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Original investigation

Genetic diversity and population structure of white-lipped peccaries (*Tayassu pecari*) in the Pantanal, Cerrado and Atlantic Forest from Brazil

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ABSTRACT

In general, habitat fragmentation is associated with a reduction in gene flow that can reduce the genetic diversity, and, consequently, a species ability to survive environmental changes. The white-lipped peccary (*Tayassu pecari*) is a Neotropical ecosystem engineer that is vulnerable throughout its distribution area and under different degrees of threat in the Brazilian Pantanal (near threatened), Cerrado (endangered) and Atlantic Forest (critically endangered). We used 13 microsatellite loci to assess the genetic diversity and population structure of 361 white-lipped peccaries sampled in four areas in the Pantanal, two areas in the Cerrado, and one area in the Atlantic Forest. We found similar levels of genetic diversity in all localities. Bayesian clustering analysis indicated the presence of two ($K = 2$; all Pantanal localities plus the Cerrado locality at the Pantanal highlands versus the other Cerrado locality plus the Atlantic Forest one) or three ($K = 3$; with the additional differentiation between the Cerrado locality and the Atlantic Forest) genetically differentiated populations. We found a pattern of isolation by distance (IBD) limited by dispersal events of up to 180 km. This IBD pattern is congruent with gene flow between the geographically closer localities in the Pantanal and the Cerrado in the Pantanal highlands (25–137 km), while dispersal between the other Cerrado locality and the Atlantic Forest, that are 500 km apart, would not be as feasible. Therefore, we considered $K = 3$ as the best scenario to represent the genetic structure of the populations analyzed. As all populations of white-lipped peccary showed moderate levels of genetic diversity, conservation actions are recommended to maintain their diversity, as it is vital to the long-term viability of these populations, especially those in more threatened biomes.

Introduction

During the last decades, anthropogenic impacts have triggered the fragmentation process by changing landscape configuration

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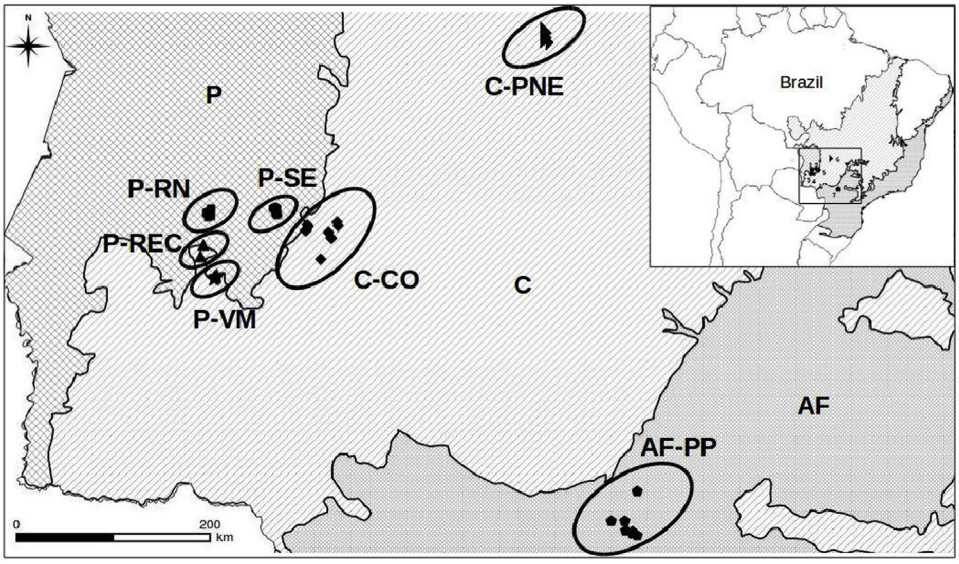

Fig. 1. Sampling sites in three Brazilian biomes. P: Pantanal, C: Cerrado, AF: Atlantic Forest, P-RN (1) Fazenda Rio Negro, P-SE (2) Fazenda Santa Emília, P-REC (3) Refúgio Ecológico Caiman, P-VM (4) Fazenda 23 de Março, C-CO (5) Fazenda Colorado, C-PNE (6) Emas National Park, AF-PP (7) Pontal do Paranapanema region.

and reducing habitat connectivity (Saunders et al., 1991). A species' movement through a landscape depends on an individuals' response to potential barriers and other characteristics of suitable habitats, as well as the species' dispersal capacity (Kupfer et al., 2006). Decreasing landscape connectivity usually reduces population size and gene flow between fragments, leading to a loss of genetic variability (via genetic drift and inbreeding) and to an increase in the spatial genetic structure (Keyghobadi, 2007). Several studies have found limited gene flow and low genetic diversity in natural populations of various mammalian species, as the Utah prairie dog – *Cynomys parvidens* (Brown et al., 2016), the New England cottontail – *Sylvilagus transitionalis* (Fenderson et al., 2014), and the American black bears – *Ursus americanus* (Murphy et al., 2018; Pelletier et al., 2017). In addition, a meta-analysis of microsatellite data from 108 mammalian species described a significant decrease in the genetic diversity of populations that suffered severe decline (Garner et al., 2005). Populations with low genetic diversity may have reduced survival capacity and a lowered ability to persist in face of environmental changes, which increases the extinction risk (Frankham, 1995; Reed and Frankham, 2003).

