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Jaguars from the Brazilian Pantanal: Low genetic structure, male-biased dispersal, and implications for long-term conservation

Daniel Luis Zanella Kantek [a,b], Cristine Silveira Trinca [b], Fernando Tortato [c], Allison Loretta Devlin [c,d,e], Fernando Cesar Cascelli de Azevedo [f,g], Sandra Cavalcanti [g], Leandro Silveira [h], Selma Samiko Miyazaki [a], Peter Gransden Crawshaw Junior [i,1], Joares Adenilson May-Junior [c,g,j,k], Carlos Eduardo Fragoso [j], Leonardo Rodrigues Sartorello [j], Lilian Elaine Rampim [j], Mario B. Haberfeld [j], Gediendson Ribeiro de Araujo [l], Ronaldo Gonçalves Morato [i], Eduardo Eizirik [b,g,m,*]

[a] Estação Ecológica de Taiamã, Instituto Chico Mendes de Conservação da Biodiversidade, Cáceres, Mato Grosso, Brazil
[b] Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Escola de Ciências da Saúde e da Vida, Laboratório de Biologia Genômica e Molecular, Porto Alegre, Rio Grande do Sul, Brazil
[c] Panthera, New York, NY, USA
[d] SUNY College of Environmental Science and Forestry, Syracuse, NY, USA
[e] Wildlife Biology Program, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, MT, USA
[f] Universidade Federal de São João del Rei, São João del Rei, Minas Gerais, Brazil
[g] Instituto Pró-Carnívoros, Atibaia, São Paulo, Brazil
[h] Instituto Onça-Pintada, Mineiros, Goiás, Brazil
[i] Centro Nacional de Pesquisa e Conservação de Mamíferos Carnívoros, Instituto Chico Mendes de Conservação da Biodiversidade, Atibaia, São Paulo, Brazil
[j] Associação Onçafari, São Paulo, São Paulo, Brazil
[k] Universidade do Sul de Santa Catarina (Unisul), Tubarão, Santa Catarina, Brazil
[l] Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil
[m] INCT-EECBio, Brazil

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ABSTRACT

Habitat loss and fragmentation are important threats to carnivores worldwide and are especially intense for large predators. Jaguars have been extirpated from over half of their original distribution, and few regions still maintain large populations. The Pantanal is among the best examples of such regions and can be used to better understand several aspects of jaguar biology that are relevant for conservation planning throughout the species' range. Thus, in this study we used microsatellite markers and field data to: (i) assess the genetic structure and gene flow of jaguars ($n = 110$) from the northern and southern Pantanal; (ii) verify if females are more philopatric than males; (iii) produce a timeline pedigree to allow the identification of distances from offspring to their mothers; and (iv) estimate the generation time for jaguars. Our results are consistent with the hypothesis that Pantanal jaguars represent a panmictic population, although exhibiting some degree of local differentiation. The Paraguay River seems to be an important factor promoting gene flow among the studied populations, highlighting its relevance for regional conservation efforts. Our data provide the first genetic evidence of female philopatry and male-biased dispersal in jaguars. In addition, we report the first timeline pedigree for a wild jaguar population and the first direct estimate of the species' generation time. Our results contribute to the construction of more realistic assessments of jaguar population dynamics and long-term genetic viability, thus contributing to the design of improved conservation strategies on behalf of this species.

* Corresponding author at: Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Escola de Ciências da Saúde e da Vida, Laboratório de Biologia Genômica e Molecular, Porto Alegre, Rio Grande do Sul, Brazil.
E-mail address: eduardo.eizirik@pucrs.br (E. Eizirik).
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1. Introduction

The conservation of large carnivores in human-dominated landscapes poses complex challenges, and require the integration of multiple approaches. A critical aspect of conservation planning for these species is the estimation of long-term projections of population trends, which in turn should incorporate realistic information on their biology and ecology (e.g. Creel et al., 2019). This includes information on population structure and dispersal patterns, which are important to include in population models to assess long-term viability and demographic connectivity over different spatial scales (e.g. Carroll et al., 2001; Creel et al., 2019). With respect to the latter, male-biased dispersal is considered common in solitary carnivores (Waser and Jones, 1983), but has not yet been directly assessed in most species. The ultimate cause of sex-biased dispersal has been proposed to be inbreeding avoidance or, in polygynous species, as a means to reduce competition for mates (local mate competition hypothesis) (Perrin and Mazalov, 2000). Sex-biased dispersal may also be a mechanism to reduce competition for resources among females (resource competition hypothesis), since females benefit from familiarity with resources within or near their natal territory, and can thus provide better parental care (Lawson Handley and Perrin, 2007). Differences in dispersal between sexes affect the spatial patterns of relatedness at the population level, and philopatric females usually present higher kinship among themselves than male dispersers (Clutton-Brock and Lukas, 2012). A necessary first step to investigate these hypotheses is to assess and compare kinship and dispersal patterns for both males and females, for each carnivoran species, allowing the incorporation of these features in more realistic population models.

The jaguar (*Panthera onca*) is a good example of such problems, as a top predator that depends on large home ranges with good-quality habitat and abundant prey (Medellín et al., 2002). Jaguars have already been extirpated from over half of their original distribution, which historically extended from the southwestern United States to south-central Argentina; most remaining populations are currently pressured by habitat loss and fragmentation, along with other human-induced threats (de la Torre et al., 2018). In this context, the Pantanal wetland is a critical biome for jaguar conservation, since it harbors the second largest population in the world and comprises one of the largest Jaguar Conservation Units (JCUs) (Sanderson et al., 2002; Rabinowitz and Zeller, 2010). It is therefore an important ecosystem for characterizing jaguar population biology, genetic diversity and social structure, all of which are critical components of conservation planning.

Although several studies have investigated jaguar ecology in the Brazilian Pantanal (e.g. Crawshaw and Quigley, 1991; Silveira, 2004; Soisalo and Cavalcanti, 2006; Azevedo and Murray, 2007; Cavalcanti and Gese, 2009; Azevedo and Verdade, 2012), so far only four genetic analyses have been published (Eizirik et al., 2008; Roques et al., 2014, 2016; Valdez et al., 2015) and all have focused on the southern portion of the biome. The initial studies indicated that southern Pantanal jaguars maintain considerable levels of genetic variability and connectivity, a pattern that must be further tested with expanded sampling into the northern portion of the biome. Furthermore, genetic studies performed so far on jaguars have neither directly evaluated kinship among individuals in a natural population, nor estimated pedigree relationships among wild-caught animals. Therefore, these aspects of the species' biology have not yet been assessed.

Thus, in this study we aimed to: (i) assess genetic structure and gene flow in jaguars sampled at seven distinct locales in the northern and southern Pantanal (Fig. 1); (ii) use genetic and geographic data to determine if females are more philopatric than males; (iii) produce a timeline pedigree based on molecular and spatial data, along with additional ecological information obtained in the field, to estimate the distances of male and female offspring from their mothers; and (iv) estimate the generation time for jaguars in the Pantanal. Accomplishing these four objectives should allow for a better understanding of jaguar biology, contribute to refined assessments of its conservation status, and

