

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

DISSERTAÇÃO DE Mestrado

GENÉTICA DE POPULAÇÕES DE ONÇA-PINTADA
(*PANTHERA ONCA*) EM BIOMAS BRASILEIROS

FERNANDA PEDONE VALDEZ

ORIENTADOR: EDUARDO EIZIRIK

PORTO ALEGRE, 2010

Livros Grátis

<http://www.livrosgratis.com.br>

Milhares de livros grátis para download.

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

**GENÉTICA DE POPULAÇÕES DE ONÇA-PINTADA
(*PANTHERA ONCA*) EM BIOMAS BRASILEIROS**

Fernanda Pedone Valdez

Orientador: Dr. Eduardo Eizirik

DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE – RS – BRASIL

2010

Sumário	
Agradecimentos.....	IV
Resumo.....	VI
<i>Abstract</i>	VII
Apresentação.....	VIII
Intrdução.....	IX
Artigo Científico.....	XI
<i>Abstract</i>	1
<i>Introduction</i>	2
<i>Materials and Methods</i>	5
<i>Results</i>	9
<i>Discussion</i>	11
<i>References</i>	17
<i>Tables</i>	22
<i>Figures</i>	31
Considerações finais.....	XII

Agradecimentos

- Agradeço em primeiro lugar aos genômicos por toda a bagunça entre um PCR e outro e por todas as risadas, que fizeram com que esses dois anos fossem mais divertidos.
- À Cladi, por todas as plaquinhas injetadas e por todos os cafés com conversa que, com certeza, renderam boas risadas e ótimas lições de vida.
- Às minhas queridas colegas e lindas amigas Tali, Flávia, Ale, Aninha, Déa, Rê, Ane, Tati (valeu pela ajuda com o Structure), Lisie (obrigada por toda a ajuda na bancada) e Mirian (um super obrigada pela aula de Diva e por todas as ajudas nessa reta final).
- À minha amada amiga Cris, valeu por toda a força e por todos os ensinamentos, tanto científicos como emocionais, e desculpa as milhões de perguntas e encheções de saco nessa última etapa.
- Ao Manoel, que desde a faculdade sempre foi o super-inteligente, valeu por todas as respostas àquelas minhas perguntas sem pé nem cabeça e por toda a paciência.
- Aos pesquisadores Fernando Azevedo, Leandro Silveira e Sandra Cavalcanti pela colaboração e pelas amostras cedidas.
- Às minha amigas, Marina, Lívia, Hosana, Martinha, Lu, Camila, Juca, Josi e Fofa por toda a compreensão e apoio nos momentos difíceis e por todas as fofocas, discussões, choros, risadas, abraços e beijos, com certeza eu não teria conseguido sem a força de vocês!
- À minha pequena grande co-orientadora Taia, que me contagiou com o amor pelas onças e me faz pensar que a gente vai conseguir salvá-las e que elas são sim os bichos mais lindos e mais poderosos do mundo. Amiga, obrigada por todo o aprendizado e todo o stress compartilhado nesses 2 anos, espero que continuemos juntas nessa batalha!
- Ao meu orientador, por todos os projetos que me colocou, com certeza eles puseram mais “emoção” nesses 2 anos, Duda, muito obrigada pela oportunidade, por todo o ensinamento e toda a confiança depositada no meu trabalho.
- À Evinha, por todos os cafezinhos e por me botar de castigo no quarto sozinha me concentrando.
- Ao Marco por todo o “apoio logístico” e pelas corridinhas matinais.
- Ao meu pai, por todo o apoio e confiança mesmo com todos os questionamentos em relação à “profissão estudante” eu sei que tu se orgulha

de mim, e à minha madrasta Ana Márcia por todo o carinho e dedicação para com essa família.

- Às minhas irmãs Carol e Júlia, que eu sei que, por mais que não entendam o porquê da minha pesquisa, me admiram pelo amor à natureza e essa vontade de salvar o mundo.
- À minha mãe pela amizade, incentivo e por toda a força durante essa difícil vida de mestrandia, obrigada por tudo, te amo!
- Ao CNPq pela bolsa concedida.
- Às oncinhas por serem esses animais tão interessantes, lindos, fortes e, principalmente, persistentes!!
- E por fim, um agradecimento especial ao meu duende com suas sempre bem escritas frases!!!!

Resumo

Atualmente, a redução do tamanho populacional constitui um problema real para muitas populações selvagens e cativas, e muitos estudos têm sido realizados nesse contexto com o intuito de identificar áreas de manejo e proteção de espécies ameaçadas. O manejo de populações naturais tem como objetivo assegurar habitats adequados para manter populações em tamanhos viáveis evitando assim que os efeitos do endocruzamento se pronunciem, além de manter corredores para facilitar a dispersão dos organismos e conseqüentemente o fluxo gênico entre populações. Os grandes carnívoros, por necessitarem de grandes áreas de vida e serem usualmente perseguidos por caçadores, são bastante vulneráveis à fragmentação de habitats. Devido ao fato de serem predadores-topo, sua extinção local geralmente leva à alteração de todo o ecossistema. Desta forma, sua conservação afeta, de maneira direta e indireta, muitos outros organismos. Os carnívoros estão entre os organismos que causam maiores desafios e preocupações às autoridades referentes à conservação. Áreas consideravelmente grandes são necessárias para englobar a área de vida de um único indivíduo, e territórios ainda maiores são necessários para abranger uma comunidade inteira deste grupo. A onça-pintada, o maior felídeo das Américas, encontra-se atualmente em menos de 50% da sua distribuição original e muitas áreas remanescentes não apresentam tamanho e disponibilidade suficiente de presas para manter uma população saudável em longo prazo. No Brasil, a Bacia Amazônica e o Pantanal são as maiores áreas de distribuição da espécie onde ainda se encontra populações grandes o suficiente para uma viabilidade por um longo período de tempo. No entanto, pouco se sabe sobre a dinâmica das populações de onça-pintada nesses biomas, havendo assim uma extrema necessidade de análises em nível genético-populacional da espécie. O presente trabalho teve como objetivo analisar 52 indivíduos provenientes do Pantanal brasileiro amostrados no período de 2001 a 2008, e fazer inferências genéticas sobre esta população, bem como compará-la com uma área previamente estudada na Mata Atlântica, onde altos níveis de estruturação foram encontrados. Foram analisados índices de diversidade genética intra-populacional e calculados índices de estruturação (F_{st} e R_{st}) assim como análise Bayesiana de estruturação no programa STRUCTURE. Os níveis de variabilidade foram bastante altos e comparáveis com aqueles encontrados para a espécie quando analisada de uma forma mais ampla (em escala filogeográfica). Quando as amostras do Pantanal foram analisadas em relação à sua estruturação, os dados indicaram a presença de apenas uma população, sugerindo que a região amostrada (Pantanal sul) consiste de uma só unidade genética. No entanto, quando as populações da Mata Atlântica foram incluídas na análise, as amostras do Pantanal foram alocadas em duas unidades genéticas incompletamente diferenciadas, devido provavelmente à influência de parentesco entre alguns dos indivíduos desta região, em combinação à provável miscigenação histórica com áreas transicionais entre os dois biomas. Este parece ter sido o caso da população amostrada na área de influência da UHE Porto Primavera (MS/SP), situada no limite interior do bioma Mata Atlântica e que atualmente encontra-se extinta por ação humana. Os resultados obtidos apóiam a inferência de que, no passado, havia conectividade genética entre populações do Pantanal e da Mata Atlântica de Interior, embasando o delineamento de possíveis ações de manejo a fim de retomar a conexão entre estes dois biomas. Além disso, os dados do Pantanal representam a primeira amostragem de uma população geneticamente saudável de onças-pintadas, podendo servir como base para a avaliação e monitoramento da variabilidade observada nesta espécie em regiões fragmentadas.

Abstract

Population size reduction currently constitutes a real problem for several natural populations, and many studies have focused on the identification and management of adequate protected areas for threatened species. The conservation of natural areas aims to guarantee good-quality habitats to keep populations at a viable size, which includes an avoidance of potentially deleterious effects of inbreeding. Additionally, conservation plans aim to keep dispersal corridors and consequently to maintain gene flow among populations that were originally connected. Large carnivores, due to their need for large home ranges and frequent persecution by hunters, are extremely vulnerable to habitat fragmentation. Since they are top-predators, their local elimination may cause the alteration of the whole ecosystem, while their protection might impact the persistence and demography of several co-occurring species. Carnivores usually cause major challenges to conservation managers, since considerably large areas are necessary to encompass the home range of a single individual and even larger regions are required to comprise an entire population. The jaguar is the largest predator in the Americas, and currently persists in less than 50% of its original distribution. Also, many remnant areas do not contain sufficient habitat and prey base to hold viable populations in the long term. In Brazil, the Amazon Basin and the Pantanal are the largest areas of the current distribution of the species, and where populations with high probability of long-term survival are still found. However, little is known about the dynamics of jaguar populations in these biomes, and thus population-genetic analyses are extremely necessary. The present study aimed to analyze 52 individuals sampled in the southern Pantanal from 2001 and 2008 to make genetic inferences on jaguar populations, as well as to compare them to previously investigated fragments in the Atlantic Forest biome, where high levels of structuring had been found. We estimated genetic differentiation among populations using an AMOVA approach to generate F_{st} and R_{st} indices. Population structuring analyses were also performed with the Bayesian approach implemented in the software STRUCTURE. Variability indices were quite high, and comparable to those found for the species when analyzed under on a phylogeographic scale. When Pantanal populations were assessed separately, a single genetic cluster was inferred, supporting the expectation of demographic connectivity throughout this area. However, when Atlantic Forest jaguars were also included in the analysis, the Pantanal population was divided into two groups, probably due to relatedness among some of the sampled individuals, combined with complex historical admixture with respect to transitional areas between the two biomes. One such area was likely the region adjacent to Porto Primavera dam, on the bank of the upper Paraná river, whose population has already been extirpated due to human activities. Results obtained in the present study support the idea that, in the past, there was genetic connectivity between populations from the Pantanal and the Upper Paraná region, which helps provide baseline data to aid in the design of adequate management actions that may reconnect these biomes. Moreover, the data shown here represent the first jaguar sampling of a genetically healthy local population, and may be used as a comparative reference for the evaluation and monitoring of the observed variability in fragmented regions.

