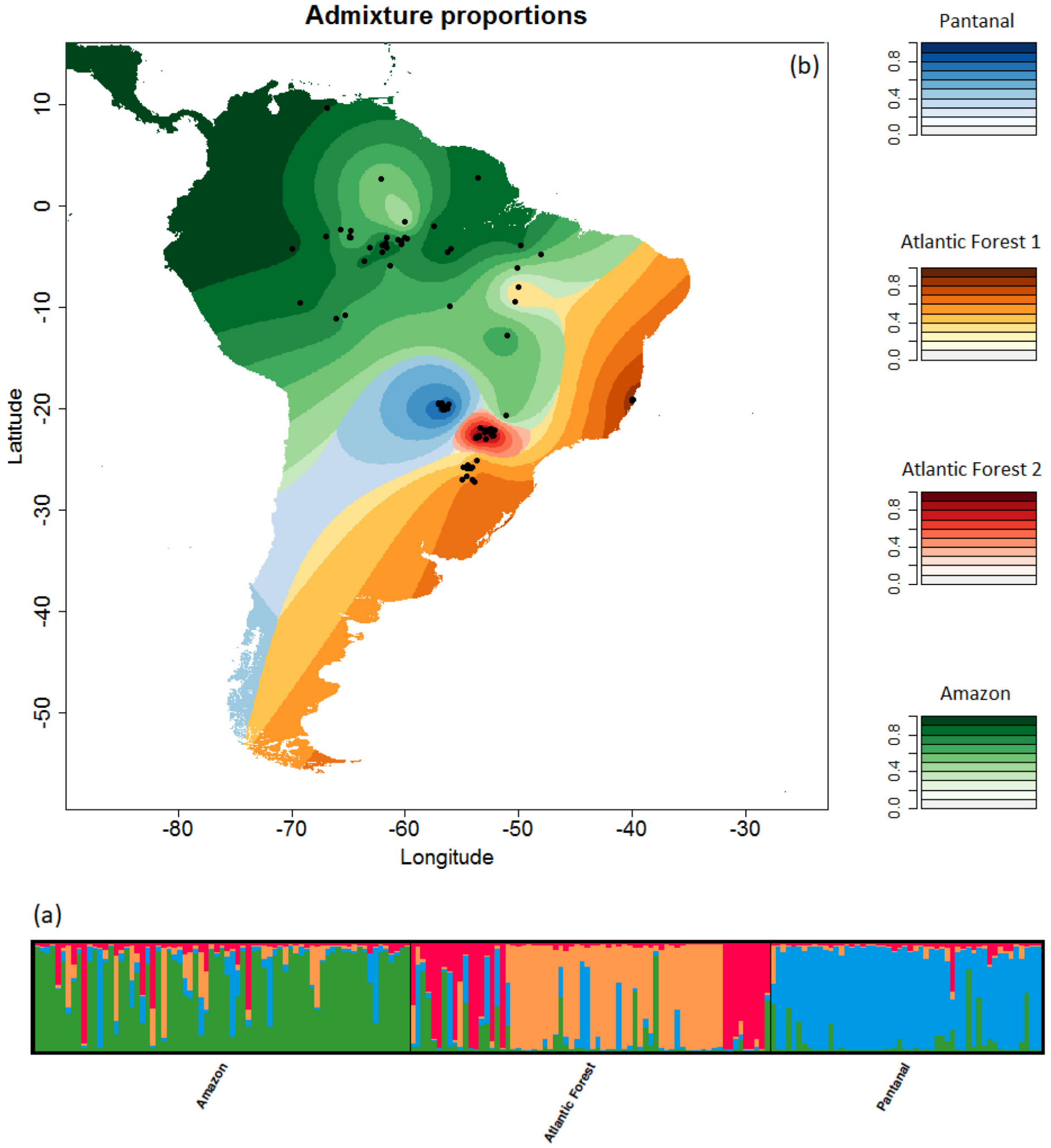

Fig. 2. Jaguar population structure in three South American biomes. The inference of genetic clusters (*k*) was based on the Puechmaille method using correlated allele frequencies. (a) Vertical bars represent each individual jaguar, and the colour of the bar shows the percentage of membership (*Q*) to the distinct clusters. (b) Spatially-explicit interpolation of admixture coefficients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

due to strong signals of anthropic-driven drift, and recent bottlenecks in two of their demes.

