Effects of habitat deterioration on the population genetics and conservation of the jaguar

S. Roques^{*1}, R. Sollman², A. Jacomo², N Torres^{2,6}, L. Silveira², C. Chavez³, C. Keller⁴, D. Mello do Prado⁴, P. Carignano Torres⁴, C. Jorge dos Santos⁴, X. Bernardes Garcia da Luz⁴, W. E. Magnusson⁴, José A. Godoy⁷, Gerardo Ceballos⁵ and F. Palomares¹

¹ Department of Conservation Biology, Estación Biológica de Doñana (EBD-CSIC). Avda. Américo Vespucio s/n. 41092, Sevilla, Spain

² Jaguar Conservation Fund/Instituto Onça-Pintada, C.P. 193, 75830-000 Mineiros - GO, Brazil

³ Departamento de Ciencias Ambientales, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Unidad Lerma, av. Hidalgo poniente 46, col. La estación, Lerma de Villada, Estado de México, 52006, México.

⁴ Instituto Nacional de Pesquisas da Amazônia - INPA, CP 2223, 69080-971 Manaus, Amazonas, Brazil

⁵ Instituto de Ecología, Universidad Nacional Autónoma de México, AP 70-275, México, D.F. 04510, México

⁶ Biology Institution, Federal University of Uberlândia – UFU, Campus Umuarama Rua Ceará, s/n, Bloco 2D, 38400-902 Uberlândia-MG, Brazil

⁷ Department of Integrative Ecology, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas

*Correspondence: Séverine Roques. Tel. +34 954466700; fax: +34 954621125. E-mail address: severineroques@hotmail.com

Short title: Jaguar genetic population structure and conservation

ABSTRACT

Over the past century, human activities and their side effects have significantly threatened both ecosystems and resident species. Nevertheless, the genetic patterns of large felids that depend heavily on large and well-conserved continuous habitat remain poorly studied. Using the largest-ever contemporary genetic survey of wild jaguars (*Panthera onca*), we evaluated their genetic diversity and population structure in natural (Brazilian Amazon) and highly modified habitats (e.g. Cerrado, Caatinga) including those close to the northern (Yucatan, Mexico) and southern (Pantanal) edge of the species' distribution range. Data from our set of microsatellites revealed a pronounced genetic structure, with four genetically differentiated geographic areas. Geographic distance was not the only factor influencing genetic differentiation through the jaguar range. Instead, we found evidence of the effects of habitat deterioration on genetic patterns: while the levels of genetic diversity in the Amazon forest, the largest continuum habitat for the species, are high and consistent with panmixia across large distances, genetic diversity near the edge of the species distribution has been reduced through population contractions. Mexican jaguar populations were highly differentiated from those in Brazil and genetically depauperated. An isolated population from the Caatinga showed the genetic effects of a recent demographic decline (within the last 20-30 years), which may reflect recent habitat degradation in the region. Our results demonstrate that the jaguar is highly sensitive to habitat fragmentation especially in human-dominated landscapes, and that in Brazil, the existing but limited genetic connectivity in the central protected areas should be maintained. These conclusions have important implications for the management of wide-ranging species with high dispersal and low population density. The restoration of ecological connectivity between populations over relatively large scales should be one of the main priorities for species conservation.

Keywords: felid, elusive, habitat deterioration, connectivity, conservation

26 INTRODUCTION

27 Human impacts on ecosystems have increased dramatically throughout the world over the
28 last century. Anthropogenic modifications of habitat (i.e., loss and fragmentation) that impact
29 population size and connectivity can result in genetic erosion, which may seriously compromise the
30 fitness of populations and increase extinction risk (Saccheri et al. 1998; Ceballos et al., 2002;
31 Frankham 2003, 2005; Reed et al. 2003; Palomares et al. 2012). In addition, the spatial distribution of
32 populations and their dynamics may also be important in shaping the patterns of genetic diversity
33 throughout a species' range. Models have suggested the vulnerability of natural populations would be
34 determined in part by their spatial distribution (peripheral vs. core populations) because it directly
35 influences the genetic variability and abundance of a species (Gyllenberg & Hanski 1992). In this
36 context, one can predict that at the scale of species spatial distribution, the most vulnerable
37 populations would be in areas impacted by both the demographic effects (i.e., location at the edge of
38 the species' range) and environmental deterioration (i.e., habitat fragmentation and loss). Genetic
39 analyses may provide early warning signals for the demographic consequences of these processes and
40 provide specific recommendations for the design of effective conservation strategies.

41 Large felids have extensive home ranges and usually depend on well-conserved continuous habitat for
42 reproduction and dispersal. They are thus particularly vulnerable to habitat degradation (Crooks
43 2002). During the last century, most of these charismatic species have experienced declines in
44 population size worldwide, and the accelerated human-mediated habitat degradation (i.e., loss and
45 fragmentation) and synergic effects of direct persecution such as hunting may be severely threatening
46 their long-term survival (Nowell & Jackson 1996; Perez 2001; IUCN 2010). While population surveys of
47 elusive carnivorous felid species are a challenge (Williams et al. 2002, Thompson 2004), genetic
48 studies are even more limited by the difficulty of obtaining an adequate number of samples. As a
49 result, the genetic patterns of many large felids and their responses to landscape scale habitat
50 disturbance, including fragmentation and degradation, remain poorly studied. Improvements to non-
51 invasive genetic testing through sampling of faeces can promote broader scale surveys in the near
52 future (Janecka et al. 2008, Roques et al. 2011, 2012). However, to date genetic studies on declining
53 populations of large carnivores are limited primarily to medium and small spatial scales, such as Amur
54 tiger (*Panthera tigris altaica*, Henry et al. 2009, Alasaad et al. 2009), jaguar (*Panthera onca*, Eizirik et
55 al. 2001, Moreno et al. 2006, Haag et al. 2010), leopards (*Panthera pardus*, Dutta et al. 2013) and tiger
56 (*Panthera tigris*, Reddy et al. 2011, Joshi et al. 2013, Sharma et al. 2013).

57 The jaguar is the largest felid in the American continent and the third-largest cat worldwide.
58 Historically, its range encompassed a large area extending from the southwestern USA through the
59 Amazon basin to the Rio Negro in Argentina, but today it occupies only about 50% of this range
60 (Mittermeier et al. 1998, Zeller 2007; Sanderson et al. 2002; Figure 1). Years of poaching and livestock
61 conflicts during the last century associated with massive rates of deforestation have reduced and
62 severely fragmented the species' habitat and distribution (Zeller 2007). As a result, the IUCN classifies
63 the jaguar as Near Threatened with declining population trends (IUCN, 2010). Most of the loss of range

64 has occurred at the edges in northern Mexico and southwestern United States, and northern
65 Argentina (Sanderson et al. 2002). In Brazil, which constitutes approximately 50% of the current jaguar
66 range (Zeller 2007), the Amazon rainforest and the Pantanal floodplains are thought to harbor the two
67 largest continuous jaguar populations worldwide (Sanderson et al. 2002). However, there is extensive
68 deforestation and development in Brazil, especially in the highly impacted southern Cerrado and
69 Caatinga biomes, at the eastern limit of the jaguar distribution.

70 The first large-scale phylogeographic study of the jaguar was based on the analyses of mitochondrial
71 DNA (mtDNA) control region sequences and 29 nuclear microsatellite loci of 44 individuals sampled
72 from Mexico to southern Brazil (Eizirik et al. 2001). It revealed a low level of genetic differentiation in
73 the species throughout its geographic range. This pattern of genetic homogeneity was interpreted as
74 the result of a rather recent population expansion, about 300,000 years ago, followed by a history of
75 demographic connectivity on a continental scale. The only partition observed between the northern
76 and southern areas of the range was attributed to a reduced historical gene flow across the Amazon
77 River, although such a reduced connectivity was not supported by a more recent study (Moreno et al.
78 2006).

79 The continued destruction and fragmentation of its habitat suggest that many jaguar populations
80 likely became demographically isolated and genetically depauperated in recent years. It appears that
81 past and recent large-scale habitat loss and fragmentation has been sufficiently strong to promote
82 genetic differentiation of jaguars in the Atlantic forest regions (Haag et al., 2010). Therefore, it is
83 critical to gain a better understanding of genetic patterns and recent demographic processes at both
84 local and large scales and to compare core and peripheral populations within the distribution range of
85 the species.

86 In this study we report on populations from Mexico and Brazil where jaguars are still found at high
87 densities and in areas representing both highly modified peripheral and well as preserved core
88 habitats. The results represent one of the most extensive genetic analyses of contemporary samples
89 of jaguars to date. We assessed the genetic structure and diversity of jaguar populations from diverse
90 areas, tested whether jaguars are still genetically connected throughout the entire distribution range,
91 and evaluated the potential genetic consequences of habitat fragmentation on populations. Finally,
92 we discuss the importance of potential corridors within Brazil and the Yucatan Peninsula in Mexico
93 and the implications for conservation priorities.

94

95 **MATERIAL AND METHODS**

96 **Study areas, samples and genotyping**

97 Non-invasive genetic samples of jaguars were obtained by collecting faeces in several areas of Mexico
98 and Brazil ([Figure 1A](#), [Table 1](#), and [Supplementary Material S1, S2](#)). We collected in six different areas

in the Yucatan Peninsula, which is close to the northern limit of the jaguar's distribution and includes the largest remaining tract of tropical forest in Mexico. In Brazil, we sampled areas with relatively high densities of jaguars and large extensions of natural or semi-natural habitat, both in Pantanal and Amazon forests, and populations in the Cerrado and Caatinga biomes where the areas are highly modified, have a high human population density, and are less suitable for jaguars. Faeces were collected in four different areas in the Brazilian Amazon, which represents the largest area of relatively continuous jaguar habitat (Sanderson et al. 2002). Pantanal is used primarily for extensive cattle ranching, there is less affected by habitat fragmentation than areas with intensive agriculture. Sampling was carried out at the Caiman Ecological Reserve (PANT), a cattle ranch and ecotourism center located in the southern Pantanal (Mato Grosso do Sul State). The Cerrado biome, originally covered by extensive areas of neotropical savannas and dry forest, has been severely fragmented by the agricultural activities of the last 50 years. Samples were obtained around three areas located within the Cerrado, and along the Araguaia river: the Emas National Park (ENP), one of Brazil's largest reserves located in the transition area with the Amazon biome; in Tocantins State, the Araguaia national Park (ANP) and the Cantão State Park (CSP), the only large conservation unit where jaguars are protected. The Caatinga of eastern Brazil represents the eastern limit of jaguar distribution in South America (Sanderson et al. 2002) and one of the most fragmented habitat remnants of the species in Brazil. Unique to Brazil, the Caatinga is a large and one of the most diverse regions of dry forests and arid scrubland of the world, but the high human population density has completely or partially transformed over 50% of its area (Casteleti et al. 2000).