Apresentação

Esta dissertação é um dos requisitos exigidos para obtenção do título de mestre pelo Programa de Pós-Graduação em Zoologia, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul.

Os resultados aqui apresentados foram gerados ao longo dos meus últimos dois anos de estudo, realizados no Laboratório de Biologia Genômica e Molecular, vinculado a esta universidade, sob orientação do Prof. Dr. Eduardo Eizirik.

O trabalho está sendo apresentado sob a forma de um artigo científico a ser submetido para a revista *Conservation Genetics*, respeitando as regras da mesma. As normas de submissão para os autores estão disponíveis no endereço eletrônico: “<http://www.springer.com/life+sci/ecology/journal/10592>”. Acompanha o artigo um resumo estendido (versão em português e inglês, ver acima). Tabelas e figuras são apresentadas após o corpo do texto.

Introdução

A conservação a longo prazo de carnívoros de grande porte apresenta-se como um grande desafio (Noss et al. 1996) devido a sua baixa densidade populacional e sua necessidade de territórios grandes e saudáveis com alta disponibilidade de presas (Marinho-Filho & Machado 2006). Além disso, o conhecimento acerca dessas espécies é bastante escasso, fato que dificulta o planejamento de estratégias de conservação efetivas (Karanth & Chellam 2009). Os grandes carnívoros são vulneráveis à perda e à fragmentação de hábitat, além de sofrerem inúmeras vezes perseguição direta e indireta por humanos devido aos seus requerimentos ecológicos conflitarem com aqueles da população local (Woodroffe & Ginsberg 1998; Inskip & Zimmermann 2008). Devido a estas características, estes animais estão entre os mamíferos mais ameaçados (Schipper et al. 2008). Devido ao fato de serem predadores de topo em muitos biomas, a sua eliminação pode causar alterações significativas no ecossistema inteiro (Noss et al. 1996, Woodroffe & Ginsberg 1998, Crooks & Soulé 1999, Crooks 2002), ao mesmo tempo, sua proteção pode impactar a persistência e a demografia de muitas espécies simpátricas (Caro 2003, Seddon & Leech 2008, Grigione et al. 2009). Por esta razão, eles são comumente conhecidos como “espécie guarda-chuva” (ou seja, mantendo estas espécies na paisagem, outras serão também protegidas – Caro 2003), e usualmente empregadas como espécies bandeira para a implementação de parques nacionais e reservas.

Desenvolvimento urbano e conversão de terras são os maiores perigos para grandes carnívoros, assim áreas saudáveis e corredores de dispersão devem ser mantidos para garantir a viabilidade a longo prazo destas populações (Grigione et al. 2009). Uma vez que os números populacionais estão diretamente ligados à disponibilidade de espaço (Marinho-Filho & Machado 2006), unidades de conservação de tamanho grande o suficiente para manter populações saudáveis são extremamente importantes para a proteção dos grandes carnívoros. Este é um problema crítico, uma vez que tem se notado que muitas áreas designadas para a conservação não são grandes o suficiente para manter uma população viável destas espécies (e.g. Sollman et al. 2008).

A onça-pintada (*Panthera onca*) é o maior predador das Américas e sua distribuição e persistência é estritamente dependente de ambientes saudáveis com presa abundante (Silveira 2004). Sua distribuição original estendia-se desde o sudeste da América do Norte, passando pela América Central e do Sul até o sul da Argentina. Atualmente esta espécie ocupa menos de 50% da sua área original, sendo que a maioria das populações remanescentes sofreu níveis severos de redução demográfica e fragmentação. A Bacia Amazônica e o Pantanal ainda mantêm as maiores populações estimadas da espécie, as quais exibem as mais altas probabilidades de sobrevivência a longo prazo (Sanderson et al. 2002).

O objetivo principal do presente trabalho foi investigar as populações de onça-pintada na porção sul do Pantanal, no estado do Mato Grosso do Sul, para testar se há continuidade genética ou se alguma subdivisão espacial pode ter ocorrido em uma escala regional neste bioma. Nós também comparamos as amostras do presente estudo com populações remanescentes do bioma Mata Atlântica. Para isto analisamos dados gerados em um estudo prévio (Haag 2009) que havia indicado a existência de diferenciação populacional devido à deriva genética gerada em um curto período de tempo (30-40 anos) causada pela fragmentação do hábitat. A comparação entre os dados genéticos dos biomas Pantanal e Mata Atlântica permitiram uma inferência sobre a conectividade histórica entre estes dois ecossistemas, e resultaram ainda na geração de informação básica que pode ser útil no futuro para investigar e monitorar populações que venham a sofrer alguma redução ou fragmentação.

Artigo Científico

“Population genetics of jaguars in the Brazilian Pantanal: molecular evidence for broad population connectivity on a regional scale”

Fernanda Pedone Valdez, Taiana Haag Fernando Azevedo, Leandro Silveira,
Sandra Cavalcanti and Eduardo Eizirik

A ser submetido ao periódico científico *Conservation Genetics*

1 Original Article

2 **Population genetics of jaguars in the Brazilian Pantanal: molecular evidence for broad**
3 **population connectivity on a regional scale**

4

5 Fernanda Pedone Valdez ¹, Taiana Haag^{1,2}, Fernando Azevedo³, Leandro Silveira⁴, Sandra
6 Cavalcanti³ and Eduardo Eizirik ^{1,3}

7

8 ¹Faculdade de Biociências PUCRS. Av. Ipiranga 6681, prédio 12. Porto Alegre, RS 90619-900,
9 Brazil

10 ² Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de
11 Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, CP 15053,
12 Porto Alegre, RS 91501-970, Brazil,

13 ³ Instituto Pró-Carnívoros. Av. Horácio Neto, 1030, Casa 10, Parque Edmundo Zandoni, Atibaia,
14 SP 12940-970, Brazil

15 ⁴ Jaguar Conservation Fund/Instituto Onça-pintada, Mineiros, GO 75830-000, Brazil

16

17 Keywords: *Panthera onca*, microsatellites, conservation, population structure, large population

18

19 Corresponding Author:

20 Eduardo Eizirik, Faculdade de Biociências PUCRS. Av. Ipiranga 6681, prédio 12. Porto Alegre,
21 RS 90619-900, Brazil. Fax: 55-51-33203612. Email: eduardo.eizirik@puers.br

22

23 *Running title:* Jaguar population structure in the Pantanal region

24

25

26 **Abstract**

27 Habitat loss and human-caused mortality are the main threats to the conservation of carnivores
28 worldwide since they lead to reduction and isolation of natural populations. Reduced
29 populations suffer the action of genetic drift and inbreeding, having as a likely consequence the
30 decline of genetic variability and adaptive fitness. Jaguar populations are extremely affected by
31 human disturbances and have already been extirpated from half of their original geographic
32 range. However, there are a few regions in the Americas that still maintain rather large jaguar
33 populations, with moderate to high probability of long-term survival. In Brazil, the Amazon
34 basin and the Pantanal are the best examples of such areas and may serve as a basis to
35 understand jaguar biology in a setting that is less impacted by human disturbances than in most
36 other regions. The current study aimed to analyze Pantanal jaguar populations sampled in the
37 southern portion of this biome. We employed 12 microsatellite loci to characterize genetic
38 diversity and population structure in this area on the basis of 52 samples collected in four
39 different ranches. Although some indication of internal structure was observed (possibly due to
40 the putative presence of related animals in the sample), our overall results supported the
41 hypothesis that this area comprises a single population unit with high levels of genetic diversity.
42 Comparisons with data previously collected from fragmented remnants of the inner Atlantic
43 Forest revealed significant but rather low levels of genetic differentiation with respect to the
44 Pantanal, and indicated the occurrence of recent connectivity between these biomes. Evidence
45 for admixture between the Pantanal and a population located on the boundary of the Atlantic
46 Forest biome corroborates the transitional nature of this area, whose jaguar population has now
47 been locally extirpated. These data can serve as a basis for understanding the natural population
48 dynamics of jaguars on a regional scale, and for supporting the design of conservation strategies
49 that maintain and restore connectivity among currently isolated areas across the species' range.

50

51

52 **Introduction**

53 The long-term conservation of large carnivores presents considerable challenges (Noss *et al.*
54 1996) due to their low population densities and the need for large and healthy habitats with high
55 prey availability (Marinho-Filho and Machado 2006). Furthermore, the knowledge on most of
56 these species is still very scarce, further hampering the planning of effective conservation
57 strategies on their behalf (Karanth and Chellam 2009). Large carnivores are highly vulnerable to
58 habitat loss and fragmentation, and often suffer direct persecution from humans because their
59 ecological requirements conflict with those of local people (Woodroffe and Ginsberg 1998;
60 Inskip and Zimmermann 2008). Due to these characteristics, they are amongst the most
61 threatened of all mammals (Schipper *et al.* 2008). Since they are top predators in many biomes,
62 their local elimination may cause major alterations in the whole ecosystem (Noss *et al.* 1996,
63 Woodroffe and Ginsberg 1998, Crooks & Soulé 1999, Crooks 2002), while their protection
64 might impact the persistence and demography of several co-occurring species (Caro 2003,
65 Seddon and Leech 2008, Grigione *et al.* 2009). For this reason, they are usually known as
66 “umbrella species” (*i.e.* by maintaining these species in the landscape, others will also be
67 protected - Caro 2003), and often employed as a flagship for the establishment of national parks
68 and reserves.

