Genetica
https://doi.org/10.1007/s10709-020-00099-y

ORIGINAL PAPER

Phylogeography of feral Monteiro pig in the Brazilian Pantanal Ecosystem

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Received: 12 September 2019 / Accepted: 29 July 2020

Abstract

The Monteiro is a feral pig found in the Brazilian Pantanal ecosystem. The goal of this research is to generate data and knolewdge related to animal populations wich can be used for management and development of an in vitro conservation program for animal resourses at Pantanal ecosystem. The present study evaluated animals sampled from 10 distinct locations within the region, using 19 microsatellite markers (N = 189) and the control region of mitochondrial DNA (mtDNA) (N = 392). Low genetic differences were found between populations with the microsatellite data. The F_{ST} range was between 0.009 and 0.063 (p-value < 0.05). The Mantel test corroborated with previous results, as low correlations between genetic and geographic distances were observed ($r^2 = 0.2309$, p = 0.06). Bayesian analysis for genetic structure identification placed the Monteiro pigs into three main clusters (MOB, Pop 1 and all others Pantanal populations). Most of the Monteiro pigs share a single European haplotype as seen by mtDNA analyses. This haplotype is not exclusive, as it is shared with other swine populations (commercial and other locally adapted breeds). Monteiro populations from different geographic locations within Pantanal are not isolated and can be considered as a large unique population. Since animals roam freely to seek food and water, or even due to seasonal flooding of their habitat, the Monteiro populations presented absence of major genetic structure and evidence of high gene flow. These results can be used to create a management plan and in situ and ex situ conservation program for conservation and use of the Monteiro breed in the Pantanal ecosystem.

Keywords Geographic distance · Genetic diversity · Microsatellites · mtDNA · *Sus scrofa*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10709-020-00099-y) contains supplementary material, which is available to authorized users.

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Published online: 07 August 2020

Introduction

The Monteiro pig, found in the Brazilian Pantanal (the world's largest inland tropical wetland), is a locally adapted, feral breed (Alderson 1999), originated from swine populations brought by European settlers to the Corumbá region in Mato Grosso do Sul State (centre-west region of the Country), in the latter half of the XVIII century. These pigs are phenotypically similar to wild boars (*Sus scrofa scrofa*), with cone-shaped heads and bodies, grey, blackish or brown hair, and small erect ears. They are fully integrated into the Pantanal ecosystem and can contribute to the preservation of native species, being an alternative protein source for the local human population, different from many other feral pig populations that are considered threats to the local environment. The Pantanal ecosystem has two well-defined contrasting seasons characterized by extreme flooding versus hot and dry conditions, when the Monteiro pigs are hunted (Desbiez et al. 2010).

Phylogeography is, in a broad sense, the study of the geographic distribution of populations using genetic variation. The variation can be studied through molecular markers such as those located on the sex chromosomes, mtDNA (Larson et al. 2005; Osei-Amponsah et al. 2017; Vilaça et al. 2014), as well as those obtained from whole-genome sequencing studies (Groenen et al. 2012; Li et al. 2014). Population genetics studies with mtDNA, Y-chromosome and autosomal markers (microsatellites and SNPs) have been published with swine domestic breeds and wild boars (Burgos-Paz et al. 2013a; Larson et al. 2005; Ramírez et al. 2009), among others reviewed by Scandura et al. (2011). Some genetic analyses using the Monteiro breed have also been conducted (Burgos-Paz et al. 2013b; Sollero et al. 2009; Souza et al. 2009). Those previous studies, with STRs, mtDNA and SNP markers, embraced several Brazilian swine breeds in order to quantify the genetic diversity within and between breeds and to explore the origin and relationship of Brazilian breeds worldwide.

The Brazilian government, represented by the Brazilian Agricultural Research Corporation (Embrapa) and some Federal Universities, support a nationwide animal genetic resources conservation program, to cryopreserve germplasm and manage in situ populations from locally adapted livestock genetic groups/breeds (Mariante et al. 2009). Considering that this program does not have any up to date genetic material cryopreserved, nor any conservation nucleus in place for Monteiro breed, it was decided to investigate genetic structure in a broad sample of feral Monteiro pigs derived from a different regions of the Pantanal, Mato Grosso do Sul, Brazil, using autosomal microsatellites and SNP markers from mtDNA. The ultimate goal is to use such data as a proxy to support the creation of a management and in vivo conservation plan to improve the sustainable use of animal genetic resources in Brazil, in the unique and important ecosystem that is the Pantanal.

Material and methods

Samples

Feral Monteiro pigs can be found scattered over the whole Pantanal ecosystem. The Nhecolândia region, a sub-region of the Pantanal, evolving the city of Corumbá, was the focus for this paper (Fig. 1).

Blood or hair samples (depending on the availability) from 181 animals from ten distinct geographic locations (PG), within Nhecolândia (Fig. 1, Table 1, Supplementary Material S1) were collected. All samples were georeferenced at the site of collection using GPS Garmin 60 (Datum-WGS 84). Eight additional samples were collected in private farms around Brasilia, Federal District (MOB) as an outgroup. Genomic DNA was extracted as previously described (Sollero et al. 2009).