Sampling was carried out in one of the most important protected areas of the Caatinga, the Serra da Capivara National Park (CAPV). Sampling of faeces in all areas was conducted mostly during the dry season between 2007 and 2009 with the exception in the Adolfo Ducke Reserve (DUCK), where samples were also collected in 2004 and 2005. In all sites, faeces were collected by inspecting roads and trails frequently used by humans or animals, except in Parque Estadual do Cantão (CANT), Araguaia (ARAG), PANT, and PNEM where scat detector dogs were used to find samples (Vynne et al. 2011b). Faeces were collected in sterilized plastic vials with approximately 30ml of absolute alcohol, subsequently transferred to 100-ml plastic jars containing silica pellets (Roeder et al. 2004), and stored at room temperature until DNA extraction. Most samples collected in the Amazon were put directly in silica gel without the first step involving an alcohol solution.

We also obtained blood samples from captured individuals (Table 1). Skin samples collected in 2007 were also obtained from ARAG, Brazil and from Ejido Caobas (CAOB) in Mexico. DNA isolation from blood, liver and skin samples followed a standard phenol–chloroform extraction protocol (Sambrook et al. 1989). DNA was extracted from faecal samples using protocols based on the GuSCN/silica method (Boom et al. 1990) as previously described in Roques et al. (2014). All scat samples collected in the wild were first screened for species identification using species-specific primers (Roques et al. 2011). Those samples belonging to jaguars were genotyped at a set of 11 microsatellite loci as described in Roques et al. (2014). Briefly, after scoring the alleles with GENEMAPPER version 4.0

137 (Applied Biosystems), a unique consensus genotype was assigned to samples given a consensus
138 criterion derived from that proposed by Taberlet et al. (1996) and based on the results of the four PCR
139 replicates. The four genotype replicates were compared to the consensus genotype and the quality
140 index value (QI) was calculated as described by Miquel et al. (2006). Full details on error rates, allelic
141 dropout and false alleles are available in a previous paper (see Supplementary Material 1 in Roques et
142 al. 2014).

143

144 **Population structure, size and gene flow**

145 To explore the genetic evidence for subdivision among jaguars, we first used the program STRUCTURE
146 over the 14 locations and to identify populations within Brazil (BRAZ) or within Mexico (MEXC).
147 Simulations were conducted by varying the number of genetic clusters ($k = 1-12$; alternatively, $k = 1-7$
148 for within BRAZ and MEXC) with 30,000 steps of the Markov chain Monte Carlo (MCMC), following a
149 burn-in period of 300,000 iterations, with and without a priori 'population' information. Twenty
150 independent runs for each k were performed under an admixture model with correlated gene
151 frequencies to determine the number of genetic clusters. The most likely number of k was calculated
152 based on Δk as described in Evanno et al. (2005) and on visual inspection of the plot of $\ln P(D)$ as a
153 function of k , using STRUCTURE HARVESTER (Earl 2011). Once the number of k was estimated, two
154 replicates of a longer run with 300,000 steps of burn-in followed by 1,000,000 steps were performed
155 to assign individuals to clusters. The partition of the total genetic variation into different genetic
156 clusters was further assessed based on a Factorial Component Analysis (FCA) in GENETIX v.4.03
157 (Belkhir et al. 2004). The extent of genetic differentiation among the populations defined based on
158 clustering approaches (see above) was estimated with F_{ST} statistics (Weir & Cockerham 1984) using
159 Genetix (5,000 permutations). Further, we tested whether patterns of neutral genetic structure were
160 the product of isolation by distance. We calculated population-level pairwise genetic differentiation
161 as $F_{ST}/(1-F_{ST})$ (Slatkin 1995) using F_{ST} values calculated in Genetix (Belkhir et al. 2004). Geographic
162 distance was calculated as the closest linear distance between pairs of sampling areas using Google
163 Earth (<http://earth.google.com>). We tested whether genetic distance was related to geographic
164 distance using Mantel tests, implemented in the program IBD (Isolation by Distance; Bohonak et al.
165 2002).

166 **Detection of migrants**

167 STRUCTURE 2.3.2 and GENECLASS 2.0 were also used to identify first-generation migrants and
168 individuals with mixed ancestry. In STRUCTURE, prior population information was used in the
169 USEPOPINFO option in to determine the individuals that were not residents of their sampled
170 population. MIGPRIOR was set to 0.05. GENECLASS 2.0 specifically identifies first generation migrants,
171 i.e. individuals born in a population different to the one it was sampled (Paetkau et al. 2004; Piry et al.
172 2004). The Bayesian criterion of Rannala and Mountain in combination with the resampling method of

173 Paetkau and an alpha level of 0.05 were used to determine critical values. We used a L_h/L_{max}
174 likelihood > 0.60 to statistically identify migrants.

175 **Genetic diversity**

176 Diversity parameters were first calculated for the pre-defined populations. Departures from linkage
177 disequilibrium and the Hardy–Weinberg equilibrium (HWE) were tested using exact tests as
178 implemented in GENEPOP on the web (Rousset 2008). Genetic diversity was assessed through the
179 observed and expected heterozygosity (H_O and H_E) estimated using GENETIX. Further, allelic richness
180 (i.e., the number of alleles per locus independent of sample size) and percentage of shared and
181 private alleles were calculated using the program HPrare (Kalinowski 2005). Differences of indices
182 among populations were tested with Wilcoxon signed-rank tests.

183 **Population size reductions**

184 We used two different approaches to test for a genetic bottleneck signature. Because violations of the
185 panmixia assumption might bias these tests, genetic homogeneity within the pre-defined population
186 units was confirmed based on both F_{ST} statistical significance (see [Supplementary Material S1](#)) and
187 Structure approaches (see above). For the first approach, the mutation-drift equilibrium test which is
188 implemented in BOTTLENECK 1.2.02 (Cornuet & Luikart 1996, Piry et al. 1999), tests whether the
189 number of loci with heterozygosity excess is significantly higher than that expected by chance at
190 mutation-drift equilibrium. In populations that have experienced a relatively recent (within the last
191 $\sim 0.2-4N_e$ generations) reduction in effective size, the number of alleles is reduced faster than gene
192 diversity, leading to a transient excess of heterozygosity (Luikart & Cornuet 1998). The program was
193 initially run under either the 100% infinite alleles model (IAM) or stepwise mutation model (SMM) of
194 microsatellites evolution. In order to test the sensitivity of the analysis to the mutation model
195 chosen, we ran the program under a two-phase mutation model (TPM model) because the
196 microsatellites in this study are dinucleotide repeats, which better fit the IAM (Cornuet & Luikart
197 1996). We ran the program with proportions of either 5% or 30% of SMM. Significance was assessed
198 from 10,000 iterations using a Wilcoxon signed-rank test which give the highest statistical power
199 when population sample size is small (30 or fewer) (Cornuet & Luikart 1996). For the second
200 approach, we used the M-ratio (Garza & Williamson 2001) which corresponds to the mean ratio of the
201 number of alleles to the allele size range across all loci, and the value is expected to decrease
202 following a population reduction. The M-ratio test is more sensitive than the other two tests and
203 would detect a bottleneck signal longer after it occurred, and thus gives insights into population
204 contractions occurring at a larger timescale. M-ratios were calculated using AGARST (Harley 2002) and
205 the critical M-ratio (M_{crit}) for each sample location was determined using the critical_M.exe software
206 (Garza & Williamson 2001). We set the mean number of non-one-stepwise mutations (μ_s) to 0.12 and
207 the mean size of larger mutation (θ_{S_0}) as 2.8 as conservative parameters (i.e., lower critical value),
208 as suggested by the authors. Pre-bottleneck values were calculated using $\alpha = 5 \times 10^{-4}$ (Garza &
209 Williamson 2001) and N_e values estimated in this study for the jaguar, as well as several N_e values

210 (i.e., 20, 50, 150, 300). Two loci with odd-sized alleles (those that did not represent multiples of the
211 recognized repeat unit) were omitted from these analyses (FC115 and FC566).

212 To estimate the effective size (N_e) in our populations, we first applied the linkage disequilibrium
213 method using the program LDNE (Waples & Do 2008), assuming random mating and excluding all
214 alleles with frequencies lower than 0.02. We also used an Approximate Bayesian Computation (ABC)
215 approach as implemented in the program ONESAMP (Tallmon et al. 2008), which is considered more
216 robust and less biased by substructure and overlapping generations than LDNE (Luikart et al. 2010).

217 In order to test the genetic effects of recent habitat degradation in the southeastern Brazilian areas
218 and especially the probable recent isolation of the Caatinga population, we used a coalescent-based
219 MCMC simulation implemented in 2MOD (Ciofi et al. 1999). This method tests whether the observed
220 population structure would better fit a gene flow-drift equilibrium model or a pure drift model; the
221 first model assumes a balance between gene flow and drift (i.e., populations at equilibrium) while the
222 second model assumes that an ancestral panmictic population has evolved into several different units
223 diverging by drift in the absence of gene flow. The MCMC search was carried out twice for 30×10^5
224 iterations with the first 3×10^4 discarded as burn-in. The posterior distribution of F (probability of co-
225 ancestry of any two genes in the putative population) was estimated for each population. Simulations
226 were run with 600,000 steps with a burn-in of 100,000 in three independent runs. We used Tracer v
227 1.40 (<http://beast.bio.ed.ac.uk/>) to evaluate the stationarity of model parameters, verify adequate
228 sample sizes, determine an appropriate amount of burn-in, and verify the consistency between runs.
229 Under the drift model, we estimated the time since isolation among the three areas relative to the
230 population size, (T/N) as $-\log(1-F)$, following Ciofi et al. (1999).