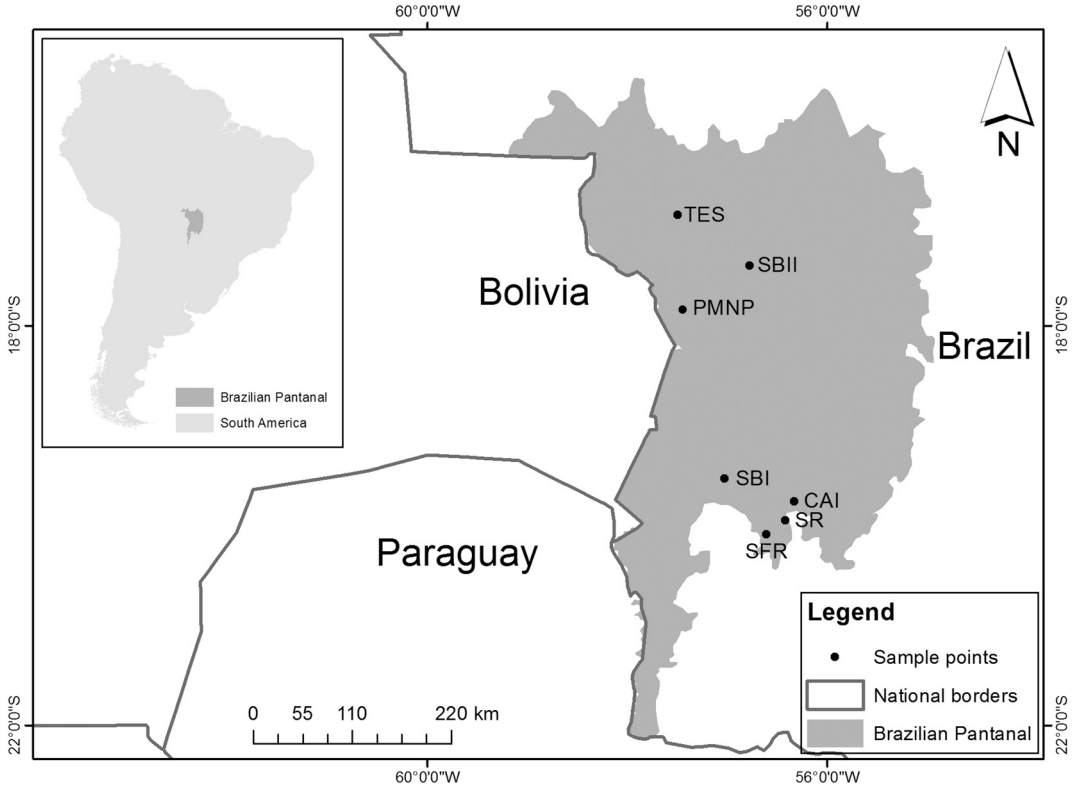

Fig. 1. Jaguar sampling locales in the Pantanal biome. The inset shows the geographic location of the Pantanal, while the main map depicts the sampling locales: northern Pantanal: TES: Taiamã Ecological Station; PMNP: Pantanal Matogrossense National Park; SBII: São Bento Ranch II; southern Pantanal: SBI: São Bento Ranch I; CAI: Caiman Ecological Refuge; SR: Sete Ranch; SFR: San Francisco Ranch. Only midpoint locations for each site are shown.

improve the design of management actions on its behalf in the Pantanal biome and across its range.

2. Materials and methods

2.1. Study area and sample collection

The Pantanal is the largest natural floodplain in the world and covers approximately 160,000 km^2 in Brazil, Paraguay, and Bolivia. The variety of vegetation and soil types makes the Pantanal one of the most diverse biomes in the Neotropics, with the Paraguay River as is its main watercourse (Nunes da Cunha et al., 2006). Despite the pressure exerted by severe retaliatory hunting due to cattle losses, as well as alteration of the original habitat, this ecosystem still harbors one of the largest remaining jaguar populations (Sanderson et al., 2002; Soisalo and Cavalcanti, 2006).

We genotyped for this study samples collected between 2008 and 2015 during field ecology and behavioral projects (Silveira, 2004; Azevedo and Murray, 2007; Cavalcanti and Gese, 2009; Azevedo and Verdade, 2012). We obtained 58 blood samples from free-ranging, captured animals inhabiting five different study sites (Fig. 1): (i) Taiamã Ecological Station [TES] (n = 19; sampled from 2011 to 2015); (ii) São Bento Ranch I [SBI] (n = 2; sampled in 2008 and 2009); (iii) São Bento Ranch II [SBII] (n = 20; from 2008 to 2014); (iv) Caiman Ecological Refuge [CAI] (n = 12; from 2012 to 2015); and (v) Pantanal Matogrossense National Park [PMNP] (n = 5; sampled in 2008, 2010 and 2011) (Supplementary Table S1).

In addition to the newly genotyped samples, our final dataset included 52 individuals previously analyzed by Valdez et al. (2015) and sampled in 4 different ranches (i.e. study sites) in the southern Pantanal: (i) San Francisco Ranch [SFR] (n = 11; sampled in 2003 in 2004); (ii) Sete Ranch [SR] (n = 10; sampled from 2001 to 2003); (iii) Caiman Ecological Refuge [CAI] (n = 21; sampled in 2003, 2005, and 2006); and (iv) São Bento Ranch I [SBI] (n = 10; sampled in 2008). The latter two sites are also represented in the newly genotyped samples, which cover different time periods relative to the previous study. Thus, the total sample (n = 110) was distributed among the study sites as follows: SFR (n = 11), SBI (n = 12), SR (n = 10), CAI (n = 33), SBII (n = 20), TES (n = 19), and PMNP (n = 5) (see Supplementary Table S1).

Our dataset encompasses the two regions within the Pantanal biome previously identified as sustaining high-density jaguar populations (Quigley and Crawshaw, 1992; Cavalcanti et al., 2012). One of them is the northern-central portion of the biome, which includes the TES, SBII, and PMNP sampling sites, while the other represents its southern portion, including sites CAI, SFR, SBI, and SR (Fig. 1). Within each of these regions, we performed detailed genetic analyses at a fine spatial scale.

2.2. Laboratory procedures

We preserved blood samples with EDTA, and in some cases subsequently mixed them with an equal volume of a salt-saturated solution (100 mM Tris, 100 mM EDTA, 2% SDS). We stored samples at −20 °C up to DNA extraction. We extracted genomic DNA using the QIAamp DNA Mini kit® (Qiagen, Hilden, Germany) according to the manufacturer's instructions, except for the final elution step, which we increased to 20 min at room temperature. We verified integrity and concentration of the extracted DNA on a 1% agarose gel stained with GelRed 10× (Freemont, California, USA). We then used each DNA sample for PCR amplifications of 11 microsatellite loci (F42, F53, F98, F124, F146, FCA391, FCA441, FCA453, FCA723, FCA740, and FCA742) originally developed for the domestic cat (*Felis silvestris catus*) (Menotti-Raymond et al., 1999, 2005), and previously optimized for amplifying jaguar DNA (Eizirik et al., 2001, 2008; Haag et al., 2010). PCR reactions followed conditions described by Haag et al. (2010). We genotyped PCR products in a 3730xl ABI DNA analyzer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and identified the resulting alleles with Peak Scanner v2.0 (Applied Biosystems, Foster City, California, USA).

To conduct the joint analysis of the seven Pantanal sites and all genotyped individuals (including those previously studied by Valdez et al. (2015)) in a single composite dataset, we selected a small subset of samples analyzed by Valdez et al. (2015) and re-genotyped them along with the new samples from the present study. This allowed us to calibrate the new genotypes relative to those previously available, resulting in a fully integrated dataset. The only locus for which this procedure yielded inconsistent results (i.e., discrepancies between the previous and current genotypes for the same animals) was FCA742, which led us to re-genotype all individuals for this marker (including all individuals reported by Valdez et al. (2015)), thus eliminating the issue.

2.3. Data analysis

2.3.1. Genetic diversity

Only individuals that were genotyped for at least eight of the 11 loci were included in the analyses. We used the software MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) to identify possible genotyping errors due to stutter peaks, as well as to assess the occurrence of null alleles and allelic dropout. We then removed loci that presented a high estimated frequency of null alleles ($nf > 0.20$) from further analyses (Chapuis and Estoup, 2007).