The high diversity and long-distance connectivity observed in the Amazon highlight the importance of this region as the most extensive stronghold for this species. It is noteworthy that genetic variability comparisons with the Pantanal population are constrained by the relatively restricted geographic area surveyed by Valdez et al. (2015), but in the case of the Atlantic Forest, our comparison was useful to confirm the effects of habitat loss and fragmentation in that biome (Haag et al., 2010; Srbek-Araujo et al., 2018). Currently, the lack of genetic studies on jaguars using historical samples from museum collections, such as those performed in other big cat species (e.g. Dures et al., 2019; Mondol et al., 2013), justifies the use of the Amazon population as a baseline. The rationale for this approach is that the Amazon region may represent a proxy for historic genetic variation in this species, assuming that it is likely to still retain most its original levels of diversity, in contrast to other areas which have undergone large-scale habitat degradation.

Accordingly, the level of genetic differentiation among jaguar populations sampled in these three biomes supports the view that this species has historically attained high levels of gene flow on a broad geographic scale (Eizirik et al., 2001). This pattern can be explained by the high dispersal potential of jaguars, favoured by high quality, continuous habitat, which in turn allowed gene flow across the Neotropics. These inferred high levels of connectivity contrast with observations of stronger population differentiation based on mtDNA markers (e.g. Eizirik et al., 2001), and support the view that this species exhibits a

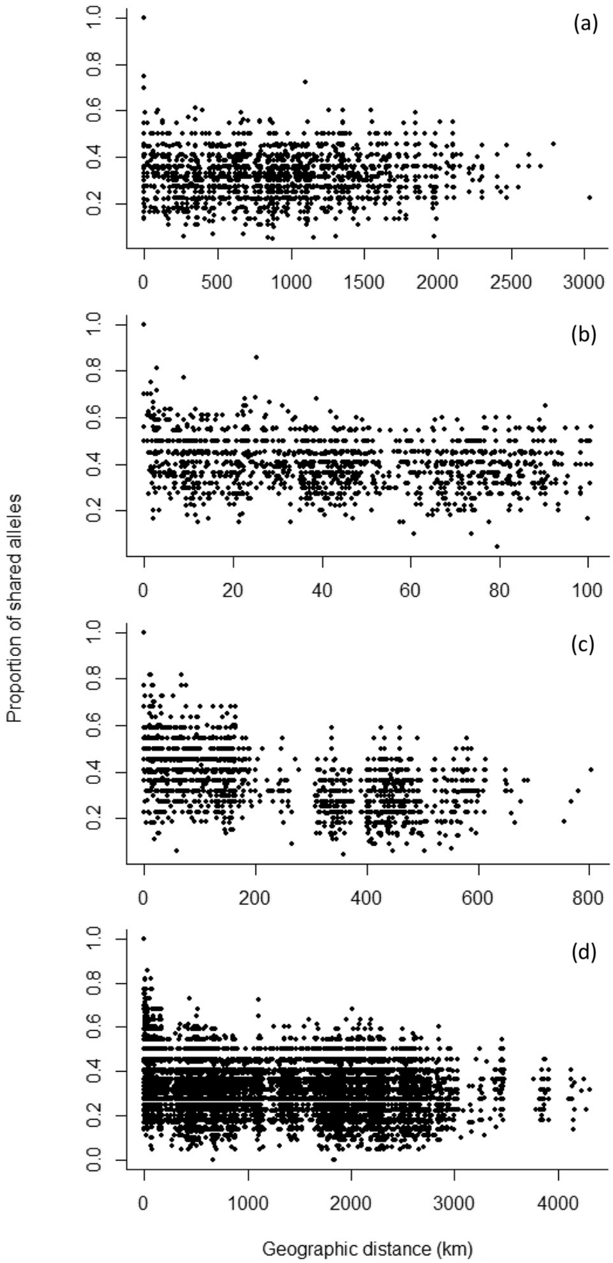

Fig. 3. Regional isolation-by-distance patterns in South American jaguars. Isolation-by-distance was assessed by plotting the pairwise proportion of shared alleles calculated in GenAlEx, versus pairwise Euclidean distances (km) across the (a) Amazon, (b) Pantanal, (c) Atlantic Forest and (d) the three populations altogether.

male-biased dispersal pattern, as has been described for other big cats (e.g. Fattebert et al., 2015; Gour et al., 2013; Smith, 1993). Similar instances of higher variability and less structured populations towards the centre of the species range had been documented elsewhere for jaguars (Roques et al., 2016) and other large mammalian carnivores such as tigers in Nepal (Thapa et al., 2018), leopards in South Africa (McManus et al., 2015), black bears in Florida (Dixon et al., 2007), and wolverines in Montana (Cegelski et al., 2003). In all of these instances, habitat fragmentation was the underlying factor causing differentiation at peripheral populations.