For mtDNA analysis a total (N) of 392 pigs was analyzed. Samples of Brazilian local pig breeds/genetic groups (N = 168), commercial (N = 29) and crossbred (N = 6), totalling 203 animals were added from previous studies (Cavalcante Neto 2010; Souza 2011). The breeds or genetic groups from these samples were: Baé, Casco de Burro, Caruncho, Canastra, Mestiços, Moura, Monteiro, Nilo, Rabo de Paixe, Piau and Tatu (local breeds/genetic groups); Landrace, Duroc, Large White, MS60 and Pietran (commercial). These sequences were aligned together with the Monteiro sequences (N = 189) to identify the primary sources of origin of Monteiro animals.

Microsatellite genotyping and data analysis

PCR amplification of nineteen microsatellites (Supplementary Material S2) was performed in multiplexed reactions using the Qiagen Multiplex PCR Kit (Qiagen Inc., Valencia, CA, USA). PCR products (1 µl) were submitted to capillary electrophoresis in an ABI 3730 genetic analyzer (Applied Biosystems, USA). Fragment analysis and genotyping calls were performed with GeneMapper software® (Applied Biosystems, USA) and sizes (bp) of each allele were classified in discrete bins using FlexiBin v2.0 (Amos et al. 2006).

Genetic diversity parameters were estimated within and among Monteiro populations with Molkin v3.0 (Gutierrez et al. 2005). Number and frequency of private alleles were estimated with GenAlex 6.3 for all populations and markers (Peakall and Smouse 2005). A kinship test (relatedness) was carried out using Ritland's method (Ritland

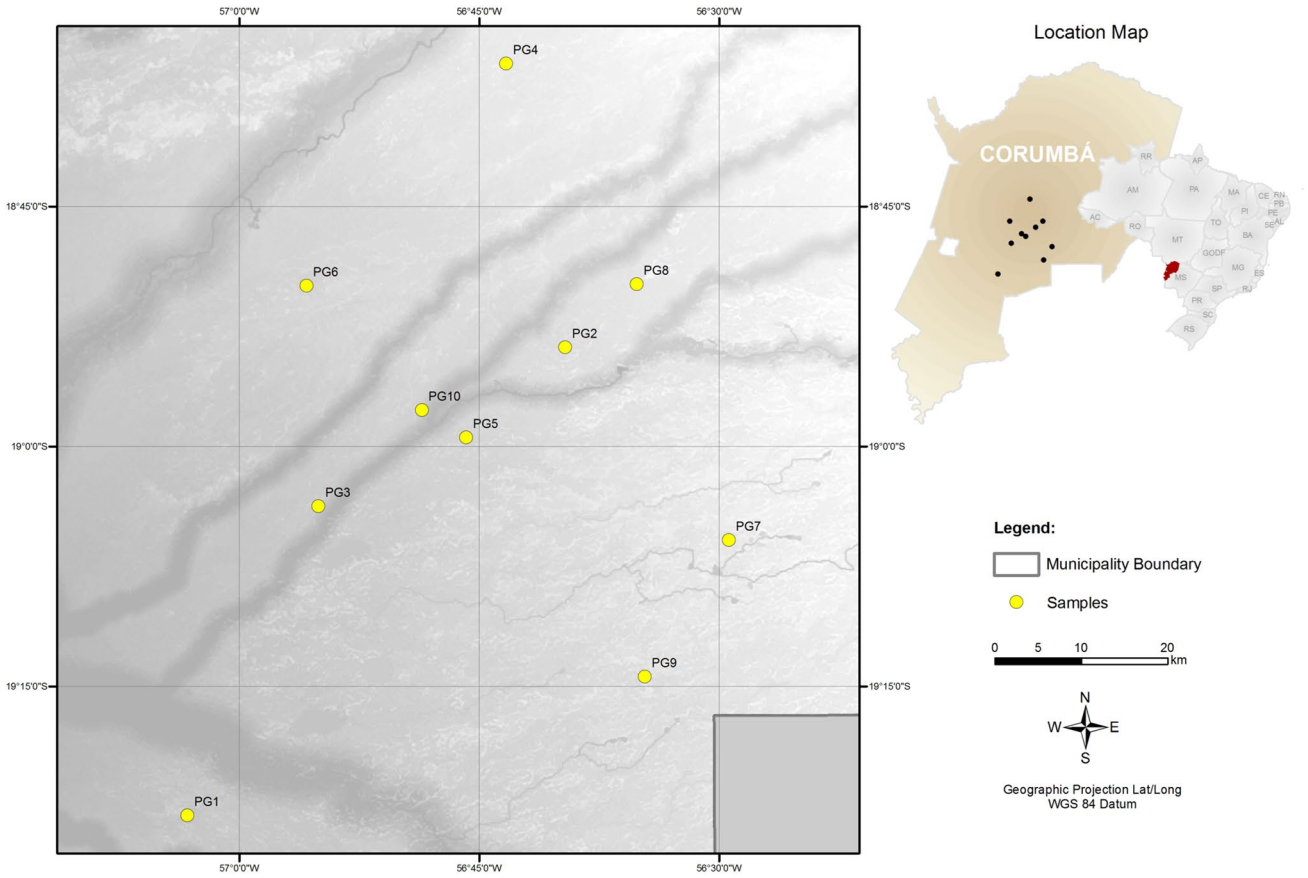

Fig. 1 The study area showing the distribution of Monteiro Feral Populations (yellow dots) in relation to Mato Grosso do Sul State and Brazil (Acronyms in Table 1). Gray areas thickness corresponds to streams and rivers