We tested the data for linkage disequilibrium (LD) among loci for each sample site and for departures from Hardy–Weinberg equilibrium (HWE) using GENEPOP (http://wbiomed.curtin.edu.au/genepop/). For all tests, we used 1000 dememorization steps and 300 batches with 10,000 iterations each. We adjusted significance levels ($\alpha = 0.05$) of departures from HWE or LD for multiple comparisons by applying the sequential Bonferroni correction (Rice, 1989). We calculated the number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon's information index (Sherwin et al., 2006) using GenAlEx 6.5 (Peakall and Smouse, 2012). Shannon's information index provides an alternative method of quantifying genetic diversity and incorporates allele numbers and frequencies. We also used FSTAT 2.9.3.2 (Goudet, 2002) to estimate allelic richness (AR; including correction for sample size). We computed the number of private alleles (i.e. alleles that are unique to a particular region) at each locus following a rarefaction method that compensates for uneven sample sizes, as implemented in the software HP-Rare (Kalinowski, 2005).

2.3.2. Population structure

To assess how genetic diversity was partitioned within and among sampling sites, we calculated Wright's F_{st} (which measures population differentiation) by using Nei's formula (Nei, 1977) in GenAlEx. We used the same software to estimate D_{est}, a more recently developed population differentiation index, which we calculated as the arithmetic mean of this metric across loci. This index has been suggested to be more reliable than traditional ones, as it would not be biased by within-population heterozygosity (Jost, 2008).

To further assess and visualize genetic relationships among regions and individuals, we performed Principal Coordinates Analyses (PCoA) via covariance matrices with data standardization using GenAlEx. This technique allowed us to explore and plot the main patterns of differentiation within the dataset. The PCoA generated major axes of variation within our multidimensional genotypic data set. Because each successive axis explains proportionately less of the total genetic variation, we used the first two axes to determine the main groupings of individuals.

We employed the Bayesian clustering approach implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to characterize population structure. We tested distinct scenarios ranging from one to seven populations to evaluate the most likely number of genetic clusters (K). We performed 10 independent runs for each K value, which consisted of two million iterations following two million burn-in steps, and assumed correlated allele frequencies. After all runs, we calculated the optimal

value of K using the method proposed by Evanno et al. (2005), as implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). An additional locus (F85) was used for a complementary analysis performed with this software, but only for the samples reported previously by Valdez et al. (2015).

We investigated population differentiation driven by isolation by distance with the Isolation By Distance Web Service software (IBDWS) 3.23 (http://ibdws.sdsu.edu/~ibdws/). We examined the relationship between $F_{ST}/1 - F_{ST}$ and log-transformed geographic distance (Ln distance) (Jensen et al., 2005), and assessed significance with 10,000 permutations.

2.3.3. Spatial organization and dispersal patterns

We obtained individual locations for 85 animals (77.27% of the total) through field research (Silveira, 2004; Azevedo and Murray, 2007; Cavalcanti and Gese, 2009; Onuma et al., 2016; Devlin, 2019), with GPS collars for 64 individuals and VHF collars for 21 individuals. For these individuals, we estimated centroids of home ranges based on the 100% Minimum Convex Polygon method (Mohr, 1947) using the *Feature to Point* tool in ArcGIS 10.1 (ESRI, Redlands, CA, USA). For 24 samples (21.82% of the total), the only available location was the point of capture, and we thus incorporated this information in the analyses. For only one individual there was no available geographic information; this individual was therefore excluded from spatial analyses.

We used home range centroids (or capture sites) to generate a matrix of geographic coordinates in GenAlEx. To obtain the distances among the sampling sites, the minimum convex polygon was estimated for each population by using the centroids and capture coordinates of its individuals. From this, we obtained another centroid from the polygon generated for each sampling site, and calculated the distances between centroids of each site.

We used two complementary genetic methods to analyze sex-specific differences in dispersal patterns. First, we conducted a spatial autocorrelation analysis with GenAlEx to assess patterns of genetic relationships among male and female jaguars. This approach calculates a spatial autocorrelation coefficient (r) by employing a multivariate analysis of the square of genetic distances against geographic distances. We plotted spatial autocorrelograms with r values against 10 predefined distance classes. For female jaguars, we plotted r coefficients against distance classes spanning 4 km (i.e., $\geq$4 km and $\geq$8 km) up to a distance of 40 km, whereas for males, we defined distance classes of 6 km up to a distance of 60 km. We delimited distance classes based on previous studies that estimated jaguar home ranges in the Pantanal (Crawshaw and Quigley, 1991; Azevedo and Murray, 2007; Cavalcanti and Gese, 2009). We estimated statistical significance ($p < 0.05$) of r values through 999 permutations and 999 bootstraps, as implemented in GenAlEx. We selected the *Multiple pops* option for the spatial autocorrelation analysis based on the distance between sampling sites (Fig. 1), the number of jaguars recorded per site, and the results obtained in the STRUCTURE software (see below). The 'multiple–populations' approach for combined data (Peakall et al., 2003) seems to be more appropriate than the pooled approach for the specific purpose of testing for heterogeneity in fine-scale spatial autocorrelation between males and females (Banks and Peakall, 2012). Thus, we divided the sampled jaguars into two groups, one containing samples from the northern Pantanal (group A: SBII, TES, and PMNP sites) and another containing samples from the southern Pantanal (group B: CAI, SFR, SR, and SBI sites; Fig. 1).

The second approach was based on assignment tests using the corrected assignment index (AIc) implemented in FSTAT. We calculated this index separately for male and female adults, and assessed both its mean and variance. This is because immigrants are expected to have negative values of AIc, whereas positive AIc values would characterize resident individuals in the sampled population (Mossman and Waser, 1999). Therefore, if there is sex-biased dispersal, the dispersing sex tends to have a lower mean AIc, relative to the more philopatric sex. Additionally, the AIc variance is expected to be larger in the dispersing sex (Lawson Handley and Perrin, 2007).

2.3.4. Pedigree reconstruction

To evaluate the ability of the microsatellite loci to estimate relatedness information from our dataset, we used the software FRANz 2.0.0 (Riester et al., 2009) to calculate three distinct indices that are informative on locus variability (Jones et al., 2010): (i) probability of identity (P_{ID}); (ii) probability of identity of full siblings (P_{IDsib}); and (iii) probability of exclusion (PE). P_{ID} and P_{IDsib} represent the probabilities that two unrelated individuals or two full-siblings, respectively, have the same genotype by chance, whereas PE is the probability of excluding one random individual as parent of a genotyped offspring. Ideally, P_{ID} and P_{IDsib} estimates should be lower than 0.001 and 0.05, respectively (Riester et al., 2009).

We reconstructed a pedigree for sampled individuals using FRANz, which applies a Markov Chain Monte Carlo (MCMC) simulation for estimating the statistical confidence of parentage inference and produces a number of output files including a maximum likelihood (ML) pedigree. We performed 10 independent runs using default settings except for the number of genotype mismatches allowed for a parent–offspring relationship (command: 'maxmismatching'), which was 1 for one parent–offspring and 2 for two parents–offspring dyad. Additionally, the age range in which females and males can reproduce (command: 'femrepro' and 'malerepro') was set between 2 and 10. Available information on approximate individual age at time of capture (estimated by the presence of milk teeth or permanent dentition, tooth color, and wear (Ashman et al., 1983)) was included in the input file. Ten mother-offspring relationships considered in this study had been previously inferred by field teams based on their observations, so this information was also incorporated in the input file. Multilocus genotypes were used to test these assumed relationships. To improve the construction of pedigrees, an additional locus (F85), genotyped for a subset of the individuals in a previous study (Valdez et al., 2015) was also included in the dataset specifically for the analysis performed in FRANz.