White-lipped peccaries (*Tayassu pecari*) belong to the family Tayassuidae (Order Artiodactyla) and are geographically distributed from southern Mexico to southern Uruguay and northern Argentina (Sowls, 1984). They live in herds that can exceed 100 individuals, which occupy large home ranges from 1600 to 20,000 ha (Fragoso, 1998; Jácomo et al., 2013; Kiltie and Terborgh, 1983; Keuroghlian et al., 2004, 2014). Herds present a periodic fission-fusion pattern, exchanging individuals between subherds in each fission-fusion cycle (Keuroghlian et al., 2004). Individuals have large dispersal ability and are able to travel long distances (3–10 km in a day, Altrichter et al., 2002; Jorge et al., 2019). Their movements are seasonal and can change according to fruit availability (Altrichter et al., 2002; Keuroghlian and Eaton, 2008a; Keuroghlian et al., 2009), as well as the presence of water bodies (Keuroghlian and Eaton, 2008b; Reyna-Hurtado, 2009). The species has an important ecological role, acting as seed predator and disperser (Keuroghlian and Eaton, 2009). Furthermore, this species can create suitable habitats for reproduction of other species, such as amphibians (Beck et al., 2010; Ringler et al., 2015), and are important prey to large felines, such as jaguars (*Pantera onca*) and pumas (*Puma concolor*) (Cavalcanti and Gese, 2010; Keuroghlian et al., 2012; Reyna-Hurtado, 2009).

White-lipped peccaries are classified as vulnerable by the IUCN (Keuroghlian et al., 2013). Their populations are currently in decline, with local extinctions, even in protected areas (Altrichter et al., 2012; Keuroghlian et al., 2012). In the present study, we analyzed white-lipped peccaries from three Brazilian biomes: Pantanal, Cerrado, and Atlantic Forest, where they are threatened to different degrees. The Brazilian Pantanal covers an area of about 140,000 km^2, from which only 3600 km^2 (2.6%) are formally protected (Junk et al., 2006). This biome is subject to pluriannual cycles of flood and drought, which influence the terrestrial and aquatic fauna (Junk et al., 2006). The species is considered near threatened in the Pantanal (Keuroghlian et al., 2012). The Cerrado originally occupied about 2 million km^2 in central Brazil (Ratter et al., 1997). In the last 35 years, more than 50% has been converted into agricultural and pasture areas (Klink and Machado, 2005). The species is considered endangered in this biome (Keuroghlian et al., 2012). The Atlantic Forest originally covered about 1,5 million km^2, and currently only 12% of its vegetation cover is left, distributed in many small (83% of patches are < 0.5 ha) and isolated fragments, with just a few large remnants (Ribeiro et al., 2009). The species is considered critically endangered in this biome (Keuroghlian et al., 2012), occupying only 8% of the remaining Atlantic Forest area (Jorge et al., 2013).

Given the current conservation status of white-lipped peccaries in these biomes, it is crucial to evaluate the genetic diversity and population structure to plan effective conservation actions that ensure population persistence (Frankham, 2003). To our knowledge, only Biondo et al. (2011) have studied the dispersal pattern and population genetic structure of white-lipped peccaries from two localities located 80 km apart in the Brazilian Pantanal. They found moderate levels of genetic diversity and weak genetic differentiation, with dispersal mediated by both sexes. Herein, we expanded the study of Biondo et al. (2011) by including two additional localities from the Pantanal and localities from two other Brazilian biomes, the Cerrado and Atlantic Forest, to assess the genetic diversity and population structure of white-lipped peccaries on a broader geographical scale. We hypothesized that: 1) as the species is less threatened in the Pantanal, its population has higher levels of genetic diversity than the Cerrado and Atlantic

Forest populations; and 2) the genetic differentiation between populations from these three biomes is related to the extension of the geographical distance between them. Thus, we tested if the dispersion movements, and consequently, gene flow are limited by the geographical distance.