Table 1 Genetic variability per population based on 19 microsatellite markers

Population	N	Nam	Nae	Nar	Ho	He	F_{IS}	HWE
MOB	8	5.42	3.89	4.16	0.61	0.71	0.19	0.005*
Pop 1 (PG1)	5	3.68	2.72	3.41	0.60	0.60	0.11	0.12
Pop 2 (PG2)	16	5.31	3.36	3.55	0.62	0.64	0.07	0.03*
Pop 3 (PG3)	16	4.63	2.98	3.32	0.59	0.62	0.05	0.07
Pop 4 (PG4)	18	4.68	3.07	3.34	0.63	0.64	0.04	0.16
Pop 5 (PG5)	26	5.05	3.20	3.37	0.67	0.65	− 0.01	0.65
Pop 6 (PG6)	10	4.26	3.13	3.43	0.65	0.65	0.04	0.15
Pop 7 (PG7)	15	5.21	3.14	3.30	0.62	0.63	0.01	0.30
Pop 8 (PG8)	36	3.47	2.73	3.47	0.67	0.59	0.01	0.46
Pop 9 (PG9)	04	4.42	3.00	3.27	0.63	0.61	− 0.03	0.75
Pop 10 (PG10)	35	6.05	3.40	3.53	0.58	0.65	0.09	0.00*
Average all populations		4.74	3.15	3.47	0.62	0.64	0.05	
Average Pantanal populations		4.67	3.07	3.40	0.63	0.63	0.04	

N number of animals within populations, *Nam* average number of alleles, *Nae* effective number of alleles, *Nar* rarefacted number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity in HWE, F_{IS} inbreeding coefficient, *HWE* Hardy–Weinberg Equilibrium

*$p < 0.05$

1996) implemented in GenAlex 6.5 (Peakall and Smouse 2012). Kinship indices (r) equal to or greater than 0.25 indicate closely related animals within populations.

Population genetic differentiation indices were estimated using Reynolds et al. (1983) linearized F_{ST} for short divergence time algorithm with Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). Bayesian analysis of population structure was performed with Structure 2.3.4 software (Pritchard et al. 2000), using the Admixture model, with K (clusters) ranging from 1 to 20, 100,000 burn-in period, 400,000 Markov chain Monte Carlo (MCMC) simulations, and five interactions for each K. To infer the ideal number of clusters, the delta K method (Evanno et al. 2005) implemented in Structure Harvester (Earl and vonHoldt 2012) was applied. Plotting and reporting clusters were carried out with Clumpak software (Kopelman et al. 2015). The genetic structure analysis was carried out to test the hypothesis of no differentiation among all sampling locales analysed from this Pantanal sub-region.

Mantel tests for matrix correlation between genetic and geographic distances (Mantel 1967) were estimated in GenAlex 6.5 (Peakall and Smouse 2012) using 10,000 permutations. Analysis of Molecular Variance (AMOVA) was performed using Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010), at two distinct levels: (1) using the 11 Monteiro populations; and (2) using the 10 Monteiro populations from Pantanal ecosystem.

Mitochondrial DNA sequencing and analysis

A 511 bp fragment within the control region of the porcine mitochondrial DNA, between the positions 15,390 and 15,900 bp was amplified from all individual with the same primer pair described by Alves et al. (2003). PCR products were generated, cleaned and sequenced using BigDye® v.3 and an ABI 3100 genetic analyzer (Applied Biosystems, USA) following previously described procedures (Alves et al. 2003; Souza et al. 2009).

All obtained mtDNA sequences were aligned and compared with AJ002189 (Ursing and Arnason 1998) as the reference sequence. A total of 363 bp of mtDNA was used for haplotype reconstruction and polymorphism detection in 392 samples with Mega v.5 (Tamura et al. 2013). The haplotypes sequences are available at NCBI, accession numbers MG837527 to MG837543.

The genetic diversity parameters of mtDNA were estimated by DnaSP (Librado and Rozas 2009). A median-joining network was constructed using the software package Network v5 (Bandelt et al. 1999) comparing haplotypes between Monteiro breed with specialized and other local swine breeds/genetic groups (Cavalcante Neto 2010; Souza 2011).

Table 2 Number of private alleles (PA) and private allele frequency (PAf) by microsatellite marker (Locus) observed in populations of feral Monteiro pigs in Brazil

Population	Locus	PA	PAf
MOB	S0155	6	0.06
MOB	S0155	7	0.06
MOB	SW1517	7	0.31
MOB	S0002	1	0.06
MOB	SW936	3	0.12
MOB	SW2406	15	0.06
MOB	S0005	15	0.18
MOB	S0005	20	0.06
MOB	S0005	22	0.06
MOB	S0068	10	0.06
MOB	S0068	12	0.12
MOB	S0090	1	0.14
MOB	SW72	7	0.06
MOB	SW72	10	0.06
MOB	SW24	13	0.07
Pop3	SW1517	9	0.06
Pop3	S0005	1	0.03
Pop8	SW936	2	0.01
Pop8	S0005	13	0.01
Pop10	SW72	5	0.08
Pop10	S0097	10	0.02

Results

Microsatellite data

The average number of alleles observed for the 19 SSR loci was 6.78 varying from four to 11 alleles per locus. SW72 and SW445 were the markers with the smallest and largest number of alleles, respectively. Nine loci (S0026, S0155, SW1517, S0002, SW2406, S0005, S0101, S0090, and S0097) deviated from the Hardy–Weinberg equilibrium (Supplementary Material S3).