4.2. High diversity in the Amazon and genetic drift in the Atlantic Forest

With the sole exceptions of observed heterozygosity (H_o) and inbreeding coefficient (F_{is}), summary statistics indicated that the Amazon rainforest sustains one of the most diverse jaguar populations in South America, as inferred from its comparison to the Atlantic Forest and the Pantanal populations (Table 2). It is expected that this pattern holds range-wide since previous studies have shown lower variability levels in other peripheral biomes not assessed in this study, such as the Caatinga in Brazil, Mesoamerican forests, and subtropical Mexico (Roques et al., 2016; Wultsch et al., 2016a, 2016b). This assertion is supported by the levels of diversity reported by Roques et al. (2016) for the Amazon [H_e (0.805) and H_o (0.848)], which were higher than those of other populations, except for the central-range Cerrado biome. Gene diversity level was similar to the value documented in this study [H_e(0.76)], although direct comparisons are hampered by the fact that different loci were employed in each assessment. Likewise, our diversity estimates are higher than those reported for the tropical rainforest in Belize ($H_e = 0.57$; $H_o = 0.57$) by Wultsch et al. (2016b), but again the set of loci is different, precluding a more direct comparison. The higher F_{is} and lower H_o values are the result of several closely related individuals, consistent in two parent-offspring dyads, three full-sibling dyads, and one full-sibling triad detected in the Amazon population (Supporting information Appendix S1).

An interesting observation was that the Atlantic forest as a whole still retain genetic diversity levels similar to those in the Pantanal, but the most isolated subpopulation (Morro do Diabo) showed even lower values ($H_o = 0.55$; $H_e = 0.50$; Haag et al., 2010) than those documented for Belize. It is remarkable that the heavily fragmented Atlantic Forest demes retain, altogether, rather high levels of diversity, likely representing a large portion of their historic variability share. However, jaguars in this highly fragmented region are under a metapopulational dynamic, where each remaining population is subject to genetic stochastic effects (Dixon et al., 2007), losing its variability by drift and even being at risk of local extirpation (Jędrzejewski et al., 2017; Thatte et al., 2018).

4.3. High connectivity in the Amazon

All the metrics were consistent in showing large-scale demographic connectivity encompassing thousands of kilometres across the Amazon basin. As a result, we infer that the lack of population subdivision in this vast region implies far-reaching amounts of gene flow throughout the landscape. A significant signal of IBD was detected from 0 to 150 km, and this pattern is expected as the individuals are more closely related in shorter distances, with genetic relatedness gradually fading away (Zanin et al., 2016). The extent of the genetic neighbourhood, where genetic correlation is negatively associated with distance, was estimated to lie between 300 and 400 km (Fig. 4). This seems biologically reasonable in terms of the high vagility and social organization of jaguars (i.e. one male overlapping the home range of three or more females), especially in a continuous, productive habitat such as the Amazon. Similar results were reported for tigers in the Sundarbans (Aziz et al., 2018).

Our results could represent one of the few possible snapshots of large-scale jaguar population connectivity before severe human intervention, illustrating the occurrence of historical panmixia throughout the tropical forested biomes across the species' range, from the Atlantic Forest in southeastern South America to the Mayan forest in Mesoamerica. Local discontinuities may occur in areas such as the Pantanal, perhaps driven by adaptive differentiation in ecological and/or behavioural traits (Figueiró et al., unpublished), but much of the interruption of long-range gene flow observed in recent studies is likely to have been exacerbated by human-driven drift. In this sense, Wultsch et al. (2016a) found signals of interruption of panmixia in northern Central America, between the Mayan forest, which is the largest tract of Neotropical rainforest outside of the Amazon, and the Honduran population, probably due to a drastic habitat loss between these two regions.