69 Land development and conversion are major threats to large carnivore species, thus
70 areas with healthy habitats and dispersal corridors should be kept to ensure long-term viability
71 of such populations (Grigione *et al.* 2009). Since population numbers are directly linked to the
72 availability of space (Marinho-Filho and Machado 2006), conservation units of sufficient size
73 are extremely important for the protection of large carnivores. This is a critical issue, as it has
74 been shown that many areas designated for conservation are not large enough to hold viable
75 populations of these species (e.g. Sollman *et al.* 2008).

76 The jaguar (*Panthera onca*) is the largest predator in the Americas, and its distribution
77 and persistence are strictly dependent upon healthy habitats and an abundant prey base (Silveira

78 2004). Its original distribution extended from southeastern North America, Central America,
79 and South America to southern Argentina. The species currently occupies less than 50% of its
80 original range, with most remaining populations suffering severe levels of demographic
81 reduction and fragmentation. Overall, the Amazon Basin and the Pantanal hold the largest
82 estimated populations of jaguars, which therefore exhibit the highest probability of long-term
83 survival (Sanderson *et al.* 2002). In Brazil, most conservation units expected to play a role in
84 jaguar conservation are insufficient to hold the 650 individuals estimated to be required for
85 long-term viability in this species (Eizirik *et al.* 2002, Sollmann *et al.* 2008). It could therefore
86 be argued that they should be connected to each other to allow for demographic connectivity
87 across broad areas and to increase the probability of long-term persistence of jaguar populations
88 (Cullen *et al.* 2005). To assess whether this strategy is adequate to maintain or restore original
89 levels of population connectivity, it is important to investigate the genetic structure and
90 demographic dynamics of remaining jaguar populations, and to infer their original patterns of
91 gene exchange. Although some initial studies have described historical patterns of population
92 subdivision and connectivity on broad geographic scales (Eizirik *et al.* 2001, Ruiz-Garcia *et al.*
93 2006), little is known with respect to the structure and dynamics of jaguar populations in a local
94 or regional context.

95 The Pantanal is the largest natural floodplain in the world, covering approximately
96 160,000 km² within Brazil, Paraguay, and Bolivia. This ecosystem is influenced by four biomes:
97 Amazon rainforest, Cerrado, Chaco, and Atlantic Forest (Adámoli 1982). The large variety of
98 vegetation and soil types makes the Pantanal one of the richest biomes in the Neotropics with
99 respect to its biodiversity (Nunes da Cunha 2006). Despite the alteration of the original habitat
100 and severe retaliation pressure because of cattle losses, this ecosystem still harbors one of the
101 largest jaguar populations on Earth, likely due to the climatic characteristics of this biome, that
102 makes it difficult to be accessed (Sanderson 2002, Soisalo and Cavalcanti 2006), especially
103 during the wet season, when most of the area is flooded.

104 Although many studies have been performed on jaguar ecology in the Brazilian
105 Pantanal (e.g. Schaller and Crawshaw 1980, Crawshaw and Quigley 1991, Quigley and
106 Crawshaw 1992, Dalponte 2002, Silveira 2004, Soisalo and Cavalcanti 2006, Azevedo and
107 Murray 2007, Cavalcanti and Gese 2009), only one preliminary assessment of genetic diversity
108 for this species in the region has been published (Eizirik *et al.* 2008). That study indicated that
109 Pantanal jaguars maintain considerably high levels of genetic variability, but did not investigate
110 the spatial distribution of this diversity, nor its historical connection to adjacent biomes such as
111 the Atlantic Forest.

112 The goal of the present study was to investigate jaguar populations in the southern
113 portion of the Pantanal, in the Brazilian state of Mato Grosso do Sul, to test whether they are
114 genetically continuous or if spatially-oriented subdivision may occur on a regional scale in this
115 biome. We also aimed to compare the sampled areas from this biome with remaining population
116 fragments from the inner Atlantic Forest. For that purpose, we reanalyzed a data set generated in
117 a previous study (Haag 2009) which had indicated that habitat fragmentation likely induced
118 drift-mediated differentiation among small forest fragments in a very short period of time (30-
119 40 years). The comparisons between Pantanal and Atlantic Forest genetic data allowed the
120 inference of historical connectivity between the two biomes, and the generation of baseline
121 information that may serve in the future to investigate and monitor additional population
122 fragments that become reduced and isolated due to human intervention.

123

124 **Materials and Methods**

125 *Sample collection and laboratory procedures*

126 Sampling was performed in the southern Pantanal region between 2001 and 2008 in the context
127 of field ecology and behavioral studies (e.g. Silveira 2004, Azevedo and Murray 2007,
128 Cavalcanti and Gese 2009). Samples from each ranch were analyzed separately, and referred to

129 initially as four different local populations. A total of 53 blood samples were collected from
130 wild-caught animals considering all four ranches: a) San Francisco Ranch (n =11) – 2003 and
131 2004; b) Sete Ranch (n =10) – from 2001 to 2003; c) Ecological Refuge Caiman (n =22) – 2003,
132 2005, and 2006; and São Bento Ranch (n =10) – 2008 (Table 1). In order to compare these
133 Pantanal populations with four fragments previously sampled in the inner Atlantic Forest (data
134 generated by Haag 2009) we analyzed a composite data set comprising 102 individuals from
135 both biomes.

136 Blood samples were preserved with EDTA and in some cases also mixed with an equal
137 volume of a salt-saturated solution (100mM Tris, 100mM EDTA, 2% SDS) to facilitate the
138 conservation of good-quality DNA content in a field setting. All samples were stored at -20°C
139 prior to DNA extraction. Genomic DNA was extracted from blood samples using a phenol-
140 chloroform protocol (Sambrook *et al.* 1989) with slight modifications. Each sample was
141 amplified for 12 microsatellite loci by the Polymerase Chain Reaction (PCR, Saiki *et al.* 1985):
142 one locus containing a dinucleotide repeat (FCA742), two loci with trinucleotide repeats (F146
143 and F98), and nine loci with tetranucleotide repeats (FCA740, FCA723, FCA453, FCA441,
144 FCA391, F124, F85, F53 and F42). These primers were originally developed for the domestic
145 cat (*Felis silvestris catus*) (Menotti-Raymond *et al.* 1999, 2005) and have been optimized and
146 standardized for jaguars (Eizirik *et al.* 2001, 2008; Haag 2009) and other wild cats (Trigo *et al.*
147 2008). Every forward primer was 5'-tailed with an M13 sequence (Boutin-Ganache *et al.* 2001)
148 and used in combination with an M13 primer that had the same sequence but was dye-labeled
149 (with FAM, HEX or NED) on its 5' end. PCR reactions were performed in a 10 ul volume
150 containing approximately 10ng of genomic DNA, 0.25U of Platinum Taq DNA polymerase
151 (Invitrogen), 1x PCR Buffer (Invitrogen), 2.0 mM MgCl₂, 200 uM of each dNTP, 0.2 uM of the
152 reverse primer, 0.0133 uM of the M13-tailed forward primer, and 0.16 uM of the M13-
153 fluorescent primer (FAM, NED or HEX). The reaction profile started with a denaturing step at
154 94°C for 3 min, followed by 10 touchdown cycles of denaturing at 94°C for 45s, annealing at
155 60-51°C for 45s, and extension at 72°C for 1.5 min. Thirty cycles of the same profile were run

156 afterwards with fixed annealing temperature of 50°C, followed by a final extension of 72°C for
157 30 min. PCR reactions were carried out for each locus separately, and products from 2 to 4 loci
158 were diluted and pooled together based on yield, size range, and fluorescent dye (Table 3).

159 PCR products were then run on a MegaBACE 1000 automated sequencer with the ET-
160 ROX 550 internal size ladder (GE Healthcare) and analyzed with the GENETIC PROFILER 2.2
161 software to determine fragment length. Negative controls, *i.e.* PCR reactions without genomic
162 DNA, were run for each batch of reactions and genotyped to monitor the presence of any
163 exogenous DNA.

164 *Data analysis*

165 The estimated frequency of null alleles was calculated with the software CERVUS 3.0.3
166 (Marshall *et al.* 1998), with large positive values potentially indicating the presence of non-
167 amplified alleles that occur most often when the microsatellite locus is cloned for one species
168 and amplified in others, due to mutations in the primer binding sites. Likewise, the software
169 MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to identify possible genotypic
170 errors that might occur during data recording due to stutter peaks, as well as to assess the
171 existence of null alleles and large-allele dropout. These genotyping mistakes, when present,
172 might lead to erroneous estimates of population genetic parameters.

173 Only individuals that had at least 70% of the loci genotyped (*i.e.* 8 out of 12) were
174 included in the analysis. The final set of genotypes was tested for any evidence of deviation
175 from Hardy-Weinberg equilibrium (HWE), employing the exact test of Guo & Thompson
176 (1992) with 10,000 dememorization steps (Excoffier *et al.* 2005) and for linkage disequilibrium
177 (LD) across pairs of loci using the software packages ARLEQUIN 3.11 (Excoffier *et al.* 2006)
178 and FSTAT 2.9.3.2 (Goudet 2002). Significance levels ($\alpha = 0.05$) for departures from HWE or
179 inferred LD were adjusted for multiple simultaneous comparisons with the sequential
180 Bonferroni approach (Rice 1989).