We generated a graphical representation of pedigree networks using the Dot program, part of the Graphviz package (Gansner et al., 2009). As in other analyses described above, two groups were assessed separately (one [A] comprising the northern [TES, SBII, and PMNP] sites, and another [B] with the southern [CAI, SR, SBI, and SFR] sites), so that two pedigrees were produced with the timeline indicating the estimated birth of the animals. Based on these pedigrees, we estimated the Euclidean distances between mother and offspring, which were used to evaluate differences between mother-male and mother-female offspring dyads using Instat 3.0.0 (GraphPad Software, San Diego, CA, USA). Additionally, we estimated jaguar generation time (T; the average time between two consecutive generations in a given population), calculated as the mean age of the parents when their offspring are born (aiming to represented the weighted average age at reproduction).

3. Results

3.1. Data quality control

Initial tests using MICRO-CHECKER indicated no evidence that the dataset was affected by allele dropout or false alleles. Possible null alleles were detected for locus FCA441 at the CAI site, for FCA742 at the SFR site, and FCA441 and F124 at the SBII site, but none presented a null allele frequency greater than 0.20. After sequential Bonferroni correction, only locus FCA742 at the CAI site and locus FCA441 at the SBII site showed evidence of deviation from HWE. This may reflect an inherent substructure within the populations, such as the presence of kin groups. While genotyping errors are a common cause of HWE deviation, results can also be influenced by inbreeding, natural selection, population substructure, or a large number of related individuals (Chen et al., 2017). The latter was in fact detected at the sampled sites (see Results). Additionally, we found no significant LD among loci after Bonferroni

correction. Given these results, we kept all loci for subsequent population genetic and kinship analyses.

3.2. Genetic diversity

Overall, *P. onca* in the Pantanal presented high levels of genetic variability (Table 1). The number of observed alleles in the total dataset (all populations together) varied from three (F98, F146) to 15 (FCA742) with a mean of 7.364 (±0.993), which was higher than the estimated effective number of alleles (4.375; see Table 1). Shannon's information index indicated most of the loci were highly informative, with an overall mean polymorphism across loci of 1.529 (±0.147). Expected heterozygosity (H_e) for the entire dataset ranged from 0.256 to 0.890 (mean of 0.713 ± 0.051), while the observed heterozygosity (H_o) varied from 0.234 to 0.867 (mean of 0.673 ± 0.054). When each local population was analyzed separately, we observed H_e values ranging from 0.638 (CAI and PMNP) to 0.679 (TES), and H_o varied from 0.639 (SBII) to 0.765 (PMNP). Allelic richness ranged from 4.034 (CAI) to 4.720 (PMNP).

3.3. Population structure and differentiation

Estimates of population differentiation based on pairwise F_{ST} and D_{EST} indices indicated low but statistically significant signs of genetic differentiation among sampled sites (Supplementary Table S2). In contrast, the PCoA did not indicate clustering among sites (Supplementary Fig. S1). In total, the first three principal axes explained 20.51% of the genetic diversity across the seven populations (PC1: 8.30%; PC2: 6.89%; PC3: 5.32%).

The assignment test performed using STRUCTURE indicated that the optimal number of genetic clusters in our dataset was $K = 2$, according to Evanno's ΔK (Evanno et al., 2005) (Fig. 2; Supplementary Table S3; Supplementary Fig. S2). Jaguars from the SBII, TES, and PMNP sites ($n = 44$) were assigned to cluster A (northern populations; average probability assignment of 0.86, 0.93, and 0.92, respectively), with only 10 individuals (25%) showing assignment probabilities lower than 0.90. Conversely, jaguars from the CAI, SR, and SFR sampling sites ($n = 54$) were all assigned to cluster B (southern populations; average assignment probability of 0.866, 0.930, and 0.923, respectively), with 16 individuals (29.62%) presenting assignment probabilities lower than 0.90. Jaguars from the SBI site seemed to be mixed, since they presented average probability of 0.464 to group in cluster A and 0.536 in cluster B (see Fig. 2).

3.4. Isolation by distance and spatial autocorrelation

There were significant associations between genetic difference and the logarithm of geographic distance at the sampling site level (Fig. 3), and a high proportion of the genetic variance was explained by geography ($r = 0.489$; $P = 0.031$). In spite of the verification of a few points distant from the line, the correlation was significant and the slope of the line is very pronounced.

Spatial autocorrelation analysis presented distinct results for female and male jaguars. For females, spatial autocorrelation was positive only up to 16 km, but was significantly so only up to 4 km ($r = 0.110$; $p = 0.001$ up to 4 km after Bonferroni correction), equivalent to approximately 50.25 km^2. In contrast, males did not show significant positive autocorrelation in any predefined distance class ($p > 0.05$; Fig. 4). Males did not show significant positive autocorrelation in the first three distance classes ($p > 0.05$). However, spatial autocorrelation was significant in the distance classes of 18.00–24.00 km ($r = 0.043$; $p = 0.040$) and 30.00–36.00 km ($r = 0.049$; $p = 0.034$; Fig. 4).

Assignment index analysis provided evidence for sex-biased dispersal. Differences in assignment index (AIc) and its variance between male (mean AIc = −0.551, AIc variance = 11.948, $n = 59$) and female adults (mean AIc = 0.637, AIc variance = 4.964, $n = 51$) were significant (AIc: $p = 0.02$, AIc variance: $p = 0.01$). The positive AIc value observed for females indicated their higher probability of originating from the population within which they were sampled, while the negative AIc value for males indicated a higher probability that the sampled individuals were migrants.

3.5. Pedigree reconstruction

The estimated P_{ID} for the two groups was extremely low ($P_{ID} = 0.000$), as well as the probability of identity of siblings ($P_{IDsib} < 0.001$ for group A and $P_{IDsib} < 0.001$ for group B). Also, the probability of parentage exclusion almost reached 1 in both geographic groups ($P_{PE} = 0.993$ for group A and $P_{PE} = 0.992$ for group B). These results indicated that our multilocus dataset was sufficiently robust to detect kinship among the animals evaluated in this study. We included 110 genotyped jaguars (51 males, 59 females) in the parentage analysis to identify mother-offspring dyads (Fig. 5). Dyads were comprised of 21 female and 15 male offspring from 19 mothers. Multilocus genotypes of all dyads were compatible with the alleged mother-offspring (only one mismatch was observed). The maximum number of offspring observed per mother during the sampling period was four. Additionally, four mother-male offspring dyads were excluded from the distance analysis since they were sub-adults at the time of blood collection, and had therefore not yet established their ultimate home range.

Euclidean distances averaged 11.02 ± 4.96 (SD) km (median = 4.06 km) for mother-female offspring dyads (88.88% < 8 km), and 19.17 ± 6.92 km (median = 8.84 km) for mother-male offspring dyads (81.81% > 5 km). Mother-female offspring dyad distances were significant lower than those observed for mother-male offspring dyads (Mann-Whitney test = 57; $p = 0.02$). Finally, based on the pedigree data, the jaguar generation time was estimated to be 4.08 years, as supported by 10 replicates in the FRANZ software.

4. Discussion

4.1. Genetic diversity

The levels of microsatellite diversity estimated here for Pantanal

Table 1
Genetic diversity of Pantanal jaguars. For each site (see Fig. 1 for identification), the sample size (N) is indicated, along with the average number of different alleles per locus (Na); allelic richness standardized by the sample size (AR); Private alleles standardized for the sample size (PA); observed heterozygosity (H_o); expected heterozygosity (H_e); Shannon's information index (I) (Sherwin et al., 2006); and the percentage of polymorphic loci (%P). For Na, H_o, H_e and I, the mean and standard error (SE) are indicated.