The mean within Pantanal Monteiro population diversity was slightly lower than that obtained for Monteiro sampled out of Pantanal region—MOB (Table 1). MOB and Pop1 populations had the highest significant ($p < 0.05$) F_{IS} (0.19 and 0.11, respectively). The average number of alleles per population ranged from 3.47 (Pop8) to 6.05 (Pop10) and the effective number of alleles ranged from 2.72 (Pop1) to 3.89 (MOB). MOB also showed the highest number of fixed alleles (Nar = 4.16). Two populations from the Pantanal (Pop5 and Pop 9) showed an excess of heterozygosity ($F_{IS} = -0.01$ and -0.03, respectively), but these were not significant ($p > 0.05$). The number of private alleles was detected in four sampling sites (MOB,

Pop3, Pop8 and Pop10) and MOB has the highest number of privative alleles (PA) across 10 distinct loci (Table 2).

The kinship analysis did not identify closely related individuals (Supplementary Material S4). For example, Pop1 had more variability between individuals (− 0.25 to 0.18), with an approximate kinship average of 0.05. Individuals from Pop2 (− 0.012) and Pop8 (− 0.045) did not share alleles by descent, and for Pop3, Pop4, Pop5, Pop7, and Pop10 the r values were significantly close to zero. Pop6 and Pop9 were the only ones that showed a mean relatedness value higher than the confidence interval.

Pairwise F_{ST} estimates have shown the highest value observed between MOB and Pop 8 (0.088) and, in general, significant F_{ST} values ($p < 0.05$) were observed when values were above 0.048 (Table 3). All pairwise F_{ST} between MOB population and Pantanal Monteiro feral populations were significant. Among the Pantanal populations, the greater differentiation was observed between Pop1 and Pop3 (0.063) (Table 3). The average distance in km between geographical points ranged from 28.42 to 63.04 km, within Pantanal populations (Fig. 1). The populations sampled from the closest and most distant geographical points were Pop5 with Pop10 (5.77 km) and Pop1 with Pop4 (93.51 km), respectively.

The Mantel test (Fig. 2) indicated that correlations between genetic and geographic distances were low, ranging from $r^2 = 0.2309$ ($p = 0.06$) using the Nei's D and $r^2 = 0.2466$ using pairwise F_{ST} ($p = 0.055$). AMOVA results (Table 4) revealed that most of the observed variation was within populations (98.16%) and a weak genetic structure ($F_{ST} < 0.02$, $p = 0.0001$) was found between Monteiro populations with or without the inclusion of MOB (Table 4). The higher genetic structure (Average $F_{ST} = 0.07$; $p = 0.0001$) was observed between MOB and Pantanal populations, as expected.

The genetic structure of the Monteiro populations was further investigated using Structure software, and the number of clusters (Ks) that best fitted the data was four (Fig. 3). The distribution was quite interesting, with one specific cluster for MOB, two for all Pantanal populations and one common to all groups. One of the specific Pantanal clusters was present in all populations except for Pop1 (PG1), that is geographically most distant from the others (Fig. 1). The delta K graph (Supplementary Material S5) presented a second peak on $K = 2$. For this, two distinct clusters were seen, formed by MOB and the Pantanal populations respectively, with proportions of assignment for MOB equal to 0.75, and the Pantanal Populations, ranging from 0.81 to 0.99 (Fig. 3).

Mitochondrial DNA data

After sequencing, the individual data of the Monteiro samples were filtered by quality and aligned with the GenBank and previous reference sequences. After analysis of quality and trimming, 61 samples were eliminated from the data, reducing the total of samples to 331. A total of 23 polymorphic sites were observed in the sequenced fragment: 20 transitions, one transversion (A ↔ T) and two deletions. The deletion, at position 15.707, was present in all animals in relation to the reference sequence, while the deletion at 15.571 was observed only in the Monteiro samples. Deletions were removed from the dataset for haplotype estimation. A total of 20 haplotypes were observed (Table 6).

Low nucleotide (0.00797 ± 0.00093) and moderate haplotype diversity (0.483 ± 0.041) was observed in the full population composed of 331 animals. Monteiro samples from the Pantanal had the lowest nucleotide (0.00045 ± 0.00044) and haplotype (0.016 ± 0.016) diversity, and commercial pigs breeds the highest (0.01874 ± 0.00186 and 0.884 ± 0.030) (Table 5).