Material and methods

Ethics statement

Animal trapping and handling were authorized by the Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (permits # 13601, 21235, 02001.001735/99-15, 067/99, 093/2000, 078/2002). The animal ethics permits were issued by the Ethics Commission on Animal Use of the Institute of Biosciences at the State University of São Paulo, Campus of Rio Claro - CEUA-IB-UNESP-CRC (Protocol # 7943) and the Bioethics Commission of the Faculty of Veterinary and Zoo Technology from the University of São Paulo (Protocol # 630/2005).

Study area and sampling

We obtained samples from seven localities in Brazil (Fig. 1) within three biomes where the species is classified at different degrees of threat: Pantanal (near threatened), Cerrado (endangered) and Atlantic Forest (critically endangered). Individuals were opportunistically captured in traps. Four localities were sampled in the Pantanal (Mato Grosso do Sul state, MS): 1) Fazenda Rio Negro (P-RN, 19°34'S, 56°14'W) with 7647 ha in the lower-middle Rio Negro (Biondo et al., 2011), sampled from January/2002 to August/2006. P-RN has forests, open grasslands, flooded grasslands ("vazantes") and lakes (e.g. "baías" and "Salinas¨) (Eaton, 2006); 2) Fazenda Santa Emília and surroundings (P-SE, 19°30'S, 55°36'W) encompass a total of 2600 ha along the upper-middle Rio Negro, including well-preserved to very disturbed areas (Biondo et al., 2011) sampled from April/2006 to July/2009; 3) Refúgio Ecológico Caiman (P-REC, 19°57'S, 56°18'W) with an area of 5600 ha of Private Natural Heritage Reserve, which has ecotourism activities and conservation programs (Refúgio Ecológico Caiman, 2019); and 4) Fazenda 23 de Março (P-VM, 20°09'S, 56°10'W) with an area of 5932 ha. Both P-REC and P-VM area located in the Miranda subregion. The vegetation encompasses flooded grasslands, savannas (*cerrados*), and semideciduos seasonal forest (Pott et al., 2011). P-REC was sampled from December/2014 to August/2015, and P-VM was sampled from October/2012 to May/2016.

Two localities were sampled in the Cerrado: 1) Fazenda Colorado and fragments (C-CO, 19°46'S, 55°14'W, MS) within the 3000 km^2 eastern highlands of the Maracaju mountain range in the Pantanal highlands. Due to agricultural expansion of cattle ranching, this region has been deforested and fragmented (Santana, 2015). Sampling was carried out from November/2009 to January/2011, March/2013, and February/2015 to February/2016; 2) Emas National Park and surroundings (C-PNE, 18°19'S, 52°45'W; Goiás state, GO), which comprise 131,864 ha of grassland plains, shrub fields, marshes and riparian forests. The National Park is surrounded by crop plantations (Jácomo et al., 2013) and was sampled from July/2001 to July/2002.

In the Atlantic Forest, we sampled the Morro do Diabo State Park (33,845 ha; a high-level priority area for Atlantic Forest conservation, Galleti et al., 2009) and two surrounding fragments (Fazenda Ponte Branca – 1195 ha, and Fazenda Santa Mônica – 584 ha) in the Pontal do Paranapanema region (AF-PP, 22°27'S, 52°10'W; São Paulo state, SP), which comprise areas of the inland Atlantic Forest. Local vegetation is comprised of semideciduos seasonal forest (Durigan and Franco, 2006). The remaining 5% of the region's original vegetation is distributed in several fragments surrounded by crops, pasture, and rural settlements (Faria, 2006). Samples were obtained from 2001 to 2005.

Blood samples were collected from 361 individuals: 134 males and 227 females (Table 1). This proportion of males to females (1̃:2) is congruent with the female-biased sex ratio of white-lipped peccaries (Biondo et al., 2011). The animals were captured using box-traps and pig pens and were immobilized with a combination of zolazepam and tiletamina (Zoletil®). Blood samples were collected in vacutainer tubes containing EDTA and stored at −20 °C. Each individual was marked with a microchip or earring. Further details about the capture and handling methods are described in Nava (2008); Biondo et al. (2011), and Jácomo et al. (2013). All samples were georeferenced with a GPS.