181 Genetic diversity indices were measured as the observed heterozygosity (H_O), expected
182 heterozygosity (H_E), and number of alleles per locus (A) employing ARLEQUIN and FSTAT.
183 The software FSTAT was also used to identify private alleles and to estimate allelic richness
184 (AR) for each population. The latter is a measure of the observed number of alleles per
185 population that is normalized to account for differences in sample size among them (Petit *et al.*
186 1998).

187 *Population differentiation and structure analysis*

188 A set of statistical tests was performed to evaluate the existence of population genetic structure
189 caused by long-term or recent isolation, such as due to historical geographic barriers or human-
190 induced habitat fragmentation. F_{st} and R_{st} indices were calculated among pairs of populations
191 using the AMOVA approach implemented in ARLEQUIN. Each test was run with 10,000
192 permutations to evaluate the statistical significance of the calculated value ($\alpha=0.05$). Pairwise
193 genetic distances were calculated for the Pantanal data set (i.e. containing the four local
194 populations sampled in this biome) as well as for the composite data set (Pantanal + Atlantic
195 Forest populations).

196 In addition, we assessed the existence of potential population subdivision using the
197 Bayesian clustering method implemented in the program STRUCTURE 2.3.1 (Pritchard *et al.*
198 2000; Pritchard and Wen 2004). We initially conducted five independent runs for each value of
199 K (number of clusters), ranging between 1 and 8 when analyzing the Pantanal samples by
200 themselves, and between 1 and 12 when also including the Atlantic Forest populations. All
201 analyses used no prior population information, along with the admixture model with correlated
202 allele frequencies (which is considered to be the best configuration in the case of subtle
203 population structure - Falush *et al.* 2003). For this initial set of analyses, an MCMC procedure
204 of 1,000,000 generations was performed following a burn-in of 500,000 steps. An additional set
205 of analysis was performed for the composite data set using 1,000,000 steps of burn-in and
206 2,000,000 steps of sampling. This was conducted to establish a chain length that was sufficient

207 to achieve convergence, which was assessed by comparing the likelihoods among different
208 replicate runs, as well as by plotting the likelihood scores along the sampled portion of each
209 chain.

210 On the basis of these initial results (in which some variation was still observed among
211 replicates with the same K, especially in the case of the composite data set), we performed a
212 final set of analyses that included a longer MCMC procedure (2,000,000 generations for burn-in
213 and another 2,000,000 for sampling). Given the ranges of probabilities for different K values
214 observed in the initial analyses, this final set included five runs for each K between 1 and 4 for
215 the Pantanal alone, and between 3 and 8 for the composite data set.

216

217 **Results**

218 *Data quality control*

219 Given the established cutoff that samples with less than 70% of the loci reliably genotyped
220 would be excluded from the analyses, we removed bPon156 from the final data set. Therefore,
221 our analyses were performed on the basis of a total sample of 52 jaguar specimens from the
222 Pantanal. We initially assessed the occurrence of deviations from HWE and linkage equilibrium
223 assuming that all individuals from the Pantanal constituted a single population, and in both tests
224 found no significant evidence of disequilibrium. We therefore accepted the null hypothesis that
225 all four local populations constituted a single genetic unit, as inferred by lack of disequilibrium
226 that could have been caused by underlying subdivision, and therefore did not test for
227 disequilibrium within each local area.

228 The estimated frequency of null alleles for all loci calculated in the software CERVUS
229 was either negative or very low (≤ 0.13) (Table 3). Furthermore, the software
230 MICROCHECKER found no evidence for null alleles, large-allele dropout, or stutter peaks

231 influencing the data set. These results allowed the use of the entire twelve-locus panel to make
232 population-level inferences.

233 *Genetic Diversity*

234 All 12 loci were polymorphic and showed high levels of diversity. The number of alleles per
235 locus ranged from 3 (F146 and F98) to 13 (FCA742) with a mean of seven. For each local
236 population, the expected heterozygosity ranged from 0.66 (Caiman) to 0.71 (São Francisco).
237 When calculated jointly for all local populations, the mean observed (H_O) and expected (H_E)
238 heterozygosity were identical (both 0.71). These indices of genetic diversity per population
239 (observed and expected heterozygosity, allelic richness, and number of alleles) are presented in
240 Table 2, while additional information on the analyzed loci is shown in Table 3. The entire
241 genotype data is available upon request.

242 *Population structure and differentiation*

243 F_{st} values among the four pairs of populations within the Pantanal region were all considerably
244 low (0.03 - 0.053) even though statistically significant (Table 4). The highest value was
245 estimated for Caiman vs. San Francisco (0.053; $P = 0.000$), while the other indices were lower
246 than 0.05. On the other hand, R_{st} values were mostly non-significant (see Table 4), except for
247 Caiman vs. San Francisco (0.07; $P = 0.0048$) and San Francisco vs. São Bento (0.10; $P =$
248 0.0016).

249 Assignment tests performed with the program STRUCTURE showed similar results
250 among different sets of analysis (see Materials and Methods), although the variance among
251 replicate runs was lowest when the longest MCMC procedure was performed; therefore, only
252 results from the latter are presented here. This Bayesian approach indicated that the Pantanal
253 data set was best explained with $K = 1$ ($\text{LnP(D)} = -1.793,98$), *i.e.* by considering all four local
254 populations as a single genetic unit (Table 5).

255 The STRUCTURE analyses of the composite data set (Pantanal + Atlantic Forest)
256 resulted in a best likelihood of six populations ($\text{LnP(D)} = -3.478,86$; see Table 6). In this case,
257 three of the four populations from the Atlantic Forest region (Iguaçu, Morro do Diabo, and
258 Ivinhema) tended to cluster separately from all others, although they presented some migrant or
259 admixed individuals (Figure 2). The fourth genetic cluster mostly contained animals from the
260 Porto Primavera population in the Atlantic Forest, but also appeared to be involved in admixture
261 with other areas. The Porto Primavera sample contained several individuals presenting genetic
262 compositions indicative of admixture with the other Atlantic Forest fragments, along with some
263 samples that displayed similar profiles to those from the Pantanal (see Figure 2). This pattern
264 suggested that the Porto Primavera population might contain admixed ancestry from both the
265 Atlantic Forest and Pantanal biomes. An intriguing observation was that, under this subdivision
266 scenario, the Pantanal population was split into two main genetic clusters, along with evidence
267 of admixture with Porto Primavera (see Figure 2). There was no obvious biological or spatial
268 pattern to the subdivision in the Pantanal, except that some individuals from Caiman ranch
269 tended to cluster more strongly into one of the clusters. One possibility to explain this pattern
270 (not seen when the Pantanal populations were analyzed by themselves) was that this Bayesian
271 approach overestimated the number of clusters in this case, due to a combination of relatedness
272 among individuals in one or more local areas (Pritchard and Wen 2004) and the complex
273 influence of admixture from the Atlantic Forest populations, especially Porto Primavera (see
274 Figure 2).

275 F_{st} and R_{st} indices comparing the Pantanal to the Atlantic Forest populations revealed
276 contrasting patterns of genetic structuring in these two biomes (Table 7). F_{st} measures of
277 differentiation were moderate to high (0.06 to 0.20) and statistically significant ($P < 0.001$) for
278 all comparisons. However, R_{st} values were only significant for Pantanal vs. each one of the
279 Atlantic Forest populations, which would be consistent with a longer period of separation
280 between these two ecosystems relative to within-biome comparisons, since the R_{st} index takes
281 into account allele size, incorporating a stepwise mutation model (Slatkin 1995).

282 **Discussion**

283 Genetic diversity indices found in the present analysis corroborate the hypothesis that Pantanal
284 jaguars maintain levels of variability expected for large healthy populations (Frankham *et al.*
285 2002). Even though loss of habitat and severe retaliation because of cattle losses have been
286 major threats in that region, population dynamics have not been considerably affected, probably
287 due to the high prey availability and difficulty to human access in some areas (Soisalo and
288 Cavalcanti 2006).

289 Levels of variability were moderate to high: expected heterozygosity (H_E) ranged from
290 0.66 to 0.71 (Table 2) and the mean number of alleles per locus was seven. These values were
291 similar to those found in a previous study that analyzed 29 microsatellite loci throughout the
292 species' geographic distribution ($H_E = 0.74$ and $A = 8.3$; Eizirik *et al.* 2001) and were quite
293 lower than those found in jaguar populations from Colombia and other South American
294 countries analyzed with 12 microsatellite loci ($H_E = 0.84$ and $A = 11.3$; Ruiz-Garcia *et al.* 2006).
295 Additionally, Uphyrkina *et al.* (2001) investigated leopard populations (recognized as
296 subspecies) across the distribution of the species and found different levels of diversity among
297 them; the mean expected heterozygosity ($H_E = 0.79$) was slightly higher than that found in the
298 current study ($H_E = 0.71$). Nevertheless, any comparison between results obtained in these
299 studies is very difficult to draw, since only 3 out of 12 loci analyzed here (FCA441, FCA391
300 and FCA453) were used in the latter work, 2 out of 12 (FCA441 and FCA453) were used by
301 Eizirik *et al.* (2001), and only 1 out of 12 (FCA391) was employed by Ruiz-Garcia *et al.* (2006).
302 Therefore, marker overlap with respect to those studies was too low to allow any meaningful
303 comparison. Moreover, the present study aimed to perform population-level analyses, while the
304 studies mentioned above sampled across a wider distribution and pursued a more
305 phylogeographic focus.