Sampling site	N	Na (S.E)	AR	PA	H_o (S.E)	H_e (S.E)	I (S.E)	%P
CAI	33	5.727 (0.541)	4.034	0.08	0.647 (0.063)	0.638 (0.054)	1.270 (0.124)	100%
SFR	11	4.273 (0.428)	4.072	0.14	0.695 (0.059)	0.667 (0.036)	1.260 (0.107)	100%
SBI	12	5.182 (0.483)	4.661	0.12	0.713 (0.062)	0.666 (0.057)	1.346 (0.134)	100%
SR	10	4.727 (0.524)	4.429	0.08	0.659 (0.077)	0.645 (0.045)	1.263 (0.125)	100%
SBII	20	5.909 (0.625)	4.549	0.14	0.639 (0.056)	0.668 (0.054)	1.384 (0.139)	100%
TES	19	5.636 (0.778)	4.495	0.22	0.696 (0.052)	0.679 (0.048)	1.377 (0.127)	100%
PMNP	5	3.909 (0.436)	4.762	0.21	0.765 (0.069)	0.638 (0.054)	1.179 (0.125)	100%

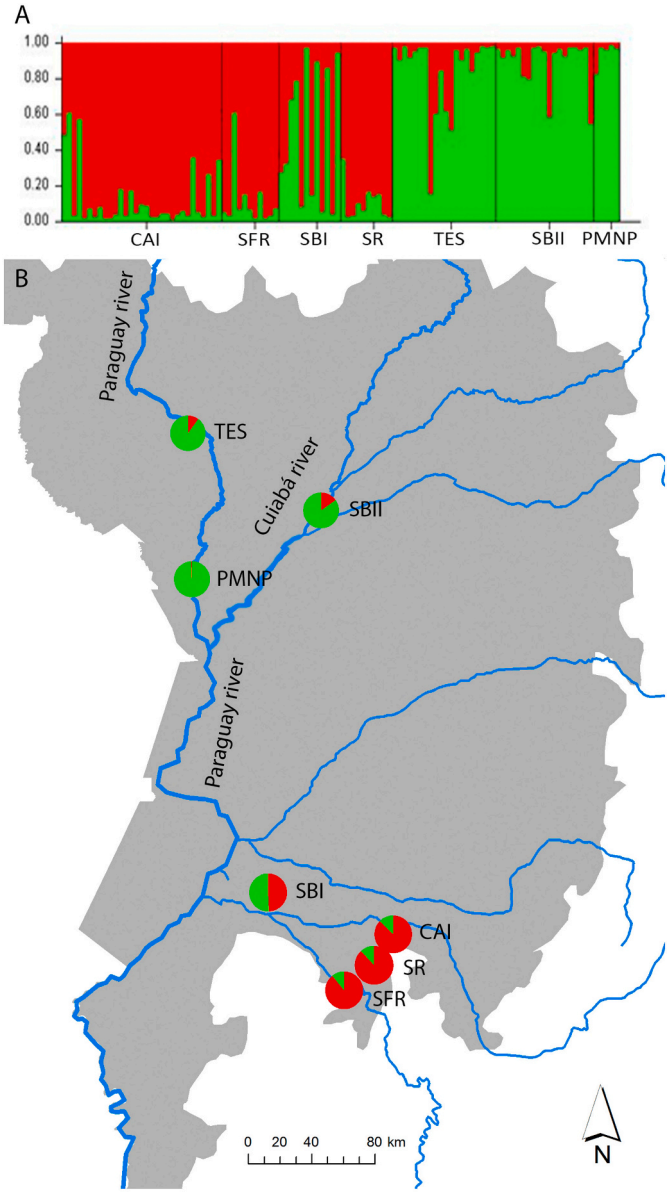

Fig. 2. Genetic structure of Pantanal jaguars estimated with the Bayesian clustering analysis implemented in the program STRUCTURE, assuming the best-fit model of two genetic clusters (K = 2). (A) Each vertical bar in the STRUCTURE barplot represents an individual. Colors in each bar represent assignment to the two inferred genetic clusters (Green = Cluster A; Red = Cluster B; see text for details), and the Y-axis represents the percentage of membership (Q) of each individual to each genetic cluster. (B) Pie charts represent the mean fractions of Q for each genetic cluster in jaguars sampled at each study site. See Fig. 1 for site names. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

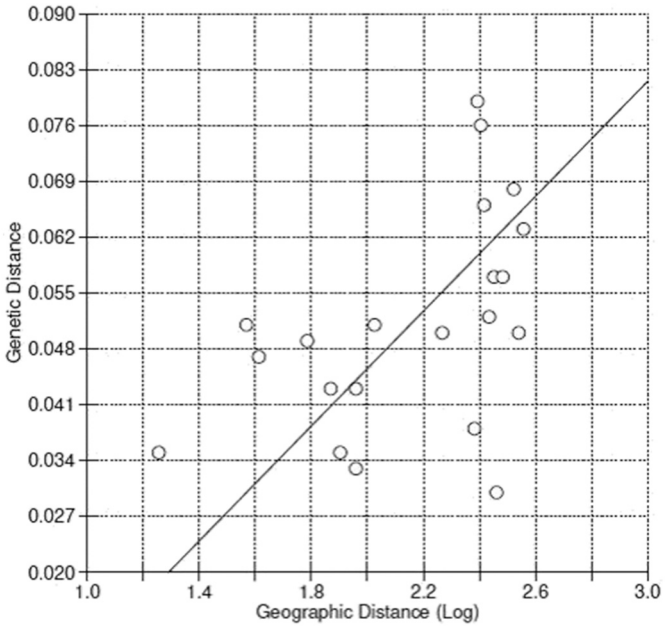

Fig. 3. Mantel test depicting the correlation between genetic distance and the logarithm of geographic distance. Genetic distance is represented by pairwise $F_{ST}/(1 - F_{ST})$ estimates among populations (F_{ST} is an index of between-population genetic differentiation; see main text for details), which is regressed against the natural logarithm of geographic distance (L_n distance), to test for isolation by distance. The Reduced Major Axis (RMA) regression line (the solid line) overlays the scatterplot.

jaguars may be considered medium to high when compared to previously reported values for the same region or to a spatially broader survey (Eizirik et al., 2001; Valdez et al., 2015; Roques et al., 2016). In addition, we observed similar levels of molecular variability among the different sampling sites in our study. This indicates that the two major regions of jaguar occurrence in the Pantanal (Quigley and Crawshaw, 1992) present similar levels of nuclear genetic variation.

So far, the population dynamics of Pantanal jaguars seems not to have been significantly affected by human disturbances such as hunting and habitat degradation, which may be due to the high availability of prey, the existence of connectivity among territories and limited human access to some areas (Soisalo and Cavalcanti, 2006). However, the regions surrounding the Pantanal are characterized by high rates of deforestation (Silva et al., 2010; Hansen et al., 2013), so the results presented here should be taken with caution given the increasing isolation of this biome. For example, jaguar populations in the southern Pantanal were historically connected southwards to the Atlantic Forest, a heavily human-impacted biome where populations of this species already show clear signs of genetic isolation and loss of genetic diversity (Haag et al., 2010). Previous genetic analyses have provided strong evidence of historical connectivity between jaguars in the southern Pantanal and a transitional population (Porto Primavera) at the southwestern boundary of the Atlantic Forest, which unfortunately has already been extirpated (Valdez et al., 2015). This illustrates the ongoing process of human-induced genetic isolation of remaining jaguar populations.