Haplotypes H1, H13, H14 to H21 and H5 to H8 were exclusive to local and commercial breeds, respectively (Table 6). The remaining haplotypes (H2, H3, H4 and H9 to H12) were shared among the different populations. H2 was the haplotype with highest frequency and distribution (234 individuals from five populations and four regions),

Table 3 Pairwise F_{ST} estimates below the diagonal and *p* values above the diagonal, calculated using 19 microsatellite loci among 11 Monteiro feral pig populations

	MOB	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
MOB	–	0.030*	0.003*	0.001*	0.001*	0.001*	0.002*	0.001*	0.043*	0.001*	0.001*
Pop1	0.080	–	0.025*	0.001*	0.067	0.006*	0.404	0.000*	0.594	0.018*	0.009*
Pop2	0.055	0.055	–	0.640	0.256	0.128	0.001*	0.464	0.957	0.353	0.420
Pop3	0.069	0.063	0.018	–	0.118	0.020*	0.004*	0.277	0.837	0.023*	0.055
Pop4	0.067	0.045	0.019	0.019	–	0.181	0.119	0.502	0.771	0.076	0.040*
Pop5	0.066	0.048	0.016	0.019	0.014	–	0.003*	0.453	0.794	0.139	0.009*
Pop6	0.070	0.044	0.040	0.034	0.024	0.029	–	0.001*	0.512	0.001*	0.001*
Pop7	0.066	0.054	0.013	0.013	0.011	0.009	0.029	–	0.828	0.268	0.003*
Pop8	0.088	0.062	0.031	0.033	0.035	0.030	0.048	0.027	–	0.646	0.820
Pop9	0.073	0.052	0.020	0.025	0.022	0.018	0.043	0.014	0.033	–	0.003*
Pop10	0.057	0.048	0.014	0.018	0.016	0.014	0.036	0.014	0.031	0.024	–

*$p < 0.05$; 10,100 permutations

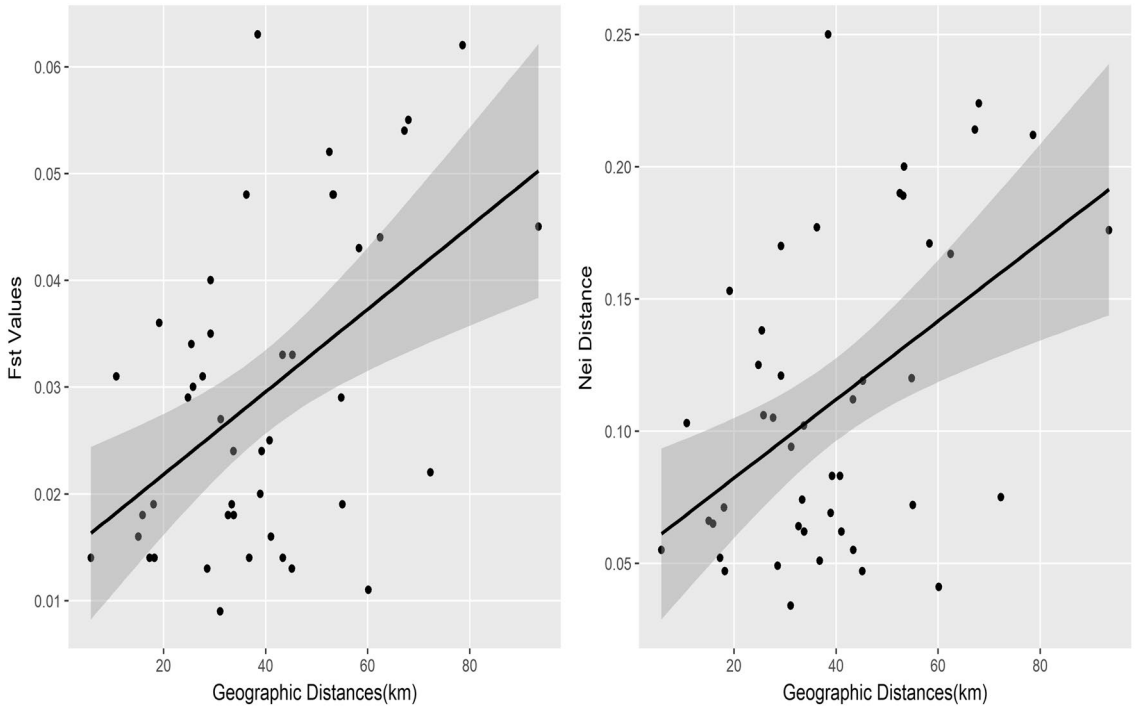

Fig. 2 Correlations between geographic distance and Nei's D_A genetic distance (**a**); geographic distance and F_{ST} (**b**) estimated by Mantel test

Table 4 Analysis of molecular variance (AMOVA) and F_{ST} using 11 populations, 10 populations, and using only the geographic information of Brazilian Monteiro breed