306 One previous study investigating jaguar genetics in Atlantic Forest fragments (Haag
307 2009) employed almost the same panel used in the present investigation, plus locus FCA741
308 that was monomorphic (*i.e.* bearing no variation) in the Pantanal samples and thus excluded

309 from the present analysis. The similarity between the sampling scheme and the geographic
310 scope in the current study and in Haag (2009) made it possible to compare them and their results
311 might lead to better conservation plans yet to be designed (see Materials and Methods for
312 detailed information). Genetic diversity in that study was high ($H_E = 0.73$) even though spatially
313 divided into four groups. The presence of private alleles in all populations but Morro do Diabo
314 supported the interpretation that very little, if any, gene flow is occurring among the habitat
315 remnants in that area. The region has suffered enormous pressure from fragmentation since
316 large tracts of land in the region were converted to farming and ranching activities. Moreover, in
317 1982 the Itaipu Binacional hydroelectric power plant was constructed, flooding thousands of
318 hectares of riverine habitat and likely eliminating altogether the gene flow between the northern
319 and southern parts of the Upper Parana region (Haag 2009). Our idea in this study was to
320 evaluate, on the same spatial scale, a set of local populations that occur in a still rather
321 continuous environment, and likely maintain levels of connectivity resembling the original
322 population dynamics of the species.

323 *Pantanal populations*

324 Among the sampled populations within the Pantanal region, genetic diversity values did not
325 change considerably. Allelic richness, calculated using a minimum sample size of eight diploid
326 individuals, varied from 4.3 to 4.7, and observed heterozygosity ranged from 0.69-0.73 (indices
327 of genetic diversity per locus and per population are presented in Table 2). A lower percentage
328 of private alleles (16%; see Table 8) than that found in the Atlantic Forest populations (25%;
329 Haag 2009) indicates that those areas in the southern Pantanal are currently more connected and
330 present some level of gene flow among themselves.

331 Our Bayesian analysis performed in the program STRUCTURE showed that the four
332 ranches investigated in the region comprise a single population from a genetic standpoint (Table
333 5 and Figure 2). Estimates of differentiation were low, although statistically significant (F_{st}
334 values ranged from 2.9% - 5.3%, $P < 0.01$; see Table 4). The estimated values were consistent

335 with the hypothesis of a panmictic population in the region, although implying some degree of
336 local differentiation, perhaps caused by the sampling of some related individuals in one or more
337 ranches, thus driving the fixation indices to significant (albeit low) values. These results might
338 be explained by some ecological and behavioral traits of the species. Jaguars exhibit territorial
339 behavior, which means they present very low degree of spatial overlap with conspecifics
340 (Azevedo and Murray 2007), juveniles tend to disperse to establish their new territory, whereas
341 adults establish large home ranges, with males setting larger areas and dispersing longer
342 distances than females (Oliveira 1994, Cavalcanti and Gese 2009). An ecological investigation
343 conducted in the Pantanal region (Cavalcanti and Gese 2009) estimated an area of 57km² during
344 the wet season and 69km² during the dry season for female jaguars. For males, size of home
345 ranges was 152km² and 170km² in the wet and dry seasons, respectively. These estimates
346 indicate that jaguars are expected to roam widely in these areas, thus likely maintaining
347 demographic and genetic connectivity across this still continuous landscape.

348 Although little is still known on jaguar dispersal patterns, recent evidence has supported
349 the notion that corridors with good-quality habitat are required for their movement, since habitat
350 fragmentation is a strict barrier for these animals (Cullen *et al.* 2005). The fact that the primary
351 activity in the Pantanal is the cattle ranching (Harris *et al.* 2005) and that the jaguar does not
352 avoid it as much as agriculture (Oliveira 1992, Hoogesteijn and Mondolfi 1992) supports the
353 hypothesis that this region still encompasses one of the largest populations of the species with
354 high probability of long-term survival (Sanderson *et al.* 2002).

355 Taking into account that the four ranches sampled in the Pantanal region may be
356 considered a single population, we compared them to four fragment sites in the Atlantic Forest
357 previously analyzed by Haag (2009). Despite the fact that the sampling scheme of both studies
358 was very similar, results found in each one of them were considerably different. That study
359 found moderate to high levels of population structuring between the four sites (Table 7) and
360 related that to strong, recent genetic drift affecting these fragments due to their small effective
361 size (N_E) (this was especially the case of two sites: Ivinhema State Park, $N_E = 12.3$ and Morro

362 do Diabo State Park $N_E = 4.6$; Haag 2009). In contrast to that pattern, the Pantanal samples
363 showed lower levels of differentiation (Table 4) and comprised a single genetic cluster (Table 5
364 and Figure 2). However, when analyzed jointly with the Atlantic Forest samples, the Pantanal
365 was divided into two groups, with no apparent biological or geographic pattern to this
366 subdivision. Our interpretation is that the presence of related individuals (as mentioned above)
367 might have led to this overestimated K , as we have observed in our previous study (Haag 2009).
368 Interestingly, this pattern of Pantanal subdivision only emerges when these samples are
369 analyzed jointly with those from the Atlantic Forest, suggesting that a mixture of two
370 phenomena is responsible for this observation. These would be local relatedness along with
371 some degree of historical admixture between the Pantanal and Atlantic Forest populations,
372 which would highlight subtle different in allele frequencies among individuals.

373 Distances among areas are very similar in both regions: within the Pantanal region
374 pairwise distances ranged from 15km (Caiman and Sete) to 80km (Sete and São Bento) and
375 between areas in the Atlantic Forest distances ranged from 80km (Morro do Diabo and Porto
376 Primavera) to 380km (Porto Primavera and Iguaçu); however, the Atlantic Forest fragments
377 have been disconnected in the few decades due to fragmentation and construction of
378 hydroelectric dams (D.A. Sana pers. obs.), leading to population size reduction and
379 consequently strong action of genetic drift (Page and Holmes 1998). The Pantanal region is still
380 rather well-preserved, allowing individuals to wander across the landscape during their lifetimes
381 following a dynamics that is likely more similar to the species' original life history. Therefore,
382 this population might serve as a model to understand jaguar genetic structure and demographic
383 dynamics, and provide valuable baseline information against which fragmented populations
384 may be compared to assess any effect of loss of diversity, inbreeding and correlated deleterious
385 processes driven by small population size and isolation due to human activities.

References

- Adámoli J (1982) O Pantanal e suas relações fitogeográficas com os cerrados: discussão sobre o conceito de complexo do Pantanal. In: Anais do 32º Congresso nacional da Sociedade Botânica do Brasil, Teresina, Universidade Federal do Piauí, p.109-119.
- Azevedo FCC and Murray DL (2007) Spatial organization and food habits of jaguars (*Panthera onca*) in a floodplain forest. *Biol Conserv* 137:391–402.
- Boutin-Ganache I, Raposo M, Raymond M and Descepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques* 31: 1-3.
- Caro TM (2003) Umbrella species: critique and lessons from East Africa. *Anim Conserv* 6: 171-181
- Cavalcanti CMC and Gese EM (2009) Spatial Ecology and Social Interactions of Jaguars (*Panthera onca*) in the Southern Pantanal, Brazil. *J Mammal* 90: 935-945.
- Crawshaw PG Jr and Quigley HB (1991) Jaguar spacing, activity and habitat use in a seasonally flooded environment in Brazil. *J Zool* 223:357-370.
- Crooks KR and Soulé ME (1999) Mesopredator release and avifaunal extinctions in a fragmented system. *Nature* 400: 563-566
- Crooks KR (2002) Relative sensitivities of mammalian carnivores to habitat fragmentation. *Conserv Biol* 16:488-502
- Cullen LJ, Abreu KC, Sana D, Nava AFD (2005) Jaguars as landscape detectives for the upper Paraná River Corridor, Brazil. *Natureza e Conservação* 3:147-161
- Dalponete JC (2002) Dieta del jaguar y depredación de ganado en el norte de Pantanal, Brasil. In: Medellín RA, Equihua C, Chetkiewicz CL, Crawshaw PG Jr, Rabinowitz A, Redford KH, Robinson JG, Sanderson EW and Taber AB (eds) *El Jaguar en el Nuevo Milenio*. Universidad Nacional Autónoma de México/Wildlife Conservation Society, México, pp 209-221.
- Eizirik E, Kim J, Menotti-Raymond M, Crawshaw PG Jr, O'Brien SJ, Johnson WE (2001) Phylogeography, population history e conservation of jaguars (*Panthera onca*, Mammalia, Felidae). *Mol Ecol* 10: 65-79.
- Eizirik E, Indrusiak CB and Johnson WE (2002) Análisis de la viabilidad de las poblaciones de jaguar: evaluación de parámetros y estudios de caso entre poblaciones remanentes del sur de Sudamérica. In: Medellín RA, Equihua C, Chetkiewicz CL, Crawshaw PG Jr,