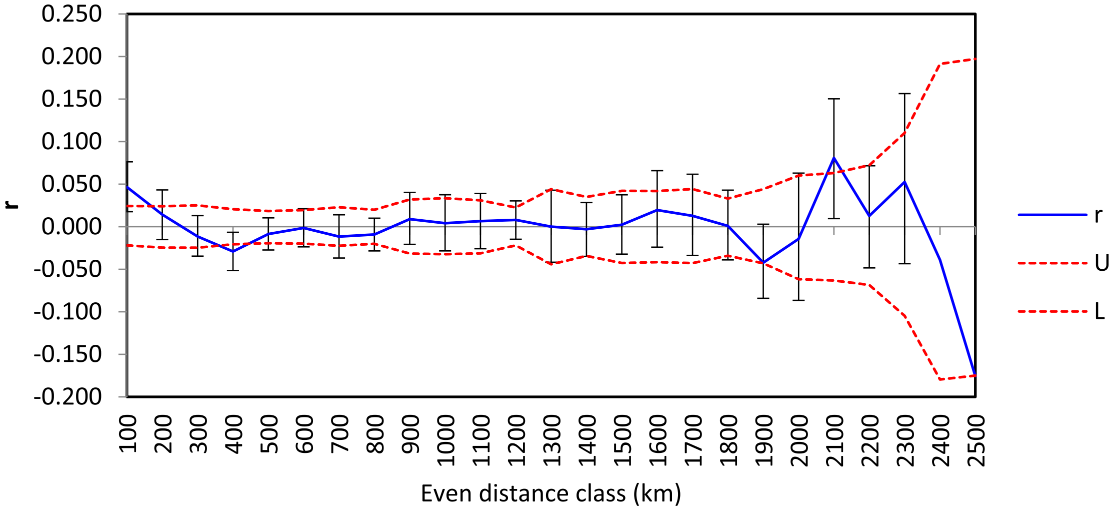

Fig. 4. Correlogram showing spatial autocorrelation for Amazon jaguars. The genetic correlation coefficient (*r*) is plotted as a function of geographic distance across defined spatial distance classes (100 km). Dashed red lines represent upper (*U*) and lower (*L*) bounds of the null hypothesis of no spatial structure based on 10,000 random permutations. Error bars represent 95% confidence intervals for *r* estimates, based on 1000 bootstrap replications. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In this context, it is extremely important to maintain the connectivity in the Amazon, as large-scale deforestation is advancing in the southern limits of the biome. Projections indicate that by 2050, the Amazon will lose 40% of its area, and the protected areas network will not be sufficient to fully protect its biodiversity (Soares-Filho et al., 2006), as deforestation, poaching, and illegal fishing and mining continue as the main threats (Kauano et al., 2017). Reversing this trend and maintaining large-scale connectivity across this biome will be critical not only for jaguars (Silveira et al., 2014) but also for many other components of Amazonian biodiversity (Lees and Peres, 2008).

In spite of their high vagility, jaguars may be more vulnerable than other species to human-induced fragmentation. For example, Figueiredo et al. (2015) identified no genetic structure between ocelots (*Leopardus pardalis*) sampled at Morro do Diabo and the Green Corridor, contrasting with the pattern observed in jaguars, suggesting that the latter are more sensitive to genetic erosion driven by anthropic disturbance. A likely explanation is that ocelots possess larger effective population sizes (due to smaller body size and higher density) in the same area, thus taking longer to show the effects of genetic drift. An additional possibility is that ocelots are more capable of navigating through the human-dominated matrix (Zimbres et al., 2018), maintaining gene flow across fragments in a way that jaguars no longer can. A similar trend was reported for ocelots, pumas, and jaguars in Belize (Wultsch et al., 2016b), as ecological and behavioural differences among these species could determine the potential and effective amounts of gene flow among populations. However, this pattern also seems to be dependent on the time elapsed since habitat perturbation and its intensity, as well as habitat productivity. Ocelots occurring in southern Texas, on the northern limit of their range, where two subpopulations occur in small blocks of semiarid habitat isolated from each other by approximately 30 km of cropland matrix, show small N_e (< 14) and high differentiation ($F_{st} = 0.163$; Janečka et al., 2011). In general, top predators are very sensitive to habitat perturbation (Dutta et al., 2012), but this sensitivity can be attenuated by differences in ecosystem productivity (Jędrzejewski et al., 2017) and their natural recolonization capability (Malaney et al., 2018).

Table 4

Contemporary effective population size (N_e) of jaguars estimated for three South American biomes (AM: Amazon; PA: Pantanal; AF: Atlantic Forest) based on linkage disequilibrium at 11 microsatellite loci, and extrapolated census size (N_c) based on the N_e point estimates.