Structure	Source of variation	d.f	SQ	Variance components	% variation	F_{ST}
11 populations	Between populations	10	99.111	0.11467	1.84	0.018*
	Within populations	367	2245.347	6.11811	98.16	
10 populations	Between populations	9	76.320	0.06875	1.12	0.012*
	Within populations	352	2142.222	6.08586	98.88	

d.f. degree of freedom

*$p < 0.05$

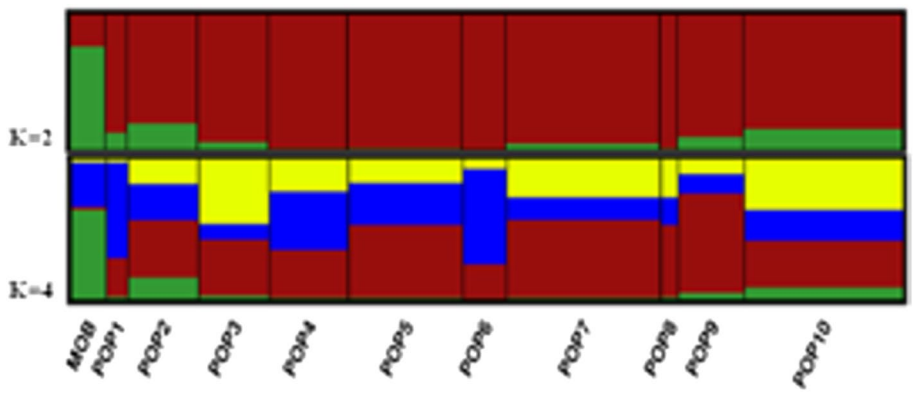

Fig. 3 Summary of estimate plots of Q for K = 2 and K = 4 in the 11 Monteiro feral pig populations. Each individual is represented by a single column portioned into K coloured segments whose length is proportional to the individual coefficient of membership in each of the K inferred clusters. Black lines separate the 11 populations

followed by H7, H4 and H13. H2 was also the most common haplotype for Monteiro breed appearing in five of six animals sampled from Brasilia (MOB) and in 121 of 122 from the Pantanal (Table 6). Regarding geographic distribution, Northeast (N = 70) and Southern regions (N = 42) showed the highest proportion of haplotypes (10), while the

Table 5 Genetic diversity at control region of Brazilian pigs by genetic groups

Genetic group	Sample size	Number of polymorphic sites	Nucleotide diversity (±SD)	Haplotype diversity (±SD)
Monteiro Pantanal	122	10	0.00045 ± 0.00044	0.016 ± 0.016
Monteiro Brasilia	6	2	0.00184 ± 0.00119	0.333 ± 0.215
Locally adapted	168	19	0.01149 ± 0.00157	0.693 ± 0.693
Crossbreds	6	10	0.01662 ± 0.00358	0.600 ± 0.129
Commercial	29	17	0.01874 ± 0.00186	0.884 ± 0.030
Total	331	21	0.00797 ± 0.00093	0.483 ± 0.041

South and Southeast concentrated higher number of unique haplotypes (H5; H8 to H11) and (H16 to H18), respectively. Network analysis showed two main haplogroups (one haplogroup contains H1, H3, H12, H13, H16, H18 and H19, while the second haplogroup contains H2, H4, H5, H6, H7, H8, H9, H11, H13, H14, H15 and H17) and it presented only one reticulation between haplotypes H5, H7, H9, and H6 (Fig. 4).

Discussion

This is one of the first studies in Brazil looking at the genetic structure and management of a feral species, with two different classes of molecular markers. Starting with the study of Crandall et al. (2000), the utilization of ecological and genetic exchangeability is well accepted as criteria to diagnose conservation or management units (Bruford 2009). Definition of management and conservation measures for a given species can be optimized based on knowledge of the genetic variability and its distribution within and between their populations so that structuring and inbreeding can be identified. Genetic structuring in populations can have natural causes or be a consequence of human action (Frankham et al. 2010). In this study, a low but significant genetic structure was seen using neutral nuclear markers (Table 4 and Fig. 3), and low haplotype diversity and distribution from mtDNA data (Fig. 4).

The within genetic diversity, observed in the present study by the Monteiro breed populations, were consistent with Sollero et al. (2009) and Silva et al. (2011) who analysed microsatellites in Brazilian pig populations. Sollero et al. (2009) mentioned that the Monteiro was one of the less diverse among the locally adapted breeds evaluated, based on estimates of the effective number of alleles and observed heterozygosity. The high number of private alleles found in Monteiro populations is also in agreement with the literature for locally adapted pig breeds (Ayizanga et al. 2016; Sollero et al. 2009). Private alleles, even at low frequencies, show a level of importance from a conservation and breeding standpoint, as they suggest the possibility of managing a repository of alternative alleles for breeding and gene bank assembly. Alleles of this type arise by mutation and can be fixed by genetic drift, selection and migration in a population. They might be an indicator to infer age or admixture level of populations (Slarkin 1985). Results from the relatedness test showed that Monteiro samples collected with unknown pedigree information had low estimated kinship levels, indicating that sampled populations in the Pantanal ecosystem region did not bias the analysis.