231

232 **RESULTS**

233 **Non-invasive genetics**

234 We successfully determined the species for 73 % ($N=473$) of 651 faecal samples collected and
235 processed (Table 1). Most of the faecal samples were from jaguars (49.7%) and pumas (41.6%), and to
236 a lesser extent, smaller felids (ocelot/margay; 8.7%). Among the 234 jaguar faecal samples, a high
237 proportion (91%) have $\geq 50\%$ quality (based on the Quality Index; QI; Miquel et al. 2006) and 71% of
238 genotypes have even higher quality ($QI \geq 75\%$). Consensus multilocus genotypes for each sample were
239 grouped into 62 different genotypes representing distinct individuals following the assignment
240 strategy described by Roques et al. (2014). Including the genotypes obtained from high quality DNA
241 sources (blood: $n=31$; liver: $n=13$, and skin: $n=7$) we gathered 102 distinct genotypes from 14 study
242 areas across the current distribution range of the jaguars (Table 1 and Supplementary Material S3).

243 **Genetic differentiation and connectivity**

244 The overall genetic differentiation was high and jaguar populations were genetically structured
245 throughout the species' range. Within Brazil, F_{st} values were low and not significantly different from
246 zero among the four Amazonian localities (DUCK, UATM, VIRU, MARA) and among all central areas
247 along the Araguaia river (CANT, ARAG, PNEM), but they were high and significant among the other
248 populations studied (see [Supplementary Material S1](#)). Based on these results, we defined four
249 differentiated genetic entities within Brazil ([Table 1](#)): AMZN (Amazon - DUCK, UATM, MARC, and
250 VIRU); PANT (Pantanal); CAPV (Caatinga); and an intermediate area in the central region, namely
251 CENTR (ARAG, CANT, and PNEM). Within the Yucatan Peninsula, estimates of genetic differentiation
252 (F_{st}) were low and not significant for any pairwise comparison, thus corroborating genetic
253 homogeneity at this scale. Differentiation among the inferred genetic units was very high and
254 significant for comparisons between Brazil (PANT, CAPV, AMZN, CENT) and MEXC ([Table 2](#); $P \leq 0.01$),
255 indicating high divergence in allele frequencies between these geographically distant areas. Within
256 Brazil, the highest value occurred with comparisons involving CAPV and the other sampling areas,
257 while differentiation between CENT and the rest of the populations was lower and the differentiation
258 between AMZN and CENT was not significant ([Table 2](#)).

259 A significant positive correlation between genetic and geographic distance was observed among the
260 *jaguar* populations at both large ([Figure 2A](#); Mantel test, $r = 0.655$, $P < 0.001$) and regional ([Figure 2B](#);
261 Mantel test, $r = 0.5232$, $P < 0.019$) scales. The result of this test showed that a considerable part of the
262 genetic variation was explained by geographic distance. Within Brazil, these results supported the
263 Factorial Component Analysis ([Figure 3B](#)) since all geographically-close populations resembled each
264 other. Also, we found that almost all comparisons involving CAPV ([Figure 2B](#), grey circles) stand
265 above the line, corroborating that this area presents more differentiation with the other areas than
266 expected by distance only.

267 The STRUCTURE analysis including all samples suggested $K = 4$ as the most likely number of genetic
268 clusters ([Figure 3A](#) and [Supplementary Material S4](#) for Evanno's output table for all K values). The
269 geographical samples with predominant membership in the four clusters were grouped into
270 Mexico (MEXC: ZAPT, EDEN, CAO, CALAK, mean $Q = 0.66$); Amazon (AMZN: MARA, VIRU, DUCK,
271 UATM, mean $Q = 0.84$), Caatinga (CAPV mean $Q = 0.71$) and Pantanal (PANT mean $Q = 0.72$). When
272 the Mexican areas were analyzed separately, a single and panmictic population (MEXC, $K = 1$) (results
273 not shown) was the most likely scenario. Within Brazil, $K = 3$ was the most likely number of genetic
274 clusters. These three clusters correspond to the three distinct geographical areas of PANT, AMZN and
275 CAPV. The individuals from the central localities CENT, namely CANT, ARAG, PNEM, cluster with
276 individuals from AMZN, but show some ancestry in the other two populations ([Figure 3A](#)).

277 The representation of all individuals in the Factorial Correspondence Analysis was also highly
278 congruent with the above clustering, clearly depicting the divergence of Mexican areas and the
279 existence of three genetic entities in Brazil (CAPV, PANT, AMZN) and with CENT individuals occupying
280 intermediate positions between these ([Figure 3B](#)). The analyses clearly illustrated that CAPV is highly
281 differentiated from the rest of populations and that jaguars from the central admixed area are

282 genetically intermediate between those from AMZN and those from southern (PANT) and eastern
283 (CAPV) populations.

284 **Identification of migrants and admixed individuals within Brazil**

285 We identified a total of 18 migrants in Brazil (Table 4). Most of them (n=14) were sampled in central
286 areas (CENT), while two in PANT, one in AMZN and one in CAPV. STRUCTURE and GENECLASS were
287 concordant in detecting six first-generation migrants (i.e. not born in the sampled area), all from
288 CENTR (n=2 in ARAG and 4 in PNEM). STRUCTURE also identified two individuals (CANT_H3-28 and
289 PANT_SGH27) that were neither readily classified as migrants nor as residents (Q-values < 0.60)
290 suggesting that they might be of admixed ancestry (Table 3).

291

292 **Genetic diversity and population demography**

293 None of the populations showed significant HWE disequilibrium after Bonferroni correction ($P \leq 0.001$).
294 Also, only two out of 55 tests for Linkage disequilibrium LD were statistically significant after applying
295 the Bonferroni correction. Those tests involved different pairs of loci and occurred in different
296 populations, suggesting that the assayed loci assorted independently. Mean expected and observed
297 heterozygosities across loci and samples were 0.800 and 0.730, respectively. Both heterozygosity and
298 allele number were higher in Brazil (mean $H_e = 0.812$, mean $A = 9.45$) than in Mexico (mean $H_e =$
299 0.634 , $A = 4.45$) (Table 3). Expected heterozygosity, H_e , calculated for the genetic clusters identified
300 above, ranged from 0.654-0.805, with values significantly higher in AMZN (Wilcoxon sign-rank test, $P \leq$
301 0.03) and lower in MEXC ($P \leq 0.03$) than in the other areas. However, the difference between MEXC
302 and CAPV was not significant ($P = 0.22$). Allelic richness was also highest for AMZN ($P \leq 0.02$) and
303 lowest for MEXC and CAPV (Table 3). The allelic richness in PANT was moderate and not significantly
304 different from the values found in MEXC ($P = 0.09$) and CAPV ($P = 0.22$). The jaguar population at
305 CAPV had the lowest proportion of private alleles (4%) in Brazil, less than half of that found for AMZN,
306 and the population at CENT shared the highest proportion of alleles with the other studied
307 populations (74%, 69% and 63% for AMZN, PANT, and CAPV, respectively). While the highest effective
308 population size was estimated for AMZN (>250), the effective population sizes were much lower for
309 the remaining populations (between 13 and 30) (Table 3). When we applied BOTTLENECK, we
310 observed clear signatures of recent bottlenecks for both MEX and CAPV under IAM ($P < 0.05$) and TPM
311 with either SMM = 5% or 70% (see Table 3). However, all tests were non-significant under SMM.
312 Among all populations sampled, the M-ratio ranged from 0.670 (CI = 0.057) to 0.888 (CI = 0.041), with
313 the lowest values found in CAPV and PANT (Table 3). However, only the value for CAPV was lower
314 than almost the whole range of simulated critical values ($M_{crit20} = 0.662$, $M_{crit50} = 0.650$, $M_{crit150} =$
315 0.629 , and $M_{crit300} = 0.600$), suggesting a stronger reduction in size of this population than in the
316 other populations. In contrast, the M-ratio of MEXC was high (0.888) and contrasts with the highly
317 significant P value when BOTTLENECK was applied; these values suggest a more recent population
318 contraction event in this region (Cornuet & Luikart 1996).

319 Using the 2Mod program, we evaluated the alternative hypotheses of whether the isolation of
320 the Caatinga population was the result of a recent isolation (i.e., the pure drift model) or if this
321 reflected an equilibrium situation of an historically small and weakly connected population (i.e., the
322 gene flow-drift equilibrium model). The results of 2Mod overwhelmingly supported a pure-drift rather
323 than a migration-drift equilibrium scenario (P (drift model) = 0.9) for the CAPV, AMZN, CENT
324 populations. Under the drift model, we calculated F values ($FCAPV = 0.1481$, 95% CI: 0.1361-0.1494;
325 $FAMZN = 0.0741$, 95% CI: 0.0737 - 0.0746; $FCENT = 0.0531$, 95% CI: 0.0536 - 0.0541) and the T/N
326 was estimated to be 0.1602 ($2N_e = 28$) for CAPV; 0.0544 ($2N_e=400$) for CENT; and 0.0768 ($2N_e = 596$)
327 for AMZN. Based on a generation time of five years and the effective population size estimates
328 (reported here), these values suggest the population in CAPV has been isolated for approximately 20
329 years.

330

331 **DISCUSSION**

332 **Genetic effects of habitat deterioration and biogeography**

333 Our study examined genetic diversity and connectivity of jaguars on a large spatial scale in
334 Mexican and Brazilian ecosystems. The results indicate that despite prior evidence for historical
335 connectivity and panmixia (Eizirik et al. 2001, Table 5), the jaguar is genetically structured throughout
336 its range. While genetic differentiation of areas of the jaguar distribution range is primarily driven by
337 isolation resulting from distance (Figure 3) and putative barriers to gene flow (e.g., Amazon River,
338 Darien Straits; Eizirik et al. 2001), the recent habitat deterioration (i.e., habitat fragmentation and
339 loss) may have caused a disruption of gene flow and an intensification of genetic drift in part of its
340 range. The population of Capiwara in the eastern edge of the species distribution is separated by a
341 large area of unsuitable habitat, suggesting that such barrier may further contribute to genetic
342 divergence and to the pronounced genetic isolation found in this area.