- Rabinowitz A, Redford KH, Robinson JG, Sanderson EW and Taber AB (eds) El Jaguar en el nuevo milenio. Universidad Nacional Autonoma de Mexico/Wildlife Conservation Society, México, pp 501-518.
- Eizirik E, Haag T, Santos AS, Salzano FM, Silveira L, Azevedo FCC and Furtado MM (2008) Jaguar Conservation Genetics. *Cat News* 4(Special issue): 31-34.
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinformatics Online* 1: 47-50.
- Excoffier L, Laval G and Schneider S (2006) ARLEQUIN: An Integrated Software Package for Population Genetics Data Analysis, Version 3.1. Bern, Switzerland, Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, Univ. of Berne. 145pp.
- Falush D, Stephens M and Pritchard JK (2003) Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* 164:1567-1587.
- Frankham R, Ballou JD and Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, 617 pp.
- Goudet J (2002) FSTAT: a program to estimate and test gene diversities and fixation indices. Lausanne: Institute of Ecology, Switzerland.
- Grigione MM, Menke K, Lopez-González C, List R, Banda A, Carrera J, Carrera R, Giordano AJ, Morrison J, Sternberg M, Thomas R, Van Pelt B (2009) Identifying potential conservation areas for felids in the USA and Mexico: integrating reliable knowledge across an international border. *Oryx* 43(1): 78-86
- Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiples alleles. *Biometrics*, 48, 361-372.
- Haag T (2009) *Genética da Conservação e Ecologia Molecular de Onças-pintadas (Panthera onca, Felidae)*. Ph.D. Thesis, Federal University of Rio Grande do Sul.
- Harris MB, Arcangelo C, Pinto ECT, Camargo G, Neto MBR and Silva SM (2005) Estimativa de perda da área natural da Bacia do Alto Paraguai e Pantanal Brasileiro. *Conservation International*, Campo Grande, MS pp35.
- Hoogesteijn R and Mondolfi E (1992) *El jaguar, Tigre Americano*. Armitano, Caracas, Venezuela.
- Inskip C and Zimmermann A (2008) Human-felid conflict: a review of patterns and priorities worldwide. *Oryx* 43(1): 18-34

- Karanth KU and Chellam R (2009) Carnivore conservation at the crossroads. *Oryx* 43(1): 1-2
- Marinho-Filho J and Machado RB (2006) Metapopulações, ecologia de paisagens e a conservação dos carnívoros brasileiros
- Marshall TC, Slate J, Kruuk LEB and Pemberton JM (1998) Statistical confidence for likelihood based paternity inference in natural populations. *Mol Ecol* 7: 639-655.
- Menotti-Raymond MM, David VA, Lyons LA et al. (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics* 57: 9-23.
- Menotti-Raymond MM, David VA, Wachter LL, Butler JM, O'Brien SJ (2005) An STR forensic typing system for genetic individualization of domestic cat (*Felis catus*) samples. *J Forensic Sci Soc* 50: 1061-1070.
- Noss RF, Quigley HB, Hornocker MG, Merrill T, Paquet PC (1996) Conservation Biology and Carnivore Conservation in the Rocky Mountains. *Conserv Biol* 10:949-963
- Nunes da Cunha, C.; Rawiel, C. P.; Wantzen, K. M.; Junk, W. J. & Lemes do Prado, A. 2006. Mapping and characterization of vegetation units by means of Landsat imagery and management recommendations for the Pantanal of Mato Grosso (Brazil), north of Poconé. *Amazoniana* XIX(1):1-32.
- Oliveira TG (1994) Neotropical Cats: Ecology and Conservation. EDUFMA, São Luís 220 pp.
- Page RDM and Holmes E (1998) *Molecular Evolution – A Phylogenetic Approach*. Blackwell Science, Cambridge.
- Petit RJ, Mousadik EL, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv Biol* 12: 844-855
- Pritchard JK, Stephens P, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- Pritchard JK, Wen W (2004) Documentation for STRUCTURE software: version 2. Department of Human Genetics, University of Chicago, Chicago.
- Quigley HB and Crawshaw PG Jr (1992) The conservation plans for the jaguar *Panthera onca* in the Pantanal region of Brazil. *Biol Conserv* 61:149-157.
- Rice WR (1989) Analyzing table of statistical tests. *Evolution* 43: 223-225
- Ruiz-Garcia M, Payán E, Murillo A, Alvarez D (2006) DNA microsatellite characterization of the jaguar (*Panthera onca*) in Colombia. *Genes & Genetic Systems* 81: 115-127.

- Saiki RK, Scharf S, Faloona F et al. (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.
- Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning*, 2nd edn. Cold Spring Harbor Laboratory Press, New York
- Sanderson EW, Redford KH, Chetkiewicz CB, Medellín RA, Rabinowitz AR, Robinson JG and Taber AB (2002) Planning to save species: the Jaguar as a model. *Conserv Biol* 16:58-72.
- Schaller GB and Crawshaw PG Jr (1980) Movement patterns of jaguar. *Biotropica* 12:161-168.
- Schipper J, Chanson JS, Chiozza F et al. (2008) The Status of the World's Land and Marine Mammals: Diversity, Threat, and Knowledge. *Science* 322: 225-230
- Seddon PJ and Leech T (2008) Conservation short cut, or long and winding road? A critique of umbrella species criteria. *Oryx* 42(2): 240-245
- Silveira L (2004) *Ecologia Comparada e Conservação da Onça-pintada (Panthera Onca) e Onça-parda (Puma Concolor), no Cerrado e Pantanal*. Ph.D. Thesis, University of Brasilia
- Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.
- Soisalo MK and Cavalcanti SMC (2006) Estimating the density of a jaguar population in the Brazilian Pantanal using camera-traps and capture-recapture sampling in combination with GPS telemetry. *Biol Conserv* 129:487-496.
- Sollman R, Tôrres NM and Silveira L (2008) Jaguar Conservation in Brazil: The Role of Protected areas. *Cat News* 4(Special issue): 15-20
- Trigo TC, Freitas TRO, Kunzler G, Cardoso L, Silva JCR, Johnson WE, O'Brien SJ, Bonatto SL and Eizirik E (2008) Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L.geoffroyi* and *L.tigrinus* in southern Brazil. *Mol Ecol* 17:4317-4333.
- Uphyrkina O, Johnson WE, Quigley H, Miquellett, Marker L, Bush M and O'Brien SJ (2001) Phylogenetics, genome diversity and origin of modern leopard, *Panthera pardus*. *Mol Ecol* 10:2617-2633.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4: 535-538.

Woodroffe R and Ginsberg JR (1998) Edge Effects and the Extinction of Populations Inside Protected Areas. *Science* 280: 2126-2128

Table 1: Jaguar samples analyzed in the present study, including the geographic location of each ranch, individual ID, sex of each individual, and contact information.

Population name	Geographic Coordinates	Individual	Sex	Contact
Caiman Ecological Refuge	19°48'00''S 56°16'12''W	bPon333	M	Jaguar Conservation Fund
		bPon339	M	
		bPon340	F	
		bPon341	M	
		bPon342	F	
		bPon343	F	
		bPon344	F	
		bPon345	F	
		bPon349	M	
		bPon350	M	
		bPon351	F	
		bPon352	F	
		bPon155	F	
		bPon156	F	
		bPon157	F	
		bPon158	F	
San Francisco Ranch	20°04'60''S 56°36'00''W	bPon67	M	CENAP/ICMBio; I. Pró-Carnívoros
		bPon68	F	
		bPon69	F	
		bPon70	M	
		bPon71	F	
		bPon72	F	
		bPon73	M	
		bPon74	F	
		bPon75	M	
		bPon76	M	
São Bento Ranch	19°28'05''S 56°59'26''W	bPon77	M	CENAP/ICMBio; I. Pró-Carnívoros
		bPon144	F	
		bPon145	M	
		bPon146	F	
		bPon147	F	
		bPon148	F	
		bPon149	F	
		bPon150	M	
Sete Ranch	19°56'60''S 56°24'60''W	bPon151	M	CENAP/ICMBio; I. Pró-Carnívoros
		bPon152	F	
		bPon153	F	
		bPon360	M	CENAP/ICMBio; I. Pró-Carnívoros
		bPon361	F	

	bPon362	F	
Table 1 : Continued.			
Sete Ranch	bPon363	M	CENAP/ICMBio; I. Pró-
	bPon364	M	Carnívoros
	bPon379	M	
	bPon384	M	
	bPon385	F	
	bPon386	M	
	bPon388	M	

Table 2: Measures of diversity at 12 loci in the four populations analyzed in this study. Sample size (N), observed number of alleles (A), observed (Ho) and expected (He) heterozigoties were calculated with the program ARLEQUIN, while allelic richness (AR) was calculated with the software package FSTAT.

Locus	Caiman Ecological Refuge (n=22)					San Francisco Ranch (n=11)					São Bento Ranch (n=10)					Sete Ranch (n=10)				
	N	A	AR ^a	Ho	He	N	A	AR ^a	Ho	He	N	A	AR ^a	Ho	He	N	A	AR ^a	Ho	He
FCA742	20	9	6.46	0.95	0.81	11	7	6.78	0.82	0.87	10	9	8.30	0.80	0.90	9	7	6.88	1.00	0.89
FCA723	18	6	4.31	0.78	0.69	11	33	2.93	0.64	0.54	10	5	4.76	0.70	0.71	10	4	3.60	0.40	0.54
FCA740	20	5	3.45	0.55	0.60	11	5	4.92	0.73	0.80	10	4	3.80	0.70	0.73	10	3	3.00	0.30	0.65
FCA441	20	5	3.67	0.35	0.49	11	3	3.00	0.73	0.63	9	5	4.89	0.67	0.80	8	3	3.00	0.50	0.54
FCA391	21	5	4.07	0.76	0.62	11	6	5.91	1.00	0.86	10	5	4.93	0.90	0.77	9	5	4.88	0.89	0.80
F98	21	3	3.00	0.67	0.66	11	3	3.00	0.55	0.68	10	3	2.99	0.60	0.49	9	3	3.00	0.78	0.58
F53	18	5	4.12	0.67	0.67	11	4	3.93	0.73	0.74	10	6	5.73	0.90	0.81	10	5	4.96	1.00	0.81
F124	21	6	4.52	0.71	0.74	11	5	4.70	0.91	0.75	10	5	4.57	0.70	0.68	10	6	5.56	0.70	0.78
F146	20	3	2.19	0.20	0.19	11	2	2.00	0.27	0.45	9	2	1.99	0.22	0.21	9	3	2.88	0.22	0.38
F85	20	8	6.62	0.80	0.85	11	6	5.59	0.82	0.81	10	4	3.80	0.80	0.68	9	6	5.66	0.89	0.74
F42	19	7	6.15	0.89	0.84	11	6	5.37	0.82	0.72	10	6	5.95	0.90	0.85	8	7	7.00	0.75	0.83
F453	16	6	5.48	0.94	0.79	11	4	3.92	0.73	0.65	10	6	5.54	0.70	0.75	10	5	4.76	0.80	0.71
Overall		5.7	4.50	0.69	0.66		4.5	4.34	0.73	0.71		5	4.77	0.71	0.70		4.7	4.60	0.69	0.69

^aAllelic Richness calculated for the minimum sample size of eight diploid individuals.