DNA extraction, PCR and genotyping

We extracted the DNA from the samples collected in P-RN and P-SE, using a standard proteinase K and phenol-chloroform protocol (Sambrook et al., 1989). DNA from C-PNE samples was extracted using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific®), following the manufacturer's instructions. We extracted the DNA from the remaining samples with a salting out protocol (Lahiri and Nurnberger, 1991) that was modified as follows: we used TTKM1 to lyse the cells and added proteinase K together with TKM2 and SDS 10%, we did not use 10 mM KCl to prepare TKM1 and TKM2, and used NaCl 5 M instead of NaCl 6 M. We amplified 13 microsatellite markers by Polymerase Chain Reaction (PCR) using seven primer-pairs specifically developed for white-lipped peccary, *Tayassu pecari* (Tpec3, Tpec4, Tpec5, Tpec10, Tpec12, Tpec16, Tpec18; Dalla Vecchia et al., 2011), one primer-pair designed for collared peccary, *Pecari tajacu* (PT0226; Biondo et al., 2011), and five primer-pairs developed for domestic pig, *Sus scrofa* (SW444, SW857, SW957, ACTG2, IGF1; Rohrer et al., 1994, 1996). All the heterologous primers were previously tested in white-lipped peccaries (Silva et al., 2010; Biondo et al., 2011). All forward primers included an additional 5'-M13 tail (5'CACGACGTTGTAAAACGAC3') following Boutin-Ganache et al. (2001). PCRs were carried out at a 12 μl total volume containing: 4̃5 ng of DNA, 1x PCR buffer, 0.2 mM of dNTPs, 2.5 mM of $MgCl_2$, 3 pmol of the reverse primer, 2 pmol of the M13 fluorescent primer (FAM or HEX), 1 pmol of the forward primer, 0.5 U of *Taq* polymerase, and 6.8 μl of ultrapure water. Amplification cycle conditions were: initial denaturation at 95 °C for 5 min; 35 cycles of 94 °C for 30 s; annealing temperature specific to each primer (Tpec16 at 52 °C; Tpec18 at 54 °C; PT0226 at 55 °C; IGF, SW444, SW857, Tepc3, Tpec4, Tpec5, Tpec10 and Tpec12 at 58 °C; ACTG and SW957 at 62 °C) for 30 s; and 72 °C for 30 s; followed by a final extension at 72 °C for 10 min. PCR products were genotyped in an ABI 3730 (Applied Biosystems) automatic sequencer. Genotypes were scored using GeneMarker software (Softgenetics). PCR and genotyping procedure were repeated until we obtained a consistent genotype for each sample and locus. Genotypes obtained for each locus were analyzed using MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) to detect null alleles and other genotyping errors. We excluded the loci with significant evidence of null alleles (IGF1, SW444, and Tpec5; see Results) from the population genetic structure analyses.

Genetic diversity

In order to test the hypothesis that Pantanal populations have a higher level of genetic diversity than the Cerrado and the Atlantic Forest populations, we calculated the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, and the number of private alleles (P_A) using GenAlex version 6.5 (Peakall and

Table 1
Indices of genetic diversity for 361 white-lipped peccaries (*Tayassu pecari*) from seven localities in Brazil (Fazenda Rio Negro = P-RN; Fazenda Santa Emília = P-SE; Refúgio Ecológico Caiman = P-REC; Fazenda 23 de Março = P-VM; Fazenda Colorado = C-CO; Emas National Park = C-PNE; Pontal do Paranapanema region = AF-PP). The values reported represent the number of individuals (n), the mean (standard deviation) number of alleles (N_A), allelic richness (A_R), observed (H_O) and the expected (H_E) heterozygosities and private alleles (P_A) per locality and in total. The inbreeding coefficients (F_{IS}) reported represent the global estimation for each location based on all 13 loci (before the slash) and the estimation for each location that exclude the three loci with evidence of null alleles (IGF1, SW444, and Tpec5, after the slash). * significantly higher than zero ($p < 0.0157$, after Benjamini and Yekutieli correction).

Locality	n	N_A	A_R	H_O	H_E	P_A	F_{IS}
P-RN	53	4.92 (2.90)	4.42 (2.49)	0.54 (0.19)	0.57 (0.20)	3	0.05/0.05
P-SE	131	5.46 (3.55)	4.51 (2.56)	0.60 (0.21)	0.59 (0.21)	1	−0.01/−0.03
P-REC	22	4.46 (2.73)	4.41 (2.70)	0.60 (0.17)	0.59 (0.16)	–	0.01/−0.02
P-VM	27	4.15 (2.19)	4.01 (2.03)	0.53 (0.28)	0.52 (0.21)	1	0.01/−0.04
C-CO	39	4.85 (3.05)	4.53 (2.73)	0.54 (0.23)	0.56 (0.21)	–	0.05/0.02
C-PNE	48	5.46 (3.15)	4.89 (2.62)	0.54 (0.26)	0.57 (0.20)	6	0.07*/0.01
AF-PP	41	4.62 (2.43)	4.32 (2.24)	0.56 (0.22)	0.54 (0.19)	9	−0.03/−0.01
Total	361	4.85 (0.49)	4.44 (0.26)	0.56 (0.03)	0.56 (0.02)	20	