343 Our results are similar to those reported by Eizirik et al. (2001) for the same area and show that the
344 genetic diversity values in Mexico are some of the lowest reported for the species (Table 5). The low
345 diversity and high differentiation for this particular region may be attributable to the recent
346 colonization of jaguar populations in the northern areas and to a global pattern of isolation by
347 distance (Eizirik et al. 2001). However, the significant signs of recent bottlenecks found in this region
348 suggest that individuals from the Mexican population might be exhibiting the genetic signals of recent
349 anthropogenic perturbations and isolation. This area is situated close to the northern limit of the
350 species' range and is probably more vulnerable to stochastic demographic effects (Vucetich & Waite
351 2003; Chavéz et al. 2005). Additionally, the Yucatan Peninsula population is connected northward to
352 areas with groups of individuals that occur at the lowest densities reported for jaguars, including the
353 relict populations of Sinaloa and Baja California (Navarro-Serment et al. 2005, Rosas-Rosas & Bender
354 2012) (see Figure 1A). Jaguars have been extirpated to the south of the Yucatan, in parts of Nicaragua

355 and Honduras, and this loss may have disrupted the gene flow with individuals from further south
356 (Sanderson et al. 2002).

357 Genetic evidences for the effects of recent isolation were compelling for the Caatinga (CAPV)
358 population. All population structure analyses indicated increased genetic drift and reduced gene flow
359 between CAPV and the other regions. A significant reduction of diversity is reflected in low values of
360 allelic richness (Table 4), whereas both estimates of heterozygosity were close to those estimated
361 previously for the species ($H_e = 0.732$ in Haag et al. 2010 and $H_e = 0.724$ in Eizirik et al. 2001), but
362 lower than those in the Amazonian strongholds (Table 5). This difference may be a reflection of the
363 generally faster response of allelic richness to population contractions than heterozygosity (Cornuet &
364 Luikart 1996, Srikwan & Woodruff 2000), with the former being thus a more sensitive signal of recent
365 genetic erosion in isolated populations. The preponderance of genetic drift and the increased
366 isolation of the CAPV population in recent times are also supported by the selection of a pure-drift
367 model by the coalescent-based simulations. The Bayesian approach suggests a very recent (about 20
368 years) genetic isolation of the CAPV population, while jaguars from the Amazon and Cerrado regions
369 probably were well connected until 100 years ago. This observation, along with the low proportion of
370 private alleles in CAPV and the fact that it shares a major proportion of its alleles with the central
371 areas, corroborates historical evidence that CAPV was once part of a much larger population that
372 included the Cerrado.

373 The detection of two migrants from PNEM (assigned to CAPV), and a single one in CAPV (assigned to
374 AMZN), is thus consistent with restricted connectivity and disturbed potential corridors recently
375 described in this area (Silveira et al. 2014 and Figure 1B). The Cerrado biome, which marks the
376 transition between the Amazon and the southern populations, has been intensively modified since
377 the 1950s through extensive cattle farming and agricultural monocultures (rice, corn, soybean), and
378 today up to 80% of this region is considered degraded (Cavalcanti & Joly, 2002). The isolation of the
379 jaguar population in the Caatinga may have been driven in the last few decades by the lack of suitable
380 habitat for connectivity with surrounding populations. The relatively low estimate of effective
381 population size calculated for CAPV is supported by results of recent field studies in the region. While
382 the Capivara National Park is considered to have an important jaguar population (Silveira et al. 2010),
383 substantial contractions as the result of habitat changes, scarcity of prey and persecution have been
384 reported recently in the Brazilian Caatinga (Sollman et al. 2008). The semiarid climate and poor soil
385 limit large scale agriculture and cattle ranching, and about 60% of this area still maintains the native
386 vegetation cover, although as fragmented blocks (Castelletti et al. 2000). The low estimated effective
387 population size suggests that further genetic erosion will occur until the population size or the gene
388 flow from other regions increases (Frankham et al. 1999, England et al. 2010, Palomares et al. 2012).

389 Jaguar populations in other Brazilian areas (AMZN, CENT, PANT) were generally more diverse than the
390 ones at the northern and eastern limits of the species range (MEXC, CAPV). The Amazon was the most
391 genetically diverse region and had the highest proportion of private alleles, and variability indices
392 were comparable to values found in other tracts of forest in Colombia, Bolivia, and Peru (Table 5).

393 Many areas in the Amazon are still connected, forming enormous blocks of evergreen forest that
394 support large effective populations (Oliveira et al. 2012) and panmictic breeding, and our estimate of
395 a moderate to large effective population size agrees with that reported in this biome (Sollmann et al.
396 2008).

397 Results for the Pantanal region indicate that even though population bottlenecks were not statistically
398 detectable, this area may be showing early signs of genetic erosion and isolation. Allelic richness and
399 heterozygosity in the population from the Caiman Ecological Reserve were medium to low (Table 4)
400 and close to those found in the nearby area of the Upper Parana (Haag et al. 2010, Table 5). These
401 results were striking for several reasons: as the largest seasonally flooded landlocked area in the
402 world, the Brazilian Pantanal still is covered by native vegetation over most of its territory and
403 relatively well-connected; the extensive cattle ranching on native pastures (Harris et al. 2005) has
404 maintained some level of habitat quality for jaguars and has provided them with additional sources of
405 prey (Swartz 2000), what may explain the reported high jaguar density (Soisalo & Cavalcanti 2006),
406 even in non-protected areas. However, in some areas of this biome, the genetic patterns we detected
407 in our research support the observations made in earlier work (Altrichter et al. 2006), namely a
408 decrease in the size of some populations and increased isolation. These results are not unexpected
409 because some intensive cattle ranching practices have resulted in a major loss of native habitat and
410 increased direct persecution (i.e., hunting) of jaguars resulting from the increased conflict with cattle
411 ranchers (Crawshaw & Quigley 2002). Additionally, populations in the southern Pantanal are
412 connected southwards with the Atlantic forest region, a heavily human-impacted biome where jaguar
413 populations also show clear signs of genetic isolation and loss of genetic diversity (Haag et al. 2010).
414 The results of our work can serve as a starting place for discussion and evaluation of the role of the
415 Pantanal as a secure refuge for jaguars.

416 **The importance of connectivity for jaguar conservation**

417 The population structure observed at this scale intimate that connectivity with the extreme eastern
418 (i.e., Caatinga) and southern areas (i.e., Pantanal) is limited (Table 2) and that much of the existing
419 connectivity may be at risk because of continued habitat erosion, and might be enhanced through
420 habitat restoration or genetic exchange among them.

421 Interestingly, our research suggests that the central areas of Brazil within the Cerrado region (PNEM,
422 ARA and CANT) (Figure 1A), may act as “stepping stones” to maintain connectivity between the
423 Amazon and the surrounding eastern and southern populations. The identification of at least 6 first-
424 generation migrants in these central areas coming from all others areas (2 from CAPV, 2 from AMZN
425 and 2 from PANT) pointed out that movements and reproduction while limited, may have occurred in
426 the recent past at this scale. The significant Isolation by Distance pattern, along with the lowest
427 genetic differences observed between the populations in the central areas and other areas in Brazil
428 (Table 2 and Figure 2B, 3B) also suggests that CENT, AMZN and CAPV populations were probably
429 connected recently. Our study thus highlights the significant potential of the Araguaia River,

430 considered as the most important biodiversity corridor in central Brazil, which flows from the center
431 of the Cerrado to the Amazon and into the Tocantins River (see [Figure 1A](#)), for the maintenance of
432 diversity and connectivity among jaguar populations in Brazil, as suggested recently (Silveira et al.
433 2014) and in earlier works (Negroes et al. 2011, Vynne et al. 2011a).

434 The restoration of ecological connectivity between populations over relatively large scales should be
435 one of the main priorities for the conservation of the jaguar and for other wide-ranging species with
436 high dispersal, low population density and that are particularly vulnerable to anthropogenic impacts.
437 We stress the importance of ambitious programs to conserve a continuous north to south habitat
438 corridor through the range of the species (Rabinowitz & Zeller 2010 and [Figure 1B](#)) and to evaluate
439 the potential for large scale jaguar corridors in Brazil (Silveira et al, 2014).

440 **Implications for species viability, conservation and management**

441 Our work showed that genetic patterns differed among jaguar populations and biomes but were
442 highly consistent with the known status of the populations as well as with the degree of habitat
443 deterioration and connectivity with neighboring populations. Large continuous forested areas, such as
444 the Amazon, still maintain genetically healthy jaguar populations. In contrast, the geographic and
445 genetic isolation of the Caatinga population suggests that the jaguar may be at risk of extinction in
446 those areas of its range not connected, and especially those near the edge, or those which may
447 become isolated in the near future by the high rates of fragmentation. With the exception of the
448 groups in the Amazon, estimates of effective population sizes were low ($N = 13$ to 30) and much
449 below the number of 85 individuals proposed as the minimum threshold for long-term population
450 viability (>200 years; Sollmann et al. 2008). These low population values reinforce other evidence
451 showing a continued trend of declining jaguar populations. While large carnivores with widespread
452 geographic ranges should be at lower risk from habitat fragmentation, our research showed that
453 jaguar connectivity may be limited by the difficulty of dispersing in modified habitats. In a changing
454 landscape, protection and/or establishment of reserves are one of the most important tools for
455 habitat preservation as a buffer against anthropogenic impacts (Noss et al. 1996, Margules & Pressey
456 2000, Rylands & Brandon 2005, Shivik 2006). In Brazil, a system of connected protected areas
457 extensive enough to hold long-term viable jaguar populations is currently implemented in the
458 Amazon, but it is absent in other important jaguar areas such as the Caatinga biome. Long-term
459 jaguar conservation may depend on alternative strategies integrating non-protected landscapes, as
460 well as cultural and political mechanisms (Sollmann et al. 2008).