Population	Estimates		NeEstimator[a]		LDNe[b]			SPEED-NE[c]		N_c[d]	
(Sample size)			MAF 0+	MAF 0.01	MAF 0+	MAF 0.01		MAF 0+	MAF 0.01	(Range)	
AM	Point estimate	N_e	115.2	724.4	119.2	887.7		278.9	278	1152	8877
($n = 64$)	95% CIs	(1)	87.3–164.1	241.4-inf	89.6–172.2	257.4-inf	(3)	116.4-inf	116.3-inf		
		(2)	43.2-inf	133.9-inf	62.2–499.3	183.6-inf	(2)	192.9–503.8	192.4–500.8		
PA	Point estimate	N_e	79.8	79.8	81.2	81.2		49.9	50.5	499	812
($n = 51$)	95% CIs	(1)	53.3–141.4	53.3–141.4	54.0–145.6	54.0–145.6	(3)	43.3–58.9	43.8–59.6		
		(2)	41.8–291.4	41.8–291.4	48.4–189.0	48.4–189.0	(2)	44.1–57.5	44.5–58.3		
AF	Point estimate	N_e	26.1	20.4	26.2	20.5		16.9	16.9	169	262
($n = 68$)	95% CIs	(1)	22.6–30.3	17.8–23.5	22.7–30.4	17.8–23.6	(3)	14.3–20.73	14.3–20.73		
		(2)	17.2–41.6	13.8–30.9	22.7–30.4	17.9–23.5	(2)	15.8–18.2	15.8–18.2		

(1) - Parametric.
(2) - Jackknife on samples (individuals).
(3) - Jackknife on loci.
MAF - Minor allele frequency.
Inf - Infinity.

[a] NeEstimator v2.1 (Do et al., 2014).

[b] LDNe v.1.31 (Waples and Do, 2008).

[c] SPEED-NE v.2.3 (Hamilton et al., 2018).

[d] Extrapolated census size N_c, where N_e represents one-tenth of N_c.

This situation also raises the question about restoring connectivity of landscapes subjected to heavy anthropic perturbation, such as the Atlantic Forest (Ribeiro et al., 2009; Silveira et al., 2014), and constitutes a warning about the negative effects of fragmentation that could occur in less disturbed regions such as the Pantanal, whose extent is much smaller than that of the Amazon. For example, the coastal Vale population still retains some of the shared diversity present in the inner portion of the Atlantic Forest (i.e. Green Corridor), likely represented by ancestral alleles. However, its size and degree of isolation make it almost impossible to maintain gene flow with other coastal subpopulations persisting in that biome, such as that described by Souza et al. (2017), or in nearby biomes such as the Cerrado and Caatinga. This strengthens the previous evidence indicating that the marked change in allele frequencies in the inland populations of that biome has caused a genetic differentiation that reflects its contemporary, fast degradation (Haag et al., 2010; Valdez et al., 2015). In fact, the magnitude of the deforestation rates in the Atlantic Forest implies that very few jaguar demes are left in that biome (Paviolo et al., 2016) other than those surveyed by Haag et al. (2010), Souza et al. (2017), and Srbek-Araujo et al. (2018), and has already propitiated defaunation and cascade effects across the region (Jorge et al., 2013). Thus, management actions such as restoring connectivity through riparian and mountainous corridors are urgently needed (Castilho et al., 2015), using spatially-explicit approaches on gene flow (Reddy et al., 2017).

4.4. Effective population size

N_e bears major relevance for conservation biology and thus deserves careful examination and cross-validation before being applied in any practical context. We recognize that its estimation requires the fulfilment of several assumptions, namely closed populations, loci sampled at random, no population subdivision and non-overlapping generations (Luikart et al., 2010). We consider that our analyses have complied with these assumptions except for non-overlapping generations and population closure, as we were constrained by a relatively small overall sample over a huge area, which is typical for an elusive, wide-ranging carnivore such as the jaguar. In addition, our sample was slightly skewed towards males (see Table S1), which could potentially influence N_e estimates. Hence, we highlight the following caveats. On the one hand, given the vast extension of the Amazon rainforest and the fact that our sampling was still sparse in some areas, it is possible that the point estimate for the effective population size we calculated for this region represents an underestimate. This issue has been identified when calculating N_e using linkage disequilibrium estimators (Wang, 2005; Waples and Do, 2010), as this method has a better performance when population size is small, as is the case for the Atlantic Forest in our assessment. Also, our inference that the Amazon population approaches panmixia indicates that our sample may be sufficiently representative of the biome as a whole to allow an inference of its overall effective size. On the other hand, we stress that, for management purposes, caution is needed to avoid over-optimism based on our estimates, which exhibited a large variance. Such over-optimism could hamper ongoing and future conservation actions, and thus we advocate the use of Ne and Nc (census size) figures on the lower end of our estimates (or more specifically, the SPEED-NE approximation) for the Amazon and the Pantanal populations. In that way, one would reduce the risks associated with potential biases induced by factors such as panmixia violations, skewed sex ratios, or initial DNA sample quality, which could cause estimates being higher than the true N_e (Luikart et al., 2010). Further studies using high throughput sequencing techniques and hundreds or thousands of genomic markers (SNPs) in a large set of individuals per population should help improve the precision and robustness of such estimates.