In this context, the Cerrado biome surrounds the Pantanal on most of its eastern borders, not only on its southern end (separating it from the Atlantic Forest areas mentioned above) but also northwards. It therefore mediates the remaining connectivity between the still healthy Amazonian jaguar populations (H_e = 0.81 - Roques et al., 2016; H_e = 0.76 - Lorenzana et al., 2020) and the Pantanal ones studied here (H_e = 0.71). The Cerrado has been intensely modified since the 1950s through extensive cattle ranching and agricultural monocultures (e.g. rice, corn, and soybean), and 65%–80% of this biome have been found to be degraded (Tomas et al., 2019). In addition, the small portion of the Amazonian biome that is located nearer to the Pantanal is almost completely deforested. In the partial areas of the Cerrado and Amazon biomes present in the Upper Paraguay River basin, the suppression of natural vegetation has reached 58.30% and 66.70% of their territories, respectively (Silva et al., 2010). This is alarming, as it suggests that ongoing gene flow between the jaguar populations from the Amazon and the Pantanal may be very limited. Given current rates of deforestation throughout that region (Hansen et al., 2013), the same concern extends to other ecosystems located to the west of the Pantanal, such as the Dry Chaco, Humid Chaco and Chiquitano dry forests. Although gene flow

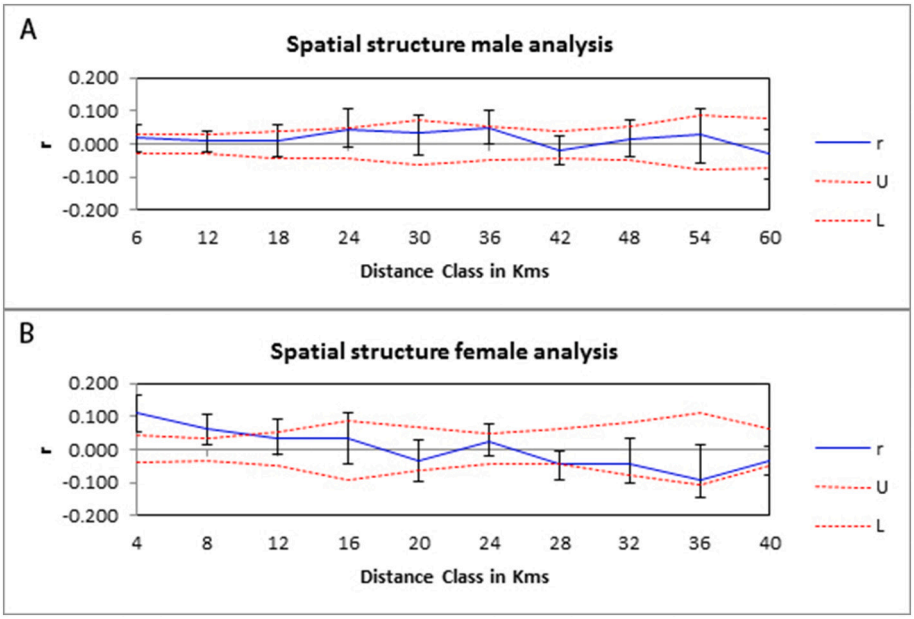

Fig. 4. Correlogram plot of the genetic correlation coefficient (*r*) as a function of distance for (A) male and (B) female jaguars. The permuted 99.9% confidence intervals (broken lines) and bootstrapped 99.9% confidence error bars are also shown.

among jaguar populations in those areas has so far not been directly estimated, it is likely that their connectivity is being negatively impacted by current loss and fragmentation of habitats. It is therefore a priority to genetically assess those jaguar populations in the context of regional conservation planning that includes maintenance of historical patterns on gene flow.

Despite the similarity in estimated H_e and allelic richness among Pantanal populations, we observed that the private allelic richness at the TES and PNP sampling sites (which are federally protected areas) was relatively higher than at other sites. Since the richness of private alleles may be a useful criterion in the conservation of genetic diversity, it is interesting to hypothesize that this observation may be related to anthropogenic actions that occur at higher magnitude in the other five studied areas. Moreover, the northern Pantanal populations exhibited the highest proportion of private alleles, possibly due to the proximity (and historical demographic connection) of this region to the Amazon biome (Roques et al., 2014).

4.2. Genetic structure

In order to effectively conserve and manage threatened carnivore species that occur in complex and heterogeneous landscapes, we must assess the genetic connectivity of natural populations. Patterns of genetic structure in wide-ranging carnivores such as jaguars are shaped by a multitude of contemporary and historical factors such as ecological, environmental, or anthropogenic influences (Creel et al., 2019). Wild jaguars prefer well-preserved closed-canopy forest habitat in proximity to riparian areas (Crawshaw and Quigley, 1991; Morato et al., 2018) and have a tendency to avoid areas of high human impact (Morato et al., 2018). The low (albeit mostly statistically significant) estimated values of F_{ST} and D_{EST} indices (Supplementary Table S2), along with the results of the PCoA, indicated that the sampled populations still present a considerable level of gene flow. Our results were consistent with the hypothesis that jaguars from the Pantanal represent a panmictic population, although we found some degree of local differentiation. This differentiation may be due to sampling related individuals at each locale, which would bias fixation indices upward. In addition, the isolation by distance (IBD) results indicated that at least part of the observed population differences can be explained simply by the Euclidean distances between sampled sites. In this context, future analyses incorporating landscape features and/or more realistic path distances are likely to further refine the observed pattern of gradual differentiation across the Pantanal.

Results obtained with the Bayesian structuring analysis identified two as the most probable number of subpopulations in our sample (i.e. $K = 2$), with all individuals assigned in different proportions to one of two genetic clusters. In addition, when assessing the average assignment probability of samples from each sampling site, we observed a gradual change that occurred in a north-south direction. This result agrees with the pattern described by Eizirik et al. (2001), who did not observe marked genetic differentiation among regions sampled south of the Amazon River. Future studies with expanded sampling, especially in the center of the Pantanal, will contribute to further testing and refining this assessment of gradual change.

However, SBII, PMNP, and TES populations presented values very close to the average probability assignment, indicating the occurrence of intense gene flow among these localities despite the geographic distance among them (91–106 km). It is interesting to highlight that these regions are connected through important rivers of the upper Paraguay River basin (Fig. 2), which are still well preserved. The close association of jaguars with water has been historically described by naturalists and explorers (Perry, 1970) which have suggested that the species seems to show a preference for terrain close to rivers, streams, and dense marshes (Cullen Junior et al., 2013; Morato et al., 2018). As reported by Crawshaw and Quigley (1991) in the Pantanal, jaguars were rarely found far from water and used dense marshlands and gallery forests more often than expected based on their relative availability across the landscape.

In contrast, when the average assignment probability of the SBI, CAI, SFR, and SR populations was compared, their distances of 61–80 km were sufficient to significantly affect the results, which is mainly due to the distinction between SBI and the other southern sites (see Fig. 2). It is interesting to note that the distance between SBI and the northernmost sampled areas (cluster A, as inferred by Structure software) varied from 185 to 289 km, while it was 61–80 km between SBI and the southern sites (cluster B). Jaguars from SBI presented an average probability of assignment close to 0.50, allowing us to hypothesize this area may be a contact zone between genetic clusters A and B, as it included mixed individuals from both clusters. As there were large differences between