461

462 **ACKNOWLEDGEMENTS**

463 This study was carried out with the support of the project BIOCON 05 - 100/06 of the Fundación
464 BBVA, the project CGL2010-16902 of the Spanish Ministry of Research and Innovation, the project
465 CGL2013-46026-P of MINECO, the excellence project RNM 2300 of the Junta de Andalucía,

and projects UAM-PTC-333 and PROMEP/103.5/12/3823. Sampling in the Mexican areas under the licence SGPA/DGVVS/549 provided by Martín Vargas of the Dirección General de Vida Silvestre (Semarnat). Faecal samples were exported from Mexico to Spain under the export licences nº MX33790 and MX42916 of the Secretaria de Medio Ambiente/CITES. Sampling in Brazil was carried out in RAPELD sites installed or maintained by the Brazilian Program for Biodiversity Research (PPBio) and under licenses #131/2005 CGFAU/LIC, 13883-1 SISBIO and 15664-1 SISBIO of the Instituto Brasileiro do Meio Ambiente – IBAMA. Faecal samples were exported from Brazil to Spain for genetic analysis under IBAMA/CGEN Autorização de Acesso license #063/05 and IBAMA/CITES export licenses #0123242BR and 08BR002056/ DF". We thank the management of the Edén Ecological Reserve (Marco Lazcano) and El Zapotal Ecological Reserve (Pronatura Península de Yucatán: Juan Carlos Faller and María Andrade) for their logistical support. We are grateful to J.S. López and J. Tavares for the collection of most of the field samples in Brazil. Julia Martínez, Gloria Clemencia Amaya, Juan Carlos Faller, Meredec Calleja and Ana Alicia Morales helped with the fieldwork in Brazil and Mexico, as well as the local reserve staff of El Zapotal and El Edén (Mexico). L. Soriano and A. Piriz provided technical advice on multiple issues, and A. García, E. Marmesat, and B. Gutiérrez assisted in the analysis of samples. Logistical support was provided by Laboratorio de Ecología Molecular, Estación Biológica de Doñana, CSIC (LEM-EBD). The Spanish Ministry of Education and Sciences supported the visit of S. Roques in Mexico. We thank Manuela Gonzalez-Suarez and Philip Hedrick for an early revision of the manuscript.

485

486 REFERENCES

- 487 Alasaad, S, Soriguer RC, Chelomina G, et al. (2011) Siberian tiger's recent population bottleneck in the
488 Russian Far East revealed by microsatellite markers. *Mammalian Biology* 76, 721- +.
- 489
- 490 Altrichter M, Boaglio G, Perovic P. (2006) The decline of jaguars, *Panthera onca*, in the Argentine Chaco.
491 *Oryx* 40, 302-309.
- 492
- 493 Belkhir K, Borsa P, Chikhi L, Raufaste N, et al. (2004) Genetix 4.05: logiciel sous Windows™ pour la
494 génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000.
495 Montpellier, France: Université de Montpellier II.
- 496
- 497 Bohonak, A. J. 2002. IBD (Isolation By Distance): A program for analyses of isolation by distance. *Journal*
498 *of Heredity*. 93, 153-154
- 499
- 500 Boom R, Sol CJa, Salimans MMM, et al. (1990) Rapid and Simple Method for Purification of
501 Nucleic-Acids. *Journal of Clinical Microbiology* 28, 495-503.
- 502

503 Casteleti CHM, Silva JMC, Tabarelli M, Santos AMM (2000) Quanto resta da Caatinga? Uma estimativa
504 preliminar. In: J. M. C. Silva & M. Tabarelli (coord.), Workshop Avaliação e identificação de ações
505 prioritárias para a conservação, utilização sustentável e repartição de benefícios da biodiversidade
506 do bioma Caatinga, www.biodiversitas.org.br/caatinga.
507

508 Cavalcanti RB, Joly CA (2002) Biodiversity and Conservation Priorities in the Cerrado Region. In: The
509 Cerrados of Brazil. Ecology and Natural History of a Neotropical Savanna. Oliveira, PS and Marquis,
510 RJ (Eds.). Columbia University Press, New York, pp 351-367.
511

512 Ceballos, G., C. Chávez, A. Rivera y C. Manterola. 2002. Tamaño poblacional y conservación del jaguar
513 (*Panthera onca*) en la Reserva de la Biosfera Calakmul, Campeche, México. Pp. 403 – 481, en:
514 Jaguares en el nuevo milenio: Una evaluación de su estado, detección de prioridades y
515 recomendaciones para la conservación de los jaguares en América. (Medellin, R. A., C.
516 Chetkiewicz, A. Rabinowitz, K. H. Redford, J. G. Robinson, E. Sanderson, y A. Taber, Eds.).
517 Universidad Nacional Autónoma de México/Wildlife Conservation Society. México D. F.
518

519 Chávez C, Arana M, Ceballos G (2005) *Panthera onca*. Pp. 367–370. In: Los mamíferos silvestres de
520 México. Ceballos G and Oliva G (Eds.). CONABIO – UNAM – Fondo de Cultura Económica, México
521 D.F.
522

523 Ciofi C, Beaumont MA, Swingland IR, et al. (1999) Genetic divergence and units for conservation in the
524 Komodo dragon, *Varanus komodoensis*. Proceeding of the Royal Society of London Series B.
525 Biological Sciences 266, 2269–2274.
526

527 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent
528 population bottlenecks from allele frequency data. *Genetics* 144, 2001-2014.
529

530 Crawshaw Jr. PG, Quigley HB (2002) Hábitos alimentarios del jaguar y el puma en el Pantanal, Brasil, con
531 implicaciones para su manejo y conservación. In: El Jaguar en El Nuevo Milenio. Medellín RA,
532 Equihua C, Chetkiewicz C LB, Crawshaw Jr. PG, Rabinowitz A, Redford KH, Robinson JG,
533 Sanderson EW and Taber AB (Eds.). Fondo de Cultura Económica, México, Universidad Nacional
534 Autónoma de México, México, Wildlife Conservation Society, New York, pp. 223-236.
535

536 Crooks KR (2002) Relative sensitivities of mammalian carnivores to habitat fragmentation. *Conservation*
537 *Biology* 16,488–502.
538
539

540 Dutta, T., Sharma, S., Maldonado, J. E., Wood, T. C., Panwar, H. S., Seidensticker, J. (2013) Gene flow and
541 demographic history of leopards (*Panthera pardus*) in the central Indian highlands. *Evolutionary*
542 *Applications*. doi: 10.1111/eva.12078 (Selected as cover page article).
543

544 Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing
545 STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources DOI:
546 10.1007/s12686-011-9548-7. Version: v0.6.8.

547

548 Eizirik E, Kim JH, Menotti-Raymond M, et al. (2001) Phylogeography, population history and
549 conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Molecular Ecology 10, 65-
550 79.

551

552 England PR, Luikart G, Waples RS (2010) Early detection of population fragmentation using linkage
553 disequilibrium estimation of effective population size. Conservation Genetics 11, 2425-2430.

554

555 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software
556 STRUCTURE: a simulation study. Molecular Ecology 14, 2611-2620.

557

558 Frankham R, Lees K, Montgomery ME, et al. (1999) Do population size bottlenecks reduce evolutionary
559 potential? Animal Conservation 2, 255-260.

560

561 Frankham R (2003) Genetics and conservation biology. Comptes Rendus Biologies 326, S22-S29.

562

563 Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite
564 loci. Molecular Ecology 10, 305-318.

565

566 Gyllenberg M, Hanski I (1992) Single-Species Metapopulation Dynamics: A structured model. —
567 Theoretical Population Biology 42, 35-62.

568

569 Haag T, Santos AS, Sana DA, et al. (2010) The effect of habitat fragmentation on the genetic structure of
570 a top predator: loss of diversity and high differentiation among remnant populations of Atlantic
571 Forest jaguars (*Panthera onca*). Molecular Ecology 19, 4906- 4921.

572

573 Harley EH (2002) AGARST, version 2.8. A program for calculating allele frequencies, GST and RST from
574 microsatellite data. Wildlife Genetics Unit, University of Cape Town, South Africa.

575

576 Harris MB, Tomas W, Mourão G, et al. (2005) Safeguarding the Pantanal Wetlands: Threats and
577 Conservation Initiatives. Conservation Biology 19, 714–720.

578

579 Henry P, Miquelle D, Sugimoto T, et al. (2009) In situ population structure and ex situ representation of
580 the endangered Amur tiger. Molecular Ecology 18, 3173-3184.

581

582 IUCN, 2010. "IUCN SSC/Cat Specialist Group" (On-line). Accessed March 29, 2011 at

http://www.catsg.org/catsgportal/20_catsg-website/home/index_en.htm.

Janecka JE, Jackson R, Yuquang Z, et al. (2008) Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. *Animal Conservation* 11, 401-411

Joshi A, Vaidyanathan S, Mondol S, Edgaonkar A, Ramakrishnan U (2013) Connectivity of Tiger (*Panthera tigris*) Populations in the Human-Influenced Forest Mosaic of Central India. *PLoS ONE* 8(11): e77980. doi:10.1371/journal.pone.0077980

Kalinowski ST (2005) HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes* 5, 187-189.

Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* 12, 228-237.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics* 11, 355-373.

Margules CR, Pressey RL (2000) Systematic Conservation Planning. *Nature* 405, 243-253.

Miquel C, Bellemain E, Poillot C, et al. (2006) Quality indexes to assess the reliability of genotypes in studies using non-invasive sampling and multiple-tube approach. *Molecular Ecology Notes* 6, 985–988.

Mittermeier RA, Myers N, Thomsen JB, da Fonseca GAB, Olivieri S (1998) Biodiversity hotspots and major tropical wilderness areas: Approaches to setting conservation priorities. *Conservation Biology* 12, 516-520.

Moreno VR, Grisolia AB, Campagnari F, et al. (2006) Genetic variability of *Herpailurus yagouaroundi*, *Puma concolor* and *Panthera onca* (Mammalia, Felidae) studied using *Felis catus* microsatellites. *Genetics and Molecular Biology* 29, 290-293.

Navarro-Serment CJ, Lopez-Gonzalez CA, Gallo-Reynoso JP (2005) Occurrence of jaguar (*Panthera onca*) in Sinaloa, Mexico. *Southwestern Naturalist* 50, 102-106.

Negroes N, Revilla E, Fonseca C, et al. (2011) Private forest reserves can aid in preserving the community of medium and large-sized vertebrates in the Amazon arc of deforestation. *Biodiversity and Conservation* 20, 505-518.