Table 3: Fluorescent label and multiplex panel employed for each locus within the optimized four-panel multiplex used in this study. Observed heterozygosity (Ho) and expected heterozygosity (He) was calculated with the software FSTAT, while the number of alleles (A), allelic size range in base pairs, and estimated frequency of null alleles (F(Null)) were calculated with CERVUS for each locus. The overall mean across loci is shown at the bottom.

Locus	Label	Panel	A	Range (bp)	Ho	He	F(Null)
FCA740	NED	1	5	300-316	0.57	0.70	0.094
FCA453	HEX	1	6	192-216	0.81	0.76	-0.032
F85	NED	1	9	139-183	0.82	0.80	-0.024
F124	FAM	1	8	207-231	0.75	0.75	-0.006
FCA441	NED	2	7	149-177	0.52	0.62	0.059
FCA391	NED	2	6	215-243	0.86	0.76	-0.070
F98	FAM	2	3	189-195	0.65	0.67	0.014
FCA723	FAM	3	7	200-244	0.65	0.63	-0.028
FCA742	NED	3	13	142-180	0.90	0.87	-0.023
F146	FAM	3	3	173-182	0.22	0.30	0.128
F42	NED	4	7	243-275	0.85	0.85	-0.007
F53	FAM	4	6	164-196	0.80	0.78	-0.016
Mean	-	-	7	-	0.71	0.71	-

Table 4: Pairwise Fst (below the diagonal) and Rst values (above the diagonal) for the four populations analyzed in the Pantanal region.

	Caiman	San Francisco	São Bento	Sete
Caiman	–	0.074*	0.031	-0.010
San Francisco	0.053*	–	0.103*	0.033
São Bento	0.042*	0.039*	–	-0.027
Sete	0.029*	0.039*	0.046*	–

Significant values: *P<0,01

Table 5: Mean values of likelihood and likelihood variance across 5 runs for each assumed number of populations (K) calculated with the program STRUCTURE using a MCMC procedure of 2,000,000 generations following a burn-in of 2,000,000 steps for the Pantanal populations.

K	LnP(D)	Var [LnP(D)]
1	-1.793,98	31,84
2	-1.903,36	268,74
3	-2.388,00	1.300,96
4	-2283,14	1.176,36

Table 6: Mean values of likelihood and likelihood variance across 5 runs for each assumed number of populations (K) calculated with the program STRUCTURE using a MCMC procedure of 2,000,000 generations following a burn-in of 2,000,000 steps for the composite data set (Pantanal + Atlantic Forest).

K	LnP(D)	Var [LnP(D)]
3	-3609,18	258,92
4	-3597,24	370,08
5	-3560,52	442,58
6	-3478,86	424,44
7	-3527,3	577,9
8	-3612,4	783,32

Table 7: Pairwise Fst (below the diagonal) and Rst values (above the diagonal) between the whole Pantanal population and the four Atlantic Forest populations.

	Pantanal	Iguaçu	Morro do Diabo	Ivinhema	Porto Primavera
Pantanal	–	0.137*	0.207*	0.106*	0.062*
Iguaçu	0.081*	–	0.004	0.057	0.053
Morro do Diabo	0.163*	0.203*	–	0.027	0.046
Ivinhema	0.113*	0.128*	0.122*	–	0.045
Porto Primavera	0.079*	0.062*	0.079*	0.067*	–

Significant values: *P<0.01

Table 8: Observed allele frequencies at each locus for each local population sampled in the Brazilian Pantanal. The number of genotyped individuals is shown in parentheses. Private alleles are double underlined.

		Caiman	San Francisco	São Bento	Sete
Locus	Allele	(20)	(11)	(10)	(9)
FCA742	142	<u>0.025</u>	-	-	-
	150	<u>0.050</u>	-	-	-
	152	0.275	0.091	0.200	0.111
	154	0.025	0.136	0.050	-
	156	-	0.091	0.050	-
	158	0.125	0.182	0.200	0.167
	160	-	0.091	-	0.222
	162	0.075	0.136	0.100	0.167
	164	0.100	-	0.150	0.167
	170	0.300	0.273	0.100	0.111
	174	-	-	0.100	0.056
	178	<u>0.025</u>	-	-	-
	180	-	-	<u>0.050</u>	-
Locus	Allele	(18)	(11)	(10)	(10)
FCA723	200	<u>0.028</u>	-	-	-
	208	<u>0.028</u>	-	-	-
	220	-	-	<u>0.050</u>	-
	232	0.306	0.273	0.100	0.250
	236	0.167	0.091	0.200	0.050
	240	0.444	0.636	0.500	0.650
	244	0.028	-	0.150	0.050
Locus	Allele	(20)	(11)	(10)	(10)
FCA740	300	0.050	0.091	-	-
	304	0.400	0.273	0.350	0.300
	308	0.500	0.182	0.350	0.500
	312	0.025	0.318	0.250	0.200
	316	0.025	0.136	0.050	-
Locus	Allele	(20)	(11)	(9)	(8)
FCA441	149	<u>0.025</u>	-	-	-
	157	-	-	<u>0.333</u>	-
	161	-	-	<u>0.167</u>	-
	165	0.125	0.227	0.167	0.313
	169	0.700	0.545	0.278	0.625
	173	<u>0.025</u>	-	-	-
	177	0.125	0.227	0.056	0.063
Locus	Allele	(21)	(11)	(10)	(9)
FCA391	215	0.071	0.091	0.150	0.167
	223	0.571	0.227	0.400	0.222
	231	0.119	0.136	0.100	0.056
	235	0.024	0.182	0.250	0.222

239	0.214	0.227	-	0.333
------------	-------	-------	---	-------

Table 8: Continued.

	243	-	0.136	0.100	-
Locus	Allele	(21)	(11)	(10)	(9)
F98	189	0.286	0.227	0.150	0.611
	192	0.262	0.364	0.700	0.167
	195	0.452	0.409	0.150	0.222
	F53				
Locus	Allele	(18)	(11)	(10)	(10)
F53	164	0.028	0.364	0.100	0.150
	180	-	-	<u>0.050</u>	-
	184	0.167	0.273	0.100	0.200
	188	0.500	-	0.350	0.300
	192	0.056	0.091	0.150	0.100
	196	0.250	0.273	0.250	0.250
Locus	Allele	(21)	(11)	(10)	(10)
F124	203	0.024	-	-	0.050
	207	0.333	0.136	0.300	0.400
	211	0.357	0.409	0.500	0.250
	215	0.190	0.136	-	0.150
	219	0.071	0.045	0.100	-
	223	0.024	-	0.050	0.100
	227	-	-	<u>0.050</u>	-
	231	-	0.273	-	0.050
Locus	Allele	(20)	(11)	(9)	(9)
F146	173	0.900	0.682	0.889	0.778
	176	0.075	0.318	0.111	0.167
	182	0.025	-	-	0.056
Locus	Allele	(20)	(11)	(10)	(9)
F85	139	0.150	0.091	0.050	-
	143	-	-	-	<u>0.111</u>
	147	0.250	0.273	0.450	0.444
	151	0.225	0.182	0.350	0.278
	155	0.025	-	-	0.056
	159	0.125	0.318	0.150	0.056
	163	0.050	0.045	-	-
	179	0.075	0.091	-	0.056
	183	<u>0.100</u>	-	-	-
Locus	Allele	(19)	(11)	(10)	(8)
F42	251	0.105	0.500	0.150	0.125
	255	0.184	0.045	-	0.188
	259	0.289	0.182	0.100	0.063
	263	0.026	0.091	0.300	0.063
	267	0.132	0.136	0.150	0.375
	271	0.132	0.045	0.150	0.125
	275	0.132	-	0.150	0.063

Table 8: Continued

Locus	Allele	(16)	(11)	(10)	(10)
FCA453	192	0.344	0.545	0.450	0.150
	196	0.094	0.091	0.050	0.500
	200	0.094	-	0.100	-
	208	0.125	-	0.050	0.100
	212	0.063	0.136	0.100	0.050
	216	0.281	0.227	0.250	0.200

Figure 1: Map of sampled Pantanal populations and Atlantic Forest sites. The inset shows the geographic location of the Pantanal and the Atlantic Forest biome within the Brazilian territory, while the main map depicts the sampling locales within each ecosystem. The largest distance between the Pantanal ranches is 80km, whereas the distance between Pantanal and Porto Primavera (the closest Atlantic Forest fragment) is approximately 450km.