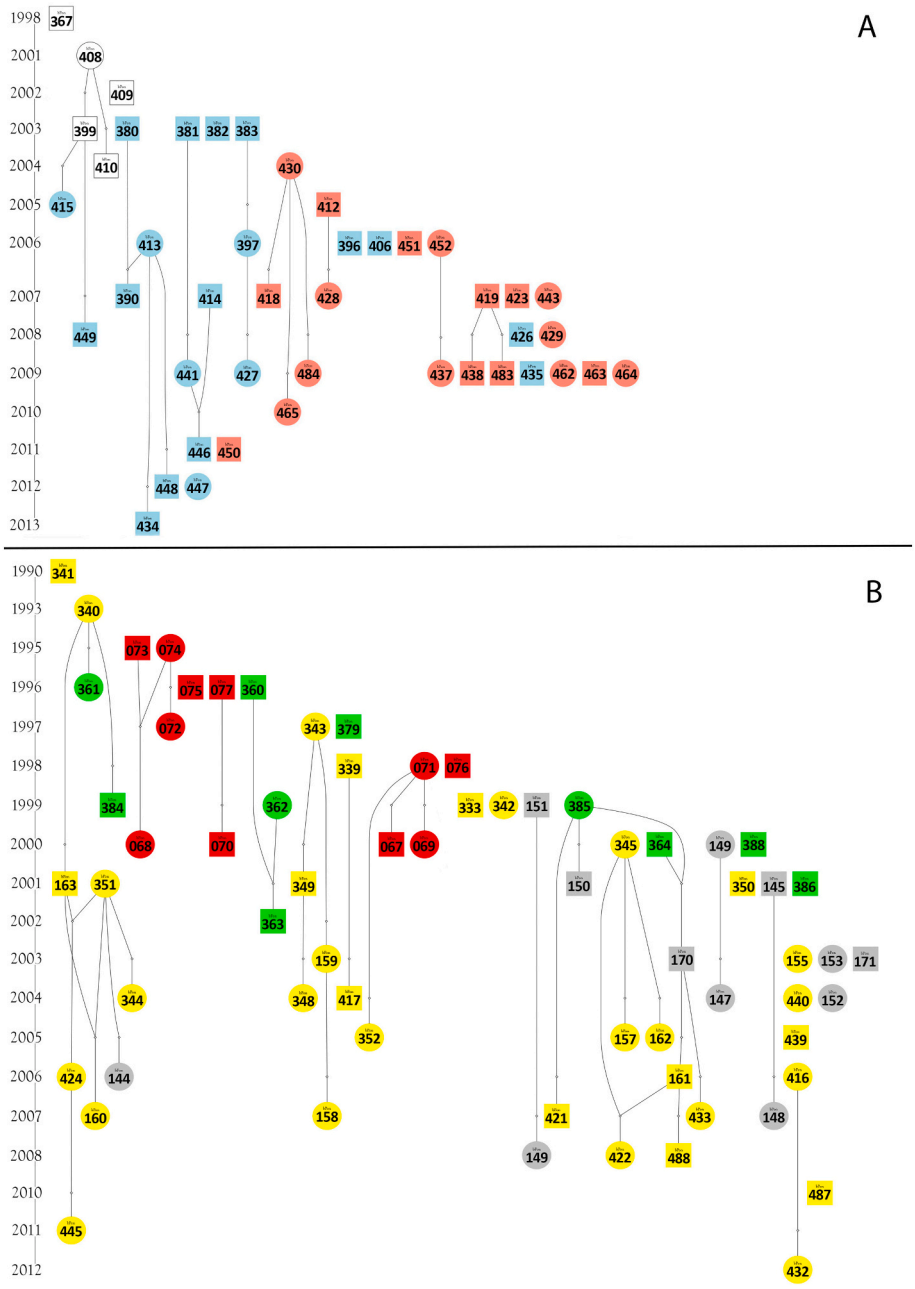

Fig. 5. Timeline pedigree depicting the genealogical relationships among jaguars from (A) northern Pantanal (TES, SBII, and PMNP sites) and (B) southern Pantanal (CAI, SFR, SR, and SBI sites). Each symbol represents one individual (squares: males; circles: females), and its color indicates its sampling site: white: PMNP; blue: SBII; pink: TES; yellow: CAI; red: SFR; green: SR; and gray: SBI. Individual symbols are placed on a line indicating their inferred year of birth (calculated based on their estimated age at the time of capture). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the distances indicated above, the Paraguay River likely supports gene flow more effectively than its tributaries in the southern region of the Pantanal.

4.3. Kinship analysis and dispersal patterns

Jaguar litter size varies from one to four cubs, with two cubs being more common (Seymour, 1989). By multiplying average litter size by the number of possibilities of reproduction during the lifetime of a given female, and considering that cubs usually remain with their mother for 2 years, more than 10 jaguars can be generated by a single female during her lifetime. In a free-ranging population of *Panthera pardus*, the mean female lifetime productivity was found to be 5.50 and reproductive success was four (Owen et al., 2010). Our kinship analysis indicated a maximum number of four descendants per female (bPon 351), and these individuals were known to have reached adulthood (based on direct field observations), which is the highest reproductive success observed in this study. Several cases ($n = 9$) of mothers with 2 and 3 cubs were also observed, and for many females ($n = 33$) no descendants were detected (Fig. 5). Thus, the low number of offspring observed in our results, when compared to the reproductive potential for jaguars, may be due to: (i) insufficient sampling, hampering the detection of the actual number of offspring per parent; (ii) cub mortality (Tortato et al., 2016); (iii) human persecution (e.g., hunting); and (iv) migration of individuals to non-sampled areas. Future studies with more exhaustive sampling should address these hypotheses.

We recovered a larger number of parent-offspring dyads in populations from group B (Fig. 5B). This may be due to the larger number of individuals sampled in this group, as well as the smaller distances between sampling sites, relative to group A (see Fig. 2). In addition, the timeline in group B spans 22 years while in group A it spans 15 years. Interestingly, no first-degree relatives were observed between the TES and SBII sites, although they are relatively close (approximately 91 km in a straight line). This may be due to the tendency of jaguars to move

close to rivers (Crawshaw and Quigley, 1991), which represents more than 200 km network (rather than Euclidean) distance between these two localities.

The mean estimated male and female home ranges of four of the populations included in this study (SFR, SR, TES, and CAI) (Azevedo and Murray, 2007; Cavalcanti and Gese, 2009; Morato et al., 2016) were 109.36 km^2 and 47.46 km^2, respectively. If we assume that home ranges are relatively circular (Cavalcanti and Gese, 2009), and that the different methodologies employed in these studies to measure them did not lead to any severe bias, we can hypothesize that the average distances for mother-male offspring dyads (19.17 ± 6.92 km) are sufficient to minimize their home range overlap. Among mother-female offspring dyads, however, average distances were smaller (11.02 ± 4.96 km), which suggests that their home ranges may indeed overlap. We must consider, however, that the variance around the average indicates substantial variation in jaguar dispersal behavior. Individual female or male jaguars may disperse over distances greater than the mean (and median), while others may not disperse from their natal range at all. Additional analyses, with expanded sampling and standardized measures of home range size and dispersal distance, will help to further clarify this issue.

4.4. Sex-biased dispersal

Although our results indicate that both sexes may disperse, dispersal behavior in Pantanal jaguars seems to be mainly male-biased, such that related females remain close to each other. Such a pattern fits the resource defense hypothesis, avoidance of kin competition by males, and inbreeding avoidance mechanisms, which have explained dispersal patterns observed in other mammal species (Perrin and Mazalov, 2000; Shields, 1987; Sandell, 1989).

In a previous home range overlap analysis performed only with the animals of the SFR ranch (Azevedo and Murray, 2007) the two highest rates of home range overlap among females (50.28% and 37.71%) were observed. Our pedigree analysis of this population revealed that both home range overlaps involved two mother-female offspring dyads. Interestingly, females from the nearby SR revealed an opposite scenario, exhibiting no home range overlap and no relatedness.