Smouse, 2012). Allelic richness (A_R), and inbreeding coefficient (F_{IS}) were calculated with FSTAT version 2.9.3.2 (Goudet, 2002). This software was also used to verify if F_{IS} values were significantly higher than zero, and the Benjamini and Yekutieli (2001) corrections were applied to adjust the critical value of p. We compared genetic diversity indices and F_{IS} values between localities using the Friedman test in SPSS 13.0 (SPSS Inc., Chicago, Illinois). To verify departures from Hardy-Weinberg, we performed probability tests using GENEPOP (Web version 4.2; Raymond and Rousset, 1995), and applied Benjamini and Yekutieli (2001) corrections to adjust the critical value of p in cases with multiple comparisons.

Population genetic structure

In order to verify if the number of molecular markers used was informative enough to detect genetic differentiation, we used POWSIM version 4.1 (Ryman and Palm, 2006). We carried out 1000 simulations, considering seven sampled populations and the sample sizes corresponding to those from our sampling localities. We used combinations of N_e (effective size) - t (generations) of 2000-20, 2000-40 and 2000-205 to verify the probability of detecting expected F_{ST} of 0.005, 0.01 and 0.05. The power to detect genetic differentiation was tested by the chi-square and Fisher's exact tests.

To test the hypothesis that the Pantanal, Cerrado, and Atlantic Forest populations are genetically differentiated, we used the Bayesian clustering method implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2000). This method infers the number of populations (K) in the dataset by probabilistically assigning individuals to clusters (Pritchard et al., 2000). We tested the number of populations (K) from 1 to 10, with 10 independent runs for each K, using 500,000 burn in runs followed by 500,000 iterations. We ran the analyses using admixture model and correlated allele frequencies. We performed two different analyses, one using sampling location (LOCPRIOR model) and another without this information. To determine the most likely number of populations (K), we estimated the mean log-likelihood values of the posterior probabilities LnP(K) (Pritchard et al., 2000) and the ΔK (Evano et al., 2005) using STRUCTURE HARVESTER version 0.6.94 (Earl and Vonholdt, 2012). Graphical displays of the clusters for the best K values were obtained using CLUMPAK, which groups independent runs to estimate a unique membership value for each individual in each cluster (Kopelman et al., 2015). In addition, we calculated pairwise F_{ST} (Wright, 1951, 1965) to infer the degree of genetic differentiation between localities using FSTAT 2.9.3.2 (Goudet, 2002). The significance of the estimated values was verified using a G test in the same software. The critical p value was corrected for multiple comparisons following Benjamini and Yekutieli (2001).

To verify the effect of geographical distance on the population genetic structure (isolation by distance, IBD), we carried out a Mantel Test with 999 permutations using GenAlex version 6.5 (Peakall and Smouse, 2012). To perform this test, we obtained a codominant genotypic matrix and a geographic distance matrix between pairs of individuals in GenAlex. We also performed a spatial autocorrelation analysis of pairwise genetic distances between individuals at different geographical distance classes to verify the spatial extent of the genetic structure. This analysis was carried out in GenAlex, and we used 9999 permutations and 10,000 bootstraps to determine the significance of spatial autocorrelation coefficients (r).

To infer recent dispersal, we determined first-generation migrants between locations using the maximum likelihood estimation L_home/L_max in GENECLASS version 2.0 (Piry et al., 2004) and applied the Bayesian criterion of Rannala and Mountain (1997), with Markov chain Monte Carlo resampling method (Paetkau et al., 2004). We simulated 10,000 individuals and used an alpha of 0.01.

Results

Genetic diversity

We observed moderate levels of genetic variability in all localities (Tables 1, S1). The mean number of alleles (N_A) and mean allelic richness (A_R) ranged from 4.15 in P-VM to 5.46 in P-SE and C-PNE, and from 4.01 in P-VM to 4.89 in C-PNE, respectively. The mean observed (H_O) and expected (H_E) heterozygosities ranged from 0.53 in P-VM to 0.60 in P-SE and P-REC, and from 0.52 in P-VM to 0.59 in P-SE and P-REC, respectively. The genetic diversity indices did not differ significantly among localities (Friedman Test; A_R, $\chi^2 = 10.57$, $p = 0.10$; H_E, $\chi^2 = 7.08$, $p = 0.31$; H_O, $\chi^2 = 6.05$, $p = 0.42$), with the exception of the number of alleles (N_A, $\chi^2 = 15.30$, $p = 0.02$). We found three private alleles in P-RN, one in P-SE and P-VM, six in C-PNE and nine in AF-PP, respectively (Tables 1, S1).