623 Noss R, Quigley HB, Hornocker MG, et al. (1996) Conservation Biology and Carnivore Conservation in the
624 Rocky Mountains. *Conservation Biology* 10, 949-963.
625

626 Nowell K, Jackson P (1996) Wild Cats: status survey and conservation action plan. IUCN/SSC Cat
627 Specialist Group, Gland, Switzerland, 406 pp.
628

629 Oliveira de TG, Ramalho EE, de Paula RC (2012) Red List assessment of the jaguar in Brazilian Amazonia.
630 CATnews Special Issue 7.
631

632 Paetkau, D., Slade, R., Burden, M. & Estoup, A. (2004) Genetic assignment methods for the direct, real-
633 time estimation of migration rate: a simulation-based exploration of accuracy and power.
634 *Molecular Ecology*, 13, 55–65.
635

636 Palomares F, Godoy JA, Lopez-Bao JV, et al. (2012) Possible Extinction Vortex for a Population of Iberian
637 Lynx on the Verge of Extirpation. *Conservation Biology* 26, 689-697.
638

639 Perez CA (2001) Synergistic effects of subsistence hunting and habitat fragmentation on Amazonian
640 forest vertebrates. *Conservation Biology* 15, 1490–1505.
641

642 Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions
643 in the effective population size using allele frequency data. *Journal of Heredity* 90, 502-503
644

645 Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. & Estoup, A. (2004) GENECLASS2: a
646 software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, 95,
647 536–539.
648

649 Rabinowitz A, Zeller KA (2010) A range-wide model of landscape connectivity and conservation for the
650 jaguar, *Panthera onca*. *Biological Conservation* 143, 939-945.
651

652 Reddy PA, Kumaraguru A, Yadav PR, et al. (2011) Studies to determine presence or absence of the
653 Indian tiger (*Panthera tigris tigris*) in Kawal Wildlife Sanctuary, India. *European Journal of Wildlife*
654 *Research* 57, 517-522.
655

656 Reed DH, Lowe EH, Briscoe DA, Frankham R (2003) Inbreeding and extinction: Effects of rate of
657 inbreeding. *Conservation Genetics* 4, 405-410.
658

659 Roeder AD, Archer FI, Poiner HN, Morin PA (2004) A novel method for collection and preservation of
660 faeces for genetic studies. *Molecular Ecology Notes* 4, 761-764.
661

662 Roques S, Adrados B, Chavez C, et al. (2011) Identification of neotropical felid faeces using RCP-PCR.
 663 Molecular Ecology Resources 11, 171-175.
 664

665 Roques S, Furtado M, Jácomo ATA, et al. (2014) Monitoring jaguar populations (*Panthera onca*) with
 666 non-invasive genetics: a pilot study in Brazilian ecosystems. *Oryx*
 667 doi:10.1017/S0030605312001640
 668

669 Rosas-Rosas OC, Bender LC (2012) Population status of jaguars (*Panthera onca*) and pumas (*Puma*
 670 *concolor*) in northeastern Sonora, Mexico. *Acta Zoológica Mexicana* (n. s.) 28, 86-101.
 671

672 Rousset F (2008) Genepop'007: A complete reimplementation of the Genepop software for Windows
 673 and Linux. *Molecular Ecology Resources* 8, 103-106.
 674

675 Rylands AB, Brandon K (2005) Brazilian protected areas. *Conservation Biology* 19, 612-618.
 676

677 Saccheri I, Kuussaari M, Kankare M, et al. (1998) Inbreeding and extinction in a butterfly
 678 metapopulation. *Nature* 392, 491–494.
 679

680 Sambrook J, Fritschi EF, Maniatis T (1989) *Molecular cloning: A laboratory manual*, Cold Spring Harbor
 681 Laboratory Press, New York.
 682

683 Sanderson EW, Redford KH, Chetkiewicz CLB, et al. (2002) Planning to save a species: the jaguar as a
 684 model. *Conservation Biology* 16, 58-72.
 685

686 Sharma S, Dutta T, Maldonado JE, Wood TC, Panwar HS, Seidensticker J. 2013 Forest corridors maintain
 687 historical gene flow in a tiger metapopulation in the highlands of central India. *Proc R Soc B* 280:
 688 20131506. <http://dx.doi.org/10.1098/rspb.2013.1506>
 689

690 Shivik JA (2006) Tools for the Edge: What's New for Conserving Carnivores. *Bioscience* 56, 253–259.
 691

692 Silveira L, Jacomo ATA, Astete S, et al. (2010) Density of the Near Threatened jaguar *Panthera onca* in
 693 the Caatinga of north-eastern Brazil. *Oryx* 44, 104-109.
 694

695 Silveira L, Sollmann R, Jacomo ATA, et al. (2014) The potential for large-scale wildlife corridors between
 696 protected areas in Brazil using the jaguar as model species. *Landscape Ecology*,.
 697

698 Soisalo MK, Cavalcanti SMC (2006) Estimating the density of a jaguar population in the Brazilian
 699 Pantanal using camera-traps and capture-recapture sampling in combination with GPS radio-
 700 telemetry. *Biological Conservation* 129, 487-496.

701

702 Sollmann R, Mundim Tôres N, Silveira L (2008) Jaguar Conservation in Brazil: The Role of

703 Protected Areas. CAT News Special Issue 4 - The Jaguar in Brazil.

704

705 Srikwan S, Woodruff DS (2000) Genetic erosion in isolated small mammal populations following rain

706 forest fragmentation. In: Genetics, Demography and Viability of Fragmented Populations. Young A.

707 and Clarke G (Eds.). Cambridge Univ. Press, Cambridge, pp. 149-172.

708

709 Swartz FA (2000) The Pantanal in the 21st century — for the planet's largest wetland, an uncertain

710 future. In: The Pantanal of Brazil, Paraguay and Bolivia. Swartz F A (Ed.). Hudson MacArthur

711 Publishers, Gouldsboro, Pennsylvania, pp. 1–24.

712

713 Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. Trends in

714 Ecology and Evolution 14, 321-325.

715

716 Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: A program to estimate effective

717 population size using approximate Bayesian computation. Molecular Ecology Resources 8, 299-

718 301.

719

720 Thompson WL (2004) Sampling rare or elusive species: Concepts, designs, and techniques for estimating

721 population parameters. Island Press, Washington, D.C., USA.

722

723 Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across

724 the species' range: null hypotheses for landscape conservation genetics. Conservation Genetics 4, 639–

725 645.

726

727 Vynne C, Keim JL, Machado RB, et al. (2011a) Resource Selection and Its Implications for Wide-Ranging

728 Mammals of the Brazilian Cerrado. Plos One 6.

729

730 Vynne C, Skalski JR, Machado RB, et al. (2011b) Effectiveness of Scat-Detection Dogs in Determining

731 Species Presence in a Tropical Savanna Landscape. Conservation Biology 25, 154-162.

732

733 Waples RS, Do C (2008) LDNE: A program for estimating effective population size from data on linkage

734 disequilibrium. Molecular Ecology Resources 8, 753-756.

735

736 Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution

737 38, 1358-1370.

738

739 Williams BK, Nichols JD, Conroy MJ (2002) Analysis and management of animal populations. Academic

740 Press, San Diego, California, USA. Wilson, K. R., and D.R. Anderson.
741
742 Zeller K (2007) Jaguars in the new millennium data base update: the state of the jaguar in
743 2006. Wildlife Conservation Society-Jaguar Conservation Program, New York, USA.
744
745

Figure 1. A. Map of the actual jaguar's geographic range (*Panthera onca*), sampling sites (black points), genetic clusters and principal ecosystems in Brazil and Mexico (see details and codes in Table 1). The map is based on information from the IUCN Red List of Threatened Species (IUCN 2013). **B.** Map of the potential corridors connecting protected jaguar populations in Brazil and degree of disturbance from Silveira et al. (2014)

Figure 2. Isolation by distance across jaguar populations. Pairwise genetic differentiation as $F_{ST}/(1-F_{ST})$ at **(A)** Multi-regional scale including Mexico (n=15 populations) and **(B)** Regional scale; Brazil (n=9 populations). In grey, genetic comparisons involving CAPV, the easternmost Brazilian sampling site.

Figure 3. A. The genetic structure of the Brazilian populations identified by the STRUCTURE analysis assuming four genetic clusters ($K = 4$; MEXC, AMAZ, PANT and CAPV) in the overall population. Individuals are represented as bars partitioned into segments corresponding to their membership in genetic clusters indicated by the colors. Individuals from the Central areas (CENT: ARAG, PNEM, CANT) show from 50% to 100% ancestry in AMAZ, and the remainder corresponding to the other two clusters **B.** Three-dimensional Factorial Component Analysis graph. Names are referred to sampling sites (see Table 1). Jaguars from the central Brazilian areas (CENT) are intermediate between three differentiated groups (PANT, CAPV and AMZN). MEXC are genetically highly differentiated from the remaining samples.

Figure 1.

Figure 2.

Figure 3.