Additionally, the much larger home ranges of males from SR (Cavalcanti and Gese, 2009) and SFR (Azevedo and Murray, 2007) covered the ranges of multiple females. The average home range estimates for males and females in SR and SFR, reported in those studies, were 91.95 km^2 and 45.35 km^2, respectively. As in the analysis performed in the previous section, we verified that average distances in mother-male offspring dyads do not indicate the occurrence of home range overlap. For mother-female offspring dyads, this estimate was lower, thus indicating that their home ranges likely overlapped. These findings are consistent with previous studies that reported dispersal in mammalian species to be primarily male-biased. For example, studies on solitary felids such as bobcats (*Lynx rufus*) (Croteau et al., 2010) and leopards (*Panthera pardus*) (Fattebert et al., 2015) have suggested that females may establish home ranges in their natal areas. Interestingly, some dispersal studies in solitary carnivores have reported varying behaviors among populations. For example, both sexes were reported to disperse over equal distances in the Eurasian lynx (*Lynx lynx*) in Switzerland (Zimmermann et al., 2005), while a study on the same species in Scandinavia showed male-biased dispersal with female philopatry (Samelius et al., 2011). Likewise, genetic studies using relatedness and spatial autocorrelation analysis on some solitary carnivores showed female philopatry and male dispersal (e.g. Bartolommei et al., 2016), whereas a study on wolverine (*Gulo gulo*) (Campbell and Strobeck, 2006) showed no sex-biased dispersal. Such heterogeneous results highlight the need for additional studies on multiple species and habitats to better understand these patterns and their underlying processes.

The quality of the home range contributes directly to female fitness for a solitary, territorial animal. Therefore, jaguar females can benefit from familiarity with the use of the territory, since food and not males is their main resource. Inclusive female fitness may further explain why related females live in nearby areas (Shields, 1987). In places where jaguar density is comparatively high, such as the Pantanal, it is quite possible that there will be overlapping home ranges for related females (Sandell, 1989). On the other hand, male dispersal appears to have evolved as a mechanism to avoid kin competition and inbreeding (Johnson and Gaines, 1990). Spatial patterns of male jaguars from SFR (Azevedo and Murray, 2007) and SR (Cavalcanti and Gese, 2009) were apparently influenced by female distribution, suggesting that the establishment of home ranges by males in the Pantanal is typically required to permit breeding opportunities. The number of females within a male's home range is correlated with male fitness (Waser and Jones, 1983), and thus adult males tend to exclude juvenile males from the areas where they were born, forcing them to disperse to prevent competition (Shields, 1987). The dispersal of young males can also occur through the encouragement of females, thus avoiding inbreeding (Shields, 1987).

The results presented here provide the first genetic evidence of female philopatry and male-biased dispersal in jaguars. When crossing unfavorable areas, there are significant mortality costs for the dispersing sex, and the survival rate in mammals can be almost 50% lower in dispersers than in individuals who remain in the natal territory (Johnson and Gaines, 1990). Remaining in the natal area, however, can also entail costs. Philopatry can lead to mating with close kin, and inbreeding depression has severe fitness costs (Lawson Handley and Perrin, 2007). Therefore, the adaptive potential may be eroded by excessive philopatry, since it decreases gene flow across the landscape, an issue that is further exacerbated by human-induced habitat fragmentation.

4.5. Generation time

Variability in rates of molecular evolution is well documented, and substitution rates can be affected by features including population size and/or life-history traits such as body size (Thomas et al., 2006). One of the most prominent explanations for variation in substitution rates resides in differences in species' generation times. The methodology used in many studies is very different from ours, and is based on species' reproductive lifespan. Species' reproductive lifespan is calculated as the difference between the age at last reproduction and the age at first reproduction, and a constant that depends on survivorship and relative fecundity of young versus old individuals in the population. Thus, even considering the potential errors resulting from the difficulty of correctly estimating the age of the animals in the context of our method (see Olifiers et al. (2010) and references therein for a discussion on this topic), the jaguar generation time estimated here may be useful for future molecular and evolutionary studies. Importantly, this is the first time that this parameter has been estimated from free-living jaguars.

From a conservation perspective, the most widely known quantitative system of classifying threatened species is the International Union for Conservation of Nature's (IUCN) Red List Categories and Criteria (IUCN 2001), which includes various categories and time horizons (in both years and generations) in its categorization system (Mace et al., 2008). Current IUCN assessments assume a generation time of 6.84 years for jaguars, which is larger than the value estimated in this study (4.08 years). It is therefore important that future IUCN assessments measure the impact of incorporating our field-based estimate of jaguar generation times. Moreover, additional field studies, especially those incorporating long-term monitoring of jaguar populations (including genetic parentage assignments across a large number of individuals) will be useful to further refine this estimate and provide a better foundation for risk assessment.

4.6. Implications for conservation and management of Pantanal jaguars

An accurate understanding of contemporary genetic connectivity is key to preserve the genetic health of jaguar populations across

landscapes. This study detected medium to high levels of genetic diversity and low genetic differentiation in Pantanal jaguars. In this context, the results presented here have important implications for the conservation of jaguar populations across the Pantanal. There seem to be no significant barriers across the Pantanal landscape that could potentially hinder jaguar dispersal. However, some specific regions could be significantly affected by geographic features. According to Cavalcanti et al. (2012), jaguars from the Pantanal could possibly be divided into two subpopulations which would be reasonably connected by the lowland corridor along the Paraguay River. The Pantanal's JCU format is strangled, so that the northern and southern populations are connected by a small corridor (Rabinowitz and Zeller, 2010). In this scenario, conservation efforts to keep the functional connectivity between northern and southern jaguar populations should be implemented, aiming to maintain natural areas conserved along the Paraguay River (apparently the main facilitator of gene flow between these areas). Our data suggest that northern populations (SBII, TES, and PMNP) seem to be more connected among themselves, and the riparian habitats of the Paraguay and Cuiabá rivers may represent the biological corridors allowing high rates of gene flow among these localities. The expansion of protected areas along these rivers, as well as the development of private conservation projects in this region, may be important strategies for maintaining connectivity among these jaguar populations.

Compared to the Amazon, the Pantanal has a smaller percentage of its total area contained in protected jaguar areas (PJAs). However, high concentrations of jaguars are also observed in non-protected areas (Silveira et al., 2014). Our genetic results lead to conclusions that agree with the jaguar corridors proposed by Silveira et al. (2014), which rely on the existence of PJAs, river courses, and their disturbance levels. In general, watercourses provide the highest potential for wildlife corridors (as they receive legal protection status in Brazil; Law 12.651/2012), especially in the case of species such as the jaguar, which are closely associated with water. We therefore recommend that increased attention should be given to protecting and characterizing the effectiveness of these corridors. More broadly, we point out that conservation initiatives for jaguars and other large carnivores should integrate interdisciplinary efforts at multiple geographic scales, enabling decision-makers to make management decisions that are strongly based on scientific data.

CRediT authorship contribution statement

Daniel Luis Zanella Kantek: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.

Cristine Silveira Trinca: Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing.

Fernando Tortato: Investigation, Resources, Writing - Review & Editing, Funding acquisition.

Allison Loretta Devlin: Investigation, Resources, Writing - Review & Editing.

Fernando Cesar Cascelli de Azevedo: Investigation, Resources, Writing - Review & Editing, Funding acquisition.

Sandra Cavalcanti: Investigation, Resources, Funding acquisition.

Leandro Silveira: Investigation, Resources, Funding acquisition.

Selma Samiko Miyazaki: Investigation, Resources.

Peter Gransden Crawshaw Junior: Investigation, Resources, Writing - Review & Editing, Funding acquisition.

Joares Adenilson May-Junior: Investigation, Resources, Writing - Review & Editing.

Carlos Eduardo Fragoso: Investigation, Resources, Writing - Review & Editing.

Leonardo Rodrigues Sartorello: Investigation, Resources, Writing - Review & Editing.

Lilian Elaine Rampim: Investigation, Resources, Writing - Review & Editing.

Mario B. Haberfeld: Investigation, Resources, Writing - Review & Editing, Funding acquisition.

Gediendson Ribeiro de Araujo: Investigation, Resources, Writing - Review & Editing.

Ronaldo Gonçalves Morato: Investigation, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Eduardo Eizirik: Conceptualization, Methodology, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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.2021.109153.

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