We observed significant departures from Hardy-Weinberg equilibrium ($p < 0.0157$) for the following loci and localities: IGF1 (P-SE, C-CO, and C-PNE), SW444 (C-PNE), SW957 (P-REC), Tpec5 (P-REC), and Tpec10 (P-VM; Table S1). Three of these loci showed evidence for the presence of null alleles, with the following frequencies: IGF1 (C-CO = 0.16, C-PNE = 0.21), SW444 (C-PNE = 0.17), and Tpec5 (P-REC = 0.18). The inbreeding coefficient (F_{IS}) ranged from −0.03 (AF-PP) to 0.07 (C-PNE, Table 1). We found F_{IS} was significantly higher than zero only in C-PNE ($p < 0.0157$), possibly due to the presence of null alleles in the loci IGF1 and SW444 in this locality (Table S1). After removing the three loci with evidence of null alleles from the entire data set, F_{IS} was not significant for any locality (Table 1), suggesting random mating between individuals.

Population genetic structure

The POWSIM results indicated that ten microsatellite loci were sufficient to detect true genetic differentiation with: i) 100% (chi-square test) and 99.6% (Fisher's exact test) probability of detecting

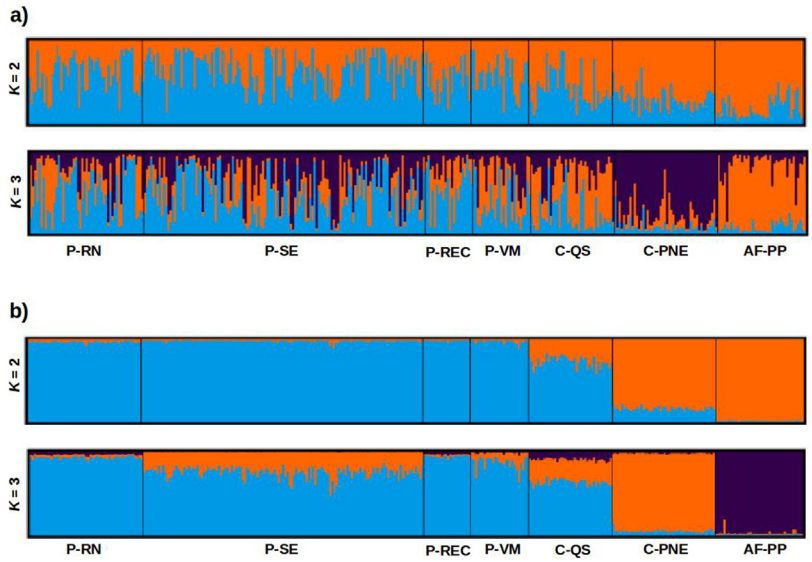

Fig. 2. Results of the Bayesian clustering analysis performed in STRUCTURE, showing the percentage of membership of each individual to each cluster. a) $K = 2$ and $K = 3$, without using the LOCPRIOR model; b) $K = 2$ and $K = 3$, using the LOCPRIOR model.

Table 2
Genetic differentiation (F_{ST} values, below diagonal) of white-lipped peccaries (*Tayassu pecari*) from seven localities in Brazil. (Fazenda Rio Negro = P-RN; Fazenda Santa Emília = P-SE; Refúgio Ecológico Caiman = P-REC; Fazenda 23 de Março = P-VM; Fazenda Colorado = C-CO; Emas National Park = C-PNE; Pontal do Paranapanema region = AF-PP). All values were significantly different from zero (p values, above diagonal) after Benjamini and Yekutieli (2001) correction, $p < 0.0192$.

	P-RN	P-SE	P-REC	P-VM	C-CO	C-PNE	AF-PP
P-RN		0.002	0.002	0.002	0.002	0.002	0.002
P-SE	0.01		0.002	0.002	0.002	0.002	0.002
P-REC	0.03	0.02		0.002	0.002	0.002	0.002
P-VM	0.04	0.04	0.04		0.002	0.002	0.002
C-CO	0.02	0.02	0.03	0.04		0.002	0.002
C-PNE	0.04	0.03	0.06	0.07	0.04		0.002
AF-PP	0.06	0.06	0.07	0.09	0.05	0.06	

a true F_{ST} value of 0.005; ii) 100% probability (for both chi-square and Fisher's exact tests) of detecting a true F_{ST} value of 0.01; and iii) 100% probability (for both chi-square and Fisher's exact tests) of detecting a true F_{ST} value of 0.05. The α error for both chi-square and Fisher's exact tests was close to 5%.