Biome	Code Biome	Sampling areas	Code area	N faeces	N other	N Species ID	N jaguar	N ind	Coordinates	
AMAZON	AMZN							12		
		Adolfo Ducke Reserve	DUCK	104	0	56	21	6	02º55' S	59º59' W
		Uatumbá Biological Reserve	UATM	29	0	19	6	3	1º46' S	-59º16' W
		Maracá Ecological Station	MARA	19	0	13	2	1	3º24'26" N	61º29'13" W
		Viruá National Park	VIRU	46	0	33	8	2	1º29'9" N	61º2'10" W
CAATINGA	CAPV	Capivara National Park	CAPV	82	0	57	53	18	8o 26' S	42o 19' W
CERRADO	CENT							14		
		Araguaia	ARAG	na	1 skin	na	na	1	3 25' 13"	53 26' 26"
			ARAG	na	11 liver	na	na	3	to 18 15' 40" S	to 47 53' 07" W
		Parque Estadual do Cantão	CANT	na	4 blood	na	na	4		
		Das Emas National Park	PNEM	61	0	49	14	3	18º 19'S	52º 45'W
			PNEM		3 blood	na	na	3		
PANTANAL	PANT	Refúgio Ecológico Caiman	PANT	98	0	79	37	34	19°57' S	56°18' W
			PANT	na	22 blood	na	na	22		
MEXICO	MEXC							24	Latitudes	Longitudes
		Ecological reserve El Zapotal	ZAPT	68	0	60	40	5	21º20'25"N	87º 36'20" W
		Ecological reserve El Eden	EDEN	64	0	44	25	3	21º 13' N	87º 11 W
		Ejido20Noviembre	EJNV	4	0	3	0	0		
		Calakmul	CALK	18	0	16	5	3	18º11'05" N	89º 44' 49" W
		Petcacab	PETC	21	0	17	10	4	19º 17' 15" N	88º 13'32.7" W
		Ejido Caobas	CAOB	34	0	27	14	9	18º 14'N	89º03' W
			CAOB	na	6 skin	na	na	6		
	CAOB	na	1 blood	na	na	0				
TOTAL				209	50	167	94	102		

Table 1: Sampling sites (n=14) in the different biomes of the jaguar distribution in Mexico and Brazil, number of field collected faeces after DNA extraction (N faeces) and other material (N other), species identification (N species ID), number of jaguar faeces (N jaguar), number of jaguar individuals (N ind) in bold, total number of jaguars after the assignment strategy for both faeces and high quality DNA sources, and geographical coordinates. na: not applicable

	MEXC	CAPV	AMZN	PANT	CENTR
MEXC	--				
CAPV	0,190	--			
AMZN	0,135	0,115	--		
PANT	0,162	0,168	0,087	--	
CENTR	0,107	0,067	0,026*	0,067	--

Table 2: F_{st} (left) indices of genetic differentiation among defined jaguar populations for Mexico (MEXC); Caatinga (CAPV); Amazon (AMZN); Pantanal (PANT); and Central areas (CENTR). All values are highly significant ($P \leq 0.01$) except * ($P \geq 0.05$)

Sample name	Sampling site	STRUCTURE Q, K=3			GENECLASS migrant	
		PANT	CAPV	AMZN	LOG(L_home)/(L_Max)> 0.60	Origin
PANT_SGM11	PANT	0.224	0.006	0.770		
PANT_SGH27	PANT	0.420	0.010	0.570		
CANT_1-5	CENT	0.013	0.036	0.950		
CANT_H2-6	CENT	0.100	0.047	0.853		
CANT_M113	CENT	0.007	0.202	0.791		
ARAG_M1	CENT	0.009	0.063	0.928		
ARAG_M2	CENT	0.188	0.031	0.782		
ARAG_H3	CENT	0.012	0.009	0.979	2.346	AMZN***
ARAG_HM4	CENT	0.135	0.172	0.693	1.514	PANT/AMZN ***
PNEM_M1	CENT	0.012	0.013	0.974	0.975	AMZN***
PNEM_M2	CENT	0.110	0.010	0.880		
PNEM_HSG18	CENT	0.018	0.018	0.964		
CANT_H3-28	CENT	0.446	0.145	0.409		
PNEM_3	CENT	0.029	0.659	0.312	0.601	CAPV***
PNEM_HSG29	CENT	0.028	0.681	0.291	1.984	CAPV***
PNEM_SG15	CENT	0.830	0.013	0.158	3.230	PANT***
DUCK_M2	AMZN	0.018	0.079	0.903	1.342	CENT

Jaguars marked with *** were identified as migrants with both methods.

Table 3: Identification of migrants performed with STRUCTURE and GENECLASS

Genetic indices	Parameters/Methods	MEXC	AMZN	PANT	CAPV	CENT
	N	24	12	34	18	14
Diversity	HE	0.654+0.147	0.805+0.084	0.726+0.097	0.709+0.133	0.837+0.0490
	HO	0.684+0.135	0.848+0.099	0.734+0.161	0.779+0.148	0.758+0.1692
	AR	5,10	6,73	5,61	5,20	7,26
Effective Pop. Size (Ne)	Onesamp	30 (22-38)	298 (na)	14 (10-17)	14 (12-16)	na
	LDNe	25 (14-45)	na (21-inf)	17 (10-28)	13 (7-28)	na
Bottleneck	Wilcoxon test					
	<i>P (SMM 5%)</i>	0.0005 ^S	0.0615 ^{NS}	0.0508 ^{NS}	0.0268 ^S	na
	<i>P (SMM 70%)</i>	0.0100 ^S	0.1302 ^{NS}	0.4410 ^{NS}	0.0500 ^S	na
	AF Distribution	<i>L-shaped</i> ^{NS}	<i>L-shaped</i> ^{NS}	<i>L-shaped</i> ^{NS}	<i>L-shaped</i> ^{NS}	na
	M Ratio	0.888+0.041 ^{NS}	0.752+0.029 ^{NS}	0.717+0.041 ^{NS}	0.670+0.057 ^{NS}	na

Table 4: Summary of genetic indices of defined populations for Mexico (MEXC); Caatinga (CAPV); Amazon (AMZN); Pantanal (PANT); and Central areas (CENT). Values are provided for number of jaguars (N), expected (HE) and observed (HO) heterozygosities, and allelic richness (AR), P values are noted as statistically significant ($P \leq 0.001$) (^S) and non significant (^{NS}); na signifies no applicable. Details of the methods are provided in the Material and Methods section.

Study sites *	Geographic scale	N ^S	N ^L	N ^A	HE	References
MEXICO (Yucatan peninsula)	Regional	24	11	5,10	0,654	<i>This study</i>
CENTRAL AMERICA (Mexico, Guatemala, Panama, Costa Rica, Nicaragua)	MultiRegional	16	29	5,20	0,622	Eizirik et al. 2001
NORTH -SOUTH AMERICA (Mexico-CA-Venezuela, French Guyana)	MultiRegional	25	29	6,80	0,695	Eizirik et al. 2001
GUATEMALA-PARAGUAY	MultiRegional	107	12	11,00	0,846	Ruiz-Garcia 2007
COLOMBIA	Regional	62	12	10,00	0,835	Ruiz-Garcia 2006
PERU	Regional	na	12	7,00	0,860	Ruiz-Garcia 2007
BOLIVIA	Regional	na	12	7,00	0,860	Ruiz-Garcia 2007
BRAZIL		59	11			
Amazon	Regional	18	11	6,90	0,805	<i>This study</i>
Cerrado	Regional	12	11	7,45	0,802	<i>This study</i>
Pantanal	Regional	34	11	7,00	0,726	<i>This study</i>
Caatinga	Regional	17	11	5,55	0,709	<i>This study</i>
NORTH ARGENTINA/SOUTH BRAZIL Atlantic Forest (Upper Parana)	Regional	13	13	6,00	0,737	Haag et al. 2010
SOUTH -SOUTH AMERICA (Brazil, Bolivia , Paraguay)	MultiRegional	17	29	6,70	0,724	Eizirik et al. 2001
MEXICO-BRAZIL	Distribution range	42	29	8,30	0,739	Eizirik et al. 2001
	Distribution range	102	11	10,55	0,800	<i>This study</i>

Table 5: Genetic surveys based on microsatellites markers that estimate the diversity of jaguar populations at different geographic scales. Study sites are ordered from north to south of the jaguar distribution range (See also Figure 1). Number of samples (N^S), loci (N^L), alleles (N^A), and expected (HE) heterozygosity. na indicates not applicable. See Supplementary Material for additional information on studied areas (codes, biomes, country, distances between sites, etc.).

S1. Fst values (below left) and Geographic distances (above right, in kms) between jaguar sampling sites. Significant values are indicated in bold ($P \leq 0.01$); na signifies not applicable (for sampling sites with $n < 3$ individuals)

	CALK	CAOB	PETC	EDEN	ZAPT	CAPV	DUCK	UATM	VIRU	MARA	CANT	PANT	PNEM	ARAG
CALK		98	142	362	281	6360	4600	4452	4000	4222	5475	5680	6130	5700
CAOB	0,0291		78	366	306	6120	4300	4152	3810	4032	5175	5480	5930	5400
PETC	0,1034	0,0467		248	208	6200	4380	4232	4420	4642	5255	5550	6000	5480
EDEN	0,0244	0,0304	-0,0397		130	6760	4980	4832	4330	4552	5855	5950	6400	6080
ZAPT	0,0926	0,0447	-0,0215	-0,0166		6600	5100	4952	4500	4774	5975	5750	6200	6200
CAPV	0,2000	0,1830	0,1847	0,1756	0,2339		1995	2143	2380	2158	1050	2100	2139	910
DUCK	0,1490	0,1140	0,1269	0,0988	0,1750	0,1255		148	600	378	875	1500	1950	1100
UATM	0,1472	0,1381	0,1371	0,1130	0,1895	0,0961	0,0200		370	230	1023	1648	2098	1248
VIRU	na	na	na	na	na	na	na	na		222	1475	2170	2630	1700
MARA	na	na	na	na	na	na	na	na	na		1253	1948	2408	1480
CANT	0,1018	0,1127	0,1085	0,0989	0,1679	0,0774	0,0197	-0,0342	na	na		1140	1160	220
PANT	0,1869	0,1541	0,1506	0,1519	0,1743	0,1729	0,0969	0,0547	na	na	0,0785		478	1200
PNEM	0,1123	0,1230	0,0923	0,0826	0,1720	0,0625	0,0354	-0,0046	na	na	-0,0261	0,0594		1000
ARAG	0,1294	0,1161	0,0483	0,0364	0,1125	0,1209	0,0650	-0,0244	na	na	0,0276	0,0925	0,0087	

S2. Sampling sites identification

Codes	Country	Biome	Sampling area
DUCK	Brazil	AMAZON	Adolfo Ducke Reserve
UATM	Brazil	AMAZON	Uatumã Biological Reserve
MARA	Brazil	AMAZON	Maracá Ecological Station
VIRU	Brazil	AMAZON	Viruá National Park
CAPV	Brazil	CAATINGA	Capivara National Park
ARAG	Brazil	CENTRAL	Araguaia
PNEM	Brazil	CENTRAL	Das Emas National Park
CANT	Brazil	CENTRAL	Parque Estadual do Cantão
PANT	Brazil	PANTANAL	Refúgio Ecológico Caiman
ZAPT	Mexico	YUCATAN	Ecological reserve El Zapotal
EDEN	Mexico	YUCATAN	Ecological reserve El Eden
EJNV	Mexico	YUCATAN	Ejido 20 Noviembre
CALK	Mexico	YUCATAN	Calakmul
PETC	Mexico	YUCATAN	Petcacab
CAOB	Mexico	YUCATAN	Ejido Caobas