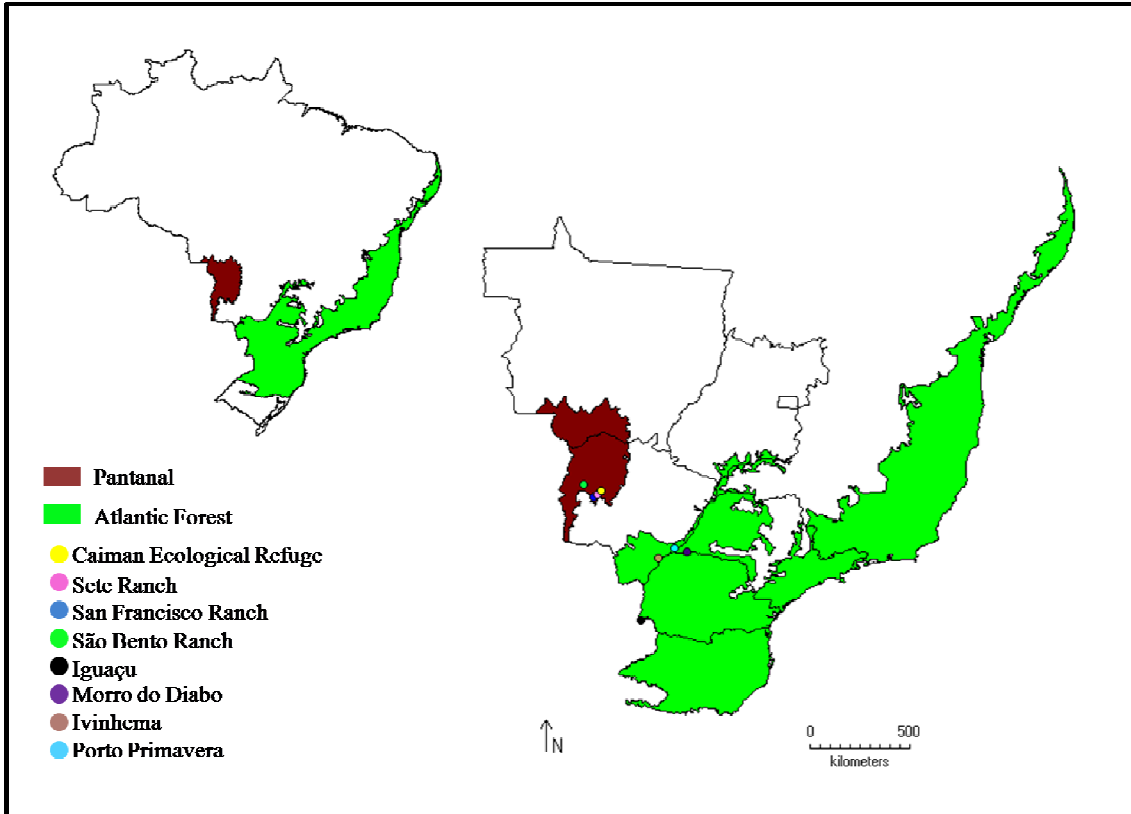
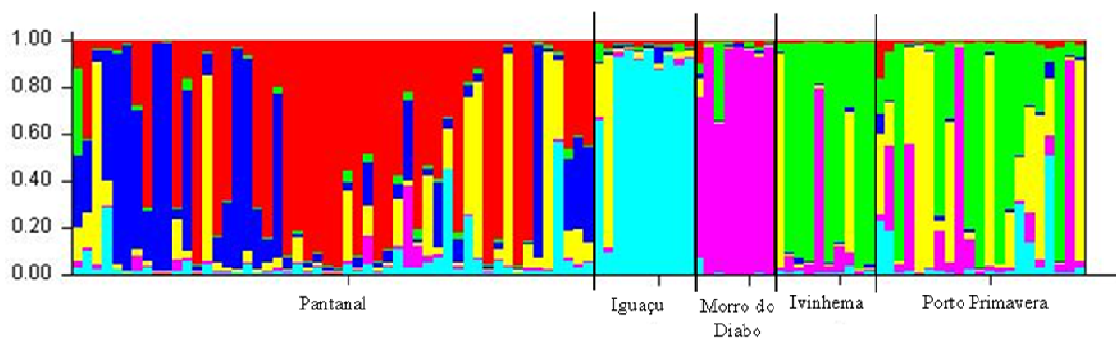


Figure 2: Proportional membership of each jaguar sample in the composite data set (Pantanal as a single population and the four Atlantic Forest fragments) inferred by STRUCTURE. Each individual is represented by a vertical bar.



Considerações finais

Os índices de diversidade genética encontrados nas populações do Pantanal corroboram a hipótese de que as onças-pintadas presentes neste bioma ainda mantêm os níveis de variabilidade esperados para uma população grande e saudável (Frankham et al. 2002). Ainda que a perda de hábitat e a retalição devido ao ataque ao rebanho tenham sido perigos constantes nesta região, a dinâmica populacional ainda não foi consideravelmente afetada, provavelmente devido à alta disponibilidade de presas e à dificuldade de acesso a muitas áreas pelos humanos (Soisalo and Cavalcanti 2006).

Os níveis de variabilidade foram de moderados a altos: heterozigosidade esperada variou entre 0.66 e 0.71 e o número médio de alelos por loco foi de sete. Estes valores são bastante similares aos encontrados em um estudo que analisou 29 locos de microssatélites ao longo da distribuição geográfica da espécie (heterozigosidade 0.74 e número médio de alelos por loco de 8,3; Eizirik et al. 2001). A análise Bayesiana realizada no programa STRUCTURE indicou a existência de apenas uma população e os índices de diferenciação foram extremamente baixos, ainda que significativos. Estes valores são consistentes com a hipótese de uma população panmítica na região, havendo, no entanto, algum nível de diferenciação local, que pode ter sido causado por amostragem ou parentesco de alguns indivíduos capturados nas áreas vizinhas.

A partir dos resultados gerados pelo programa STRUCTURE e pelos índices de diferenciação genética, comparamos as amostras do Pantanal como uma única população com os fragmentos remanescentes da Mata Atlântica. A amostragem dos dois biomas foi relativamente semelhante embora os resultados tenham sido bastante divergentes. No bioma Mata Atlântica os fragmentos indicaram níveis de diferenciação genética de moderado a alto, enquanto que no Pantanal esses valores foram bastante baixos e a análise populacional resultou em apenas uma população para toda a área. Desse modo, analisamos a população Pantaneira com a da Mata Atlântica no programa STRUCTURE e o resultado foi a subdivisão do Pantanal em duas populações e as quatro populações da Mata Atlântica se mantiveram. Porém nenhuma explicação biológica foi encontrada para este fenômeno de subdivisão das amostras do Pantanal e isto só pode ser visto quando mais amostras são incluídas na análise, o que pode indicar a presença de indivíduos mais relacionados entre si que leva o programa a superestimar o número de populações.

Conclui-se então que as onças-pintadas presentes no bioma Pantanal ainda encontram-se dispostas em uma população grande e saudável, provavelmente devido ao bom estado de preservação que este bioma se encontra, o que permite que os animais dispersem por

longas distâncias, mantendo assim o fluxo gênico entre eles. Assim, esta população deve servir como modelo para entendermos a estruturação genética e a dinâmica demográfica da espécie, gerando informação básica de extrema importância para o estudo da perda de diversidade, do endocruzamento e dos processos decorrentes do pequeno tamanho populacional e do isolamento devido à ação humana.

Livros Grátis

(<http://www.livrosgratis.com.br>)

Milhares de Livros para Download:

[Baixar livros de Administração](#)

[Baixar livros de Agronomia](#)

[Baixar livros de Arquitetura](#)

[Baixar livros de Artes](#)

[Baixar livros de Astronomia](#)

[Baixar livros de Biologia Geral](#)

[Baixar livros de Ciência da Computação](#)

[Baixar livros de Ciência da Informação](#)

[Baixar livros de Ciência Política](#)

[Baixar livros de Ciências da Saúde](#)

[Baixar livros de Comunicação](#)

[Baixar livros do Conselho Nacional de Educação - CNE](#)

[Baixar livros de Defesa civil](#)

[Baixar livros de Direito](#)

[Baixar livros de Direitos humanos](#)

[Baixar livros de Economia](#)

[Baixar livros de Economia Doméstica](#)

[Baixar livros de Educação](#)

[Baixar livros de Educação - Trânsito](#)

[Baixar livros de Educação Física](#)

[Baixar livros de Engenharia Aeroespacial](#)

[Baixar livros de Farmácia](#)

[Baixar livros de Filosofia](#)

[Baixar livros de Física](#)

[Baixar livros de Geociências](#)

[Baixar livros de Geografia](#)

[Baixar livros de História](#)

[Baixar livros de Línguas](#)

[Baixar livros de Literatura](#)
[Baixar livros de Literatura de Cordel](#)
[Baixar livros de Literatura Infantil](#)
[Baixar livros de Matemática](#)
[Baixar livros de Medicina](#)
[Baixar livros de Medicina Veterinária](#)
[Baixar livros de Meio Ambiente](#)
[Baixar livros de Meteorologia](#)
[Baixar Monografias e TCC](#)
[Baixar livros Multidisciplinar](#)
[Baixar livros de Música](#)
[Baixar livros de Psicologia](#)
[Baixar livros de Química](#)
[Baixar livros de Saúde Coletiva](#)
[Baixar livros de Serviço Social](#)
[Baixar livros de Sociologia](#)
[Baixar livros de Teologia](#)
[Baixar livros de Trabalho](#)
[Baixar livros de Turismo](#)