It is interesting to note that estimates of Amazonian jaguar census size based on other lines of evidence, such as hunting records (e.g. Antunes et al., 2016), may suggest the existence of a larger population in this region, relative to our extrapolation based on the effective size. One possible factor leading to this potential difference is the fact the effective size is very sensitive to demographic fluctuations over time (Frankham, 1995), and strongly influenced by previous periods of low population numbers. Additional research using complementary approaches will be required to reconcile these different estimates and lead to an integrated assessment of jaguar population size in the region.

In any event, our current estimate demonstrates that the Amazon sustains a much larger effective population than the other two assessed biomes, at least twice the size of the southern Pantanal's and almost eight times larger than the Atlantic Forest's. Such differences are driven by the available area in each biome, along with historical factors (such as past demographic fluctuations) and current anthropogenic disturbances. Despite the massive expanse of the Amazon basin, it is currently losing primary cover, which can lead to jaguar demographic reductions and local extirpations as the agrarian frontier continues encroaching on the rainforest. As for the Pantanal and the Atlantic Forest biomes, primary habitat currently extends over roughly equivalent areas (~100,000 km^2), although in the former case it constitutes a single, continuous block, while in the latter the remaining area is fragmented into thousands of small patches. Up to 80% of those patches are smaller than half a square kilometre (Ribeiro et al., 2009), which is too small to sustain even a single jaguar individual, partially explaining the very low N_e (17–26) estimates for that biome. Indeed, those figures put Atlantic Forest jaguars (even when treating the biome as a single unit) below the $N_e = 50$ threshold proposed by Franklin and Frankham (1998) to avoid short-term risks due to inbreeding (Rutledge et al., 2017). Given the evidence for strong human-induced isolation among remnant Atlantic Forest populations, local effective sizes are actually much lower (Haag et al., 2010; Srbek-Araujo et al., 2018).

4.5. Concluding remarks

On the basis of the results presented here, and the need to further refine these inferences, we recommend that continuous molecular surveys (including genome-wide approaches) be performed throughout the jaguar range, addressing demographic as well as adaptive questions, and providing updated information on the genetic health of natural populations. This would help to rapidly detect changes that can further compromise the persistence of jaguars throughout their distribution, enabling improved management actions in the context of long-term conservation strategies that integrate multiple spatial scales.

We conclude by stressing the importance of maintaining connectivity regionally and across the species' range to ensure that gene flow persists within and across biomes, including those that still represent large strongholds for the species, such as the Amazon rainforest. To achieve this goal, it is critical to monitor the loss of genetic diversity driven by human-induced fragmentation and population isolation and to actively restore gene flow in some cases. In this context, it is noteworthy that rampant habitat loss is currently taking place in the southeastern Amazon across the “arc of deforestation”, which represents an imminent threat (or perhaps already a reality) of gene flow interruption with adjacent biomes such as the Cerrado, Caatinga and Pantanal. Recent trends of increased deforestation and weakened enforcement of environmental protection in this region are alarming, and have been the focus of intense concern by the scientific community (e.g. Abessa et al., 2019; Kehoe et al., 2019). The situation in the Atlantic Forest is even more worrisome, as evidence has accumulated demonstrating that drastic habitat fragmentation takes only a few decades to induce negative effects (both genetic and demographic) on wildlife species such as jaguars. Urgent action is needed to avoid that the Amazon rainforest follows a similar path of environmental degradation in the next few decades, which could lead to disastrous effects on a global scale.

Data accessibility statement

Part of the data used in this paper has been published previously (Haag et al., 2010; Valdez et al., 2015; Srbek-Araujo et al., 2018). Two of these previous data sets have been deposited in the Dryad digital repository (https://doi.org/10.5061/dryad.1884/1; https://doi.org/10.5061/dryad.371c6). The third previous data set (Srbek-Araujo et al., 2018) and the novel data reported here have been deposited in the Mendeley Data Repository (DOI: doi:10.17632/gdf5fj7f2s.1; DOI: 10.17632/nwdm9f9rrj.2).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2020.108417.

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