The Bayesian approach implemented in STRUCTURE, both with and without LOCPRIOR model, supported $K = 2$ as the most likely value of K, followed by $K = 3$ (Table S2; Fig. 2a and b). The results using LOCPRIOR showed lower levels of genetic admixture, thus resulting in more differentiated groups than the results of the analysis that did not use this model (Fig. 2a and b). Assuming $K = 2$, the Pantanal localities (P-RN, P-SE, P-REC, and P-VM) plus the Cerrado locality in the Pantanal highlands (C-CO) were grouped into a genetic group, while the Cerrado of C-PNE and the Atlantic Forest (AF-PP) localities were together in another group (Fig. 2a and b). $K = 3$ showed the same clustering pattern as observed for $K = 2$, but separated C-PNE from AF-PP into two distinct groups (Fig. 2a and b).

We found a mean F_{ST} value of 0.04 ± 0.03. The lowest value was observed between the Pantanal locations P-SE and P-RN ($F_{ST} = 0.01$), and the highest was observed between P-VM and AF-PP ($F_{ST} = 0.09$; Table 2). C-PNE and AF-PP had a moderate level of genetic differentiation ($F_{ST} = 0.06$). All pairwise values were different from zero ($p < 0.0192$; Table 2), showing significant genetic differentiation between localities.

We found a significant but weak positive correlation between genetic and geographic distances (Mantel test; $R_{XY} = 0.178$, $p = 0.001$; Fig. 3). We detected significant positive spatial autocorrelation at the first distance class, 100 km ($r = 0.015$, $p = 0.0001$), suggesting that within this distance individuals are genetically more similar than more spatially distant individuals. The extension of the positive genetic structure was identified by the x intercept at 180 km (Fig. 4).

GENECLASS identified four white-lipped peccary females as first-generation migrants: one from C-CO but sampled in P-REC; one from P-SE sampled in C-CO; one from P-RN sampled in C-PNE; and one from C-PNE sampled in AF-PP (Table 3).

Discussion

We found moderate levels of genetic diversity in all localities. The values observed here were similar to those observed by Biondo et al. (2011) for P-RN and P-SE, using seven of the same microsatellite markers and some of the individuals studied here (mean values of allelic richness and observed and expected heterozygosities of 4.96, 0.56, and 0.57 for P-RN, respectively, and 4.98, 0.62, and 0.58 for P-SE). The genetic diversity indices did not differ between the biomes. This result did not support our hypothesis of a higher level of genetic diversity for white-lipped peccaries from the Pantanal in comparison to those from the Cerrado and Atlantic Forest. The lack of relationship between biome threat level and the levels of genetic diversity may be due to the presence of well-preserved areas in the sampled regions of all biomes. These areas can act as biodiversity sources, especially for species with intermediate and high dispersal capability (Tambosi et al., 2014), that can colonize smaller fragments that are far from reserves and maintain some level of gene flow. In the C-PNE, the results found by Jácomo et al. (2013) suggested that the herds use forest areas within Emas National Park and dispersed fragments outside the park. Recently, Russo et al. (*in prep.*) observed evidence of gene flow between Morro Do Diabo

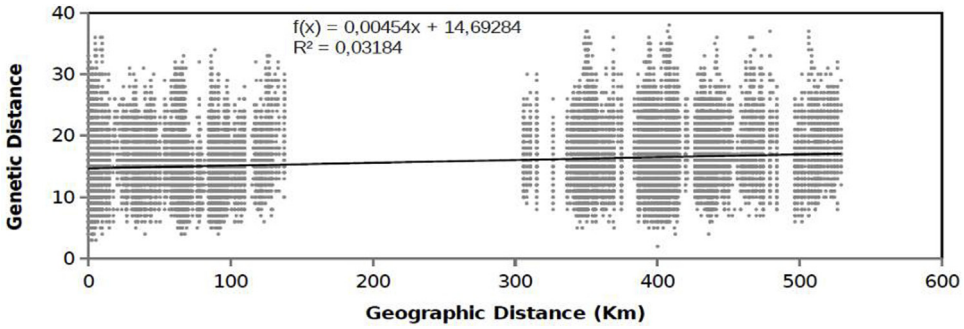

Fig. 3. Results of isolation by distance (IBD) analysis for white-lipped peccaries (*Tayassu pecari*) from seven localities in Brazil. Each point represents a pairwise comparison between codominant genotypic distance and geographic distance (km) among individuals.