The fixation index (F_{ST}), which is also a measure of the structure of genetic variation, ranges between 0 and 1, but usually in mammals, ranges from 0 to 0.25, with most values being close to 0.1 (Holsinger and Weir 2009). Low genetic structuring was observed among studied populations ($F_{ST} = 0.018$, $p = 0.018$), which has been previously reported for Brazilian pig populations collected in neighbouring geographic locations ($F_{ST} = 0.036$, $p = 0.0001$) (Silva et al. 2011). This pattern was corroborated by low Mantel test correlation ($r^2 = 0.2466$) and suggested the presence of high gene flow between the wetland (Pantanal) populations despite the geographic distance, except by Pop1 (PG1) that was the most divergent and geographically distant from the other eight Pantanal populations (Fig. 1). This difference might be explained by the presence of the Negro river natural barrier located between Pop1 (PG1) and the remaining populations (Fig. 1) besides their great capacity for dispersion in streams or habitat flooding. On the other hand, the observed difference might be due to a sampling effect, suggested by the low genetic diversity indexes for Pop1 (PG1) (Table 1).

Cowled et al. (2009), working with feral pig populations in Australia, found a significant positive correlation between geographic and genetic distances, and most of the genetic differentiation between sub-populations was explained by the distance between them. The fact that Monteiro pigs were released in the Pantanal region relatively recently (200 years ago) (Desbiez et al. 2010) and their characteristic high mobility capacity during the wet and dry seasons, could promote a high gene flow among the populations leading to the observed low genetic differentiation.

Table 6 Mitochondrial (D-loop region) haplotypes distribution in Brazilian pigs

Haplotype (F)	Breed (F)	Nucleotide positions (bp)																				
		15543	15558	15565	15579	15587	15592	15615	16618	16619	16674	16675	16682	17114	17123	17229	17236	17340	17741	17758	18254	18540
AJ002189		T	A	G	C	C	A	T	C	T	T	T	G	T	A	A	C	A	C	T	C	T
H1 (8)	SBA (1); SCT (1); SPI (1); SCB (5)	C		A	T	T	G	C								G		G	T			
H2 (168)	SBA (3); SCB (3); SCR (4); SCTA (1); SLD (1); SME (3); SMO (2); *SMT_P (121); *SMT_DF (5); SNI (9); SPI (9); SPT (3); SRP (2); STA (2)													C					T	C		
H3 (6)	*SMT_P (1) SBA (1); SLW (2); SMS (3)	C		A	T	T	G	C				C				G			T		T	
H4 (10)	SCB (1); SMO (8); SMS (1)								T					C								
H5 (4)	SDC (3); SMS (1)		T																	C		
H6 (3)	SDC (1); SMS (1); SCB (1)													C								
H7 (23)	SLD (1); SMS (6); *SMT_DF (1); SPN (2); SCB (13)		T											C						C		
H8 (3)	SLD (3)		T					C		C			A	C								
H9 (1)	SLD (1)																					
H10 (5)	SLD (2); SMO (1); SMS (2)	C		A	T	T	G	C				C		C		G					T	C
H11 (1)	SLW (1)		T											C						C		C
H12 (4)	SME (3); SPI (1)	C		A	T	T	G	C						C		G	T		T		T	
H13 (8)	SPI (3); SCB (5)		T					C						C						C		
H14 (70)	SCB (70)	C																	T	C		
H15 (2)	SCB (2)		T					C							G					C		
H16 (1)	SCB (1)	C		A	T	T	G	C								G			T			
H17 (5)	SCB (5)													C					T		T	
H18 (3)	SCB (3)	C		A	T	T	G	C			C					G			T		T	
H19 (2)	SCB (2)	C		A	T	T	G									G		G	T			
H20 (3)	SCB (3)	C		A	T	T	G	C						C		G	T		T		T	

Columns refer to the position of polymorphic sites among 20 haplotypes by breed (lines). Locally adapted breeds: SBA = Baé; SCB = casco de burro (Mule foot); SRC = Caruncho; SCT = Canastra; SME = mestiços; SMO = Moura; SMT_P = Monteiro from Pantanal; SMT_DF = Monteiro from DF; SNI = Nilo; SRP = Rabo de Peixe (fish tail); SPI = Piau; STA = Tatu, Commercial: SLD = Landrace; SDC = Duroc; SLW = Large White; SMS = MS60; SPN = Pietrain. Dots (.) and dashes (–) indicates matches and gaps, respectively, compared to the reference pig sequence GenBank assesion number AJ002189 (Ursing and Arnason 1998). *Pig sequences generated in present study

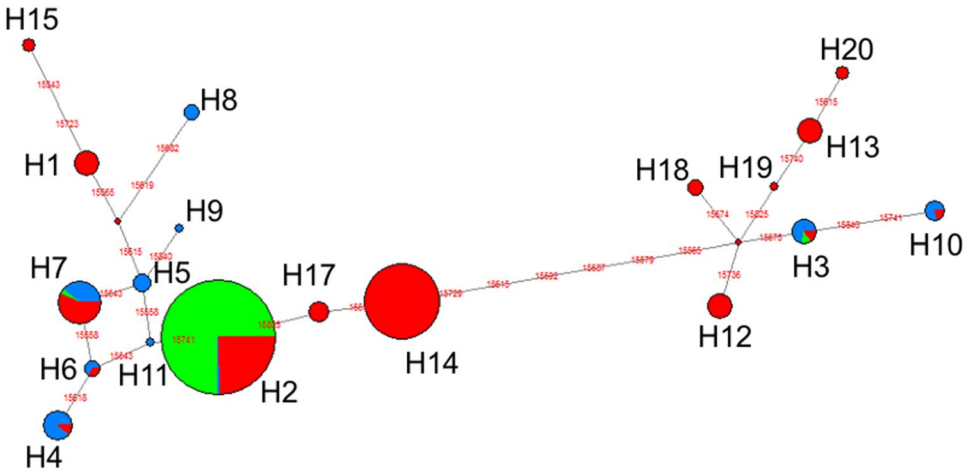

Fig. 4 A median-joining network illustrating the relationships among Monteiro breed (green), specialized breeds (blue) and locally adapted breeds (red). Node sizes are proportional to haplotypes frequegenencies. Numbers represent the nucleotide changes between node