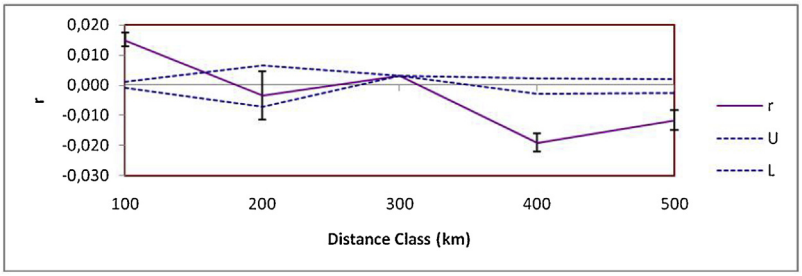

Fig. 4. Correlogram of the spatial autocorrelation analysis for white-lipped peccaries representing the spatial autocorrelation coefficient (*r*) at five distance classes. Dashed lines represent upper and lower 95% confidence intervals for the null hypotheses of no genetic structure. Error bars show 95% confidence intervals of *r* based on bootstrapping results.

Table 3
White-lipped peccary first-generation migrants (F_0) identified by GENECLASS, using likelihood computations (L_home/L_max). Origin is the sampling location of the given individual (1- P-RN, 2- P-SE, 3- P-REC, 4- P-VM, 5- C-CO, 6- C-PNE, 7- AF-PP). *P*-value < 0.01 indicates potential first-generation migrants. Values in bold indicate the best assigned locality for each individual.

				-log(L)						
Individual	Origin	-log (L_home/ L_max)	Probability	1	2	3	4	5	6	7
TC12	3	4.58	0.001	11.26	12.42	14.56	12.82	**9.98**	12.90	11.66
TT24	5	3.94	0.002	9.38	**7.48**	8.64	10.49	11.42	9.29	14.28
TE12	6	2.87	0.003	**9.42**	10.34	13.47	10.51	11.66	12.29	12.76
PT8	7	4.38	0.0003	9.49	9.85	13.55	9.93	12.66	**7.32**	11.71

State Park and a close fragment, Ponte Branca, areas that were both studied here.

STRUCTURE results supported two genetic clusters (K = 2) as the most probable scenario, and K = 3 as the second most probable scenario. As expected, the populations analyzed were genetically differentiated according to their geographic distance. The Mantel test showed a pattern of isolation by distance, indicating that the gene flow was limited by the increasing geographical distance between populations. In addition, the results obtained by the spatial autocorrelation analysis revealed that the dispersal of white-lipped peccaries is geographically limited at a distance of 180 km.

The clustering of the Pantanal localities and C-CO (Pantanal highlands) (K = 2 and K = 3) could be due to the proximity between these localities (25–137 km). This could have facilitated gene flow, as observed by Biondo et al. (2011) for P-RN and P-SE, which are located 80 km apart. Corroborating this idea, we observed evidence of recent dispersal events of individuals from the Pantanal (P-SE) to Cerrado (C-CO) and from Cerrado (C-CO) to Pantanal (P-REC), according to GENECLASS. Although significant F_{ST} values were observed between these localities, it represented a weak genetic differentiation (mean F_{ST} value of 0.03 ± 0.01).

Considering the pattern of isolation by distance found, K = 3, which split C-PNE and AF-PP into two distinct genetic groups, seems to be the most plausible scenario as these localities are 500 km apart. Even though we found evidence of a recent dispersal event from C-PNE to AF-PP, the F_{ST} value of 0.06 showed moderate levels of genetic differentiation between these localities. We also observed higher numbers of private alleles in C-PNE and AF-PP, which could indicate an effect of genetic drift in these populations. In other words, the genetic drift could be stronger than gene flow (Slatkin, 1987).

AF-PP and Pantanal localities are about 410–500 km apart and we also observed significant genetic differentiation between these localities, with the highest values of F_{ST} (mean F_{ST} value of 0.07 ± 0.01) and no first-generation migrants. Similar to our results, genetic differentiation between Pantanal and Atlantic Forest was also observed for jaguars (*Panthera onca*; Valdez et al., 2015) and for the giant anteater (*Myrmecophaga tridactyla*; Clozato et al., 2017).

Concluding, we found moderate levels of genetic diversity in all localities, and the populations were differentiated according to geographical distance. Although we observed moderate levels of genetic diversity, Pantanal, Cerrado and Atlantic Forest biomes have been suffering strong anthropogenic impacts. It is important to maintain the genetic diversity found for these white-lipped peccary populations, with special attention to the populations that are critically endangered, as those in the Atlantic Forest. To ensure the connectivity between the localities studied and surrounding

fragments within each of the three genetic groups, it seems to be important to preserve riparian zones (Biondo et al., 2011), which act as corridors for white-lipped peccaries (Keuroghlian and Eaton, 2008b). Therefore, improving the connectivity could increase the chances of gene flow, preventing the loss of the genetic diversity and maintaining the population viability of white-lipped peccary populations, as well as for other species.

Declarations of interest

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mambio.2019.03.001.

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