Average F_{ST} between MOB and Pantanal populations was moderate to high (0.07), suggesting that there were differences between Monteiro pigs from the Pantanal region and Brasilia, due to geographic distance, genetic drift or even admixture by specialized/commercial breeds in Brasilia locale (Sollero et al. 2009). These differences were enhanced by the presence of private alleles in the MOB population. F_{ST} tends to be higher when populations are physically more distant. For example, Brazilian pig populations sampled from various regions of Brazil presented F_{ST} of 14% (Sollero et al. 2009) and for pigs breeds sampled from different countries the F_{ST} was approximately 27% (Kim et al. 2005; Laval et al. 2000). The latter is the highest differentiation available in the literature for pigs using microsatellites markers.

Structure Bayesian analysis corroborated the basic genetic distance analysis ($F_{ST,}$ AMOVA, Mantel tests) and improved the identification of a fine genetic structure scale inside the Monteiro breed. As well as the analysis with nuclear markers, the results from Fig. 3 are a clear example of a phylogeographic category V according to Avise (2000). In this category, there are alleles/haplotypes specific or in high frequency present in some regions and there are common alleles spread over all regions. The genetic divergence among populations/regions is not strong (low/moderate F_{ST} values). In the present study, specific Ks for different geographic regions (MOB, Pantanal), and within regions, were identified by Bayesian analysis (Pantanal Pop1 against all other Pantanal populations). As mentioned before, this might due to the presence of a higher number of private alleles in the Brasilia population (MOB), as well as the geographic distance (more than 1000 km).

The mtDNA analyses included Brazilian breeds that already had been classified based on haplotype origin: Asian or European (Cavalcante Neto 2010; Souza 2011). Similar classifications using Brazilian or Iberian breeds were observed with Cytochrome b gene alone (Sollero et al. 2009), as well as Cytochrome b and control region (Alves et al. 2003). The low haplotype diversity within these two main haplogroups was expected and they resemble a phylogeographic category IV (Avise 2000) classification. Most of the haplotypes are spread over all regions (sympatric) and they are closely related (low number of substitutions). The star shape of the network and the existence of few haplotypes with a high frequency are strong evidence of this recent expansion of Asian and European pig haplogroups.

Most of the Monteiro samples have the European haplotype H2 and share it with other Brazilian locally adapted pig breeds sampled in different regions of the country. This reinforces the low mitochondrial genetic structure of pigs in the country, but part of this diversity is already distinct (haplotypes present only in Brazil) from the genetic diversity that they had in their original countries (Souza et al. 2009). The presence of Asian haplotypes in Brazilian breeds suggests admixture with commercial/ specialized breeds.

From a conservation standpoint, mtDNA and nuclear markers results will be useful for the implementation of ex situ and in situ management practices. Besides the shallow matrilineal phylogeographic pattern of the Monteiro breed, it can be used to certify animals that belong to the European haplogroup (e.g., all Monteiro samples from Pantanal). On the other hand, nuclear markers highlighted the need to cryopreserve germplasm (semen) from both Brasilia and Pantanal populations to conserve the full diversity of the breed observed in the country to date. Efforts are now underway to collect semen from those animals following the strict sanitary legislation of the country. The inexistence of a deep genetic structure of Monteiro in the Pantanal region suggests that there are no major concerns that could stop or mitigate gene flow among localities inside the Pantanal ecosystem.

Conclusions

There is a weak genetic structure of Monteiro pigs in Brazil, and the animals from the Pantanal ecosystem can be considered a large, unique mating population. Larger rivers might be significant geographic barriers for those pigs. Even with low genetic diversity between regions (samples from different States), ex situ measures are needed to preserve the genetic diversity of the species in the country, as well as allow for future colonization events, as necessary.

Acknowledgements This work was supported by CAPES (Coordination of Superior Level Staff Improvement), CNPq (National Council for Scientific and Technological Development), Embrapa (Brazilian Agricultural Research Corporation) and INCT Pecuaria (MCT/FINEP/CNPq/FAPEMIG). We also thank Embrapa Swine and Poultry, Embrapa Pantanal and EMATER-DF for the biological material. We wish to thank Aderbal Cavalcante Neto for the unpublished mtDNA sequences from locally adapted breeds; and Potira Hermuche from Brasilia University for the map on Fig. 1.

Author contributions CM, ARC and SRP designed study; material preparation and data collection were performed by ECS, UP and CAS; data analysis were performed by ECS; original draft preparation by CM and DAF; writing, review and editing by CM, DAF, ARC, UP and SRP.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individuals participants included in the study.

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