S4. Evanno Table output for all K values

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	20	-4137.635000	0.702083	—	—	—
2	20	-3833.175000	14.642256	304.460000	76.350000	5.214360
3	20	-3605.065000	1.781270	228.110000	125.100000	70.230810
4	20	-3502.055000	2.305251	103.010000	286.510000	124.285791
5	20	-3685.555000	175.872189	-183.500000	263.440000	1.497906
6	20	-3605.615000	114.975390	79.940000	—	—

Supplementary file S3 : Database of 102 jaguars for 11 microsatellites in 14 sampling sites in Mexico and Brazil

Samples	Code area	Code Biome	Biome	Type	FC24a	FC26a	FC43a	FC566a	FC115a	FC126a	FC547a	FC77a	FC82a	FC90a	FC176a												
CAPM1	CAPV	CAPV	CAATINGA	faeces	224	226	133	139	115	121	167	171	189	212	143	155	231	233	146	150	198	216	110	114	209	219	
CAPM2	CAPV	CAPV	CAATINGA	faeces	224	224	131	133	121	121	171	173	189	198	143	155	226	233	146	150	208	216	114	116	217	219	
CAPM3	CAPV	CAPV	CAATINGA	faeces	224	224	133	151	121	121	165	173	189	199	143	159	231	233	146	150	198	216	106	116	209	219	
CAPM4	CAPV	CAPV	CAATINGA	faeces	224	228	133	139	113	121	163	169	189	212	143	155	226	233	146	155	198	198	120	120	215	215	
CAPM5	CAPV	CAPV	CAATINGA	faeces	224	224	135	139	117	121	165	165	198	212	155	159	223	233	146	150	198	200	106	116	225	0	
CAPM6	CAPV	CAPV	CAATINGA	faeces	224	224	131	139	115	121	165	173	198	218	143	157	226	233	146	150	198	208	106	116	209	217	
CAPM7	CAPV	CAPV	CAATINGA	faeces	224	226	131	139	117	121	165	165	212	218	143	155	0	0	146	150	198	200	116	118	225	229	
CAPM8	CAPV	CAPV	CAATINGA	faeces	224	224	133	139	117	123	165	165	189	214	143	155	0	0	150	155	198	200	108	120	227	231	
CAPM9	CAPV	CAPV	CAATINGA	faeces	224	224	135	139	117	121	165	165	198	212	155	159	223	233	146	150	198	200	106	116	225	0	
CAPM10	CAPV	CAPV	CAATINGA	faeces	224	224	0	0	139	115	117	0	0	198	198	155	159	0	0	146	0	198	216	0	0	209	217
CAPM11	CAPV	CAPV	CAATINGA	faeces	224	230	131	139	115	121	167	171	198	198	155	155	230	230	146	146	194	194	106	114	209	219	
CAPH1	CAPV	CAPV	CAATINGA	faeces	224	224	139	151	117	123	163	173	189	199	155	159	223	0	146	150	216	216	106	116	225	0	
CAPH2	CAPV	CAPV	CAATINGA	faeces	224	0	139	151	0	121	165	165	208	212	155	157	227	227	146	150	198	198	106	116	209	225	
CAPH3	CAPV	CAPV	CAATINGA	faeces	224	226	135	139	115	121	165	165	189	212	155	159	0	0	146	146	198	216	106	118	209	225	
CAPH4	CAPV	CAPV	CAATINGA	faeces	224	226	131	135	115	121	165	171	198	199	155	159	227	233	145	150	198	208	106	114	219	219	
CAPH5	CAPV	CAPV	CAATINGA	faeces	224	224	139	139	115	121	165	173	189	218	155	157	0	0	146	146	198	208	106	114	219	225	
CAPH6	CAPV	CAPV	CAATINGA	faeces	224	226	139	139	115	123	165	167	199	212	155	157	231	231	145	146	198	198	106	110	209	215	
CAPH7	CAPV	CAPV	CAATINGA	faeces	224	224	139	139	115	121	163	165	218	218	155	157	226	227	146	150	198	198	106	116	209	225	
PANM1	PANT	PANT	PANTANAL	faeces	220	226	127	127	113	115	165	167	208	211	159	159	230	231	147	155	198	198	114	114	225	225	
PANM2	PANT	PANT	PANTANAL	faeces	226	226	133	133	117	121	171	175	194	200	0	159	233	233	143	147	198	198	108	114	219	221	
PANM3	PANT	PANT	PANTANAL	faeces	226	228	131	133	117	121	167	171	189	211	143	159	223	233	145	145	198	198	114	114	211	215	
PANM4SG19	PANT	PANT	PANTANAL	blood	228	228	131	133	117	117	167	167	196	212	159	161	226	230	147	147	200	208	108	110	215	219	
PANM5	PANT	PANT	PANTANAL	faeces	224	0	133	151	115	115	162	169	189	198	159	161	230	235	147	150	208	216	108	114	0	217	
PANM6	PANT	PANT	PANTANAL	faeces	0	0	133	149	113	0	165	173	0	0	155	157	231	235	135	147	198	198	110	110	0	0	
PANM7	PANT	PANT	PANTANAL	faeces	228	0	131	133	117	117	162	173	189	198	157	159	230	233	147	155	198	208	108	110	213	225	
PANM5SG16	PANT	PANT	PANTANAL	blood	228	228	133	133	117	117	167	173	196	216	143	159	227	230	143	145	198	200	106	110	215	225	
PANM8	PANT	PANT	PANTANAL	faeces	226	0	133	133	117	117	171	173	193	194	159	161	230	231	145	147	192	200	108	108	215	225	
PANM10	PANT	PANT	PANTANAL	faeces	226	0	133	133	117	117	173	173	0	0	159	161	230	231	145	147	198	204	108	114	215	219	
PANSGM01	PANT	PANT	PANTANAL	blood	220	224	133	151	115	115	162	169	196	196	159	161	230	231	147	147	204	204	108	114	215	225	
PANSGM03	PANT	PANT	PANTANAL	blood	222	228	133	151	113	117	167	173	197	213	143	157	230	230	147	147	198	200	0	114	219	0	
PANSGM04	PANT	PANT	PANTANAL	blood	226	228	129	129	113	113	167	171	197	219	143	157	230	230	147	155	196	198	108	114	219	225	
PANSGM11	PANT	PANT	PANTANAL	blood	220	230	133	133	115	117	166	173	211	214	159	163	229	230	135	145	198	208	110	114	213	215	
PANSGM20	PANT	PANT	PANTANAL	blood	220	228	133	151	113	113	162	167	196	219	143	161	230	230	147	155	198	198	114	114	219	225	
PANSGM23	PANT	PANT	PANTANAL	blood	220	220	133	133	115	117	165	166	196	211	155	159	230	235	145	145	196	198	106	110	215	215	
PANSGM16	PANT	PANT	PANTANAL	blood	228	228	133	133	117	117	167	173	196	216	143	159	227	230	145	145	198	200	106	110	215	225	
PANH1	PANT	PANT	PANTANAL	faeces	220	226	133	151	113	113	162	165	197	215	143	159	230	231	147	147	198	198	106	114	215	225	
PANH2	PANT	PANT	PANTANAL	faeces	220	228	133	133	121	0	0	167	197	215	143	159	230	230	147	147	198	198	106	114	215	219	
PANH3	PANT	PANT	PANTANAL	faeces	220	226	127	151	113	113	167	171	194	219	155	161	0	230	145	147	198	200	108	114	215	219	
PANH4	PANT	PANT	PANTANAL	faeces	220	228	133	151	113	113	162	167	192	200	143	161	0	230	147	155	198	200	114	114	219	225	
PANSGH02	PANT	PANT	PANTANAL	blood	226	228	133	151	115	117	167	173	196	213	143	161	230	231	135	145	198	200	110	114	219	225	
PANSGH07	PANT	PANT	PANTANAL	blood	220	224	0	0	115	115	162	169	196	197	159	161	230	235	147	150	198	204	108	114	215	219	
PANSGH08	PANT	PANT	PANTANAL	blood	224	226	131	133	113	113	167	169	196	196	155	161	231	235	147	150	198	198	108	108	213	225	
PANSGH10	PANT	PANT	PANTANAL	blood	224	224	133	133	113	113	169	169	197	197	161	163	230	231	147	147	198	204	108	108	219	225	
PANSGH12	PANT	PANT	PANTANAL	blood	226	228	133	133	117	117	167	173	189	196	159	159	230	230	135	147	198	200	106	110	211	213	
PANSGH14	PANT	PANT	PANTANAL	blood	220	226	131	133	113	117	165	167	194	196	155	159	231	235	147	147	198	198	106	108	211	215	
PANSGH21	PANT	PANT	PANTANAL	blood	224	228	133	133	113	117	167	169	196	216	155	159	226	233	145	147	198	200	106	108	225	225	
PANSGH22	PANT	PANT	PANTANAL	blood	226	0	133	133	113	117	162	173	189	189	143	159	230	233	135	155	200	204	106	114	215	225	
PANSGH24	PANT	PANT	PANTANAL	blood	220	226	133	151	113	113	162	171	215	219	159	161	230	231	147	147	198	198	114	114	215	225	
PANSGH25	PANT	PANT	PANTANAL	blood	226	228	133	133	113	115	167	173	196	211	155	159	229	231	145	147	196	198	108	110	211	225	
PANSGH27	PANT	PANT	PANTANAL	blood	224	226	133	133	113	117	173	173	189	214	159	163	226	230	135	135	192	200	106	114	211	215	
PANSGH30	PANT	PANT	PANTANAL	blood	220	226	133	151	113	115	162	173	196	197	159	159	231	235	147	150	198	204	110	114	215	225	
PANSGH09	PANT	PANT	PANTANAL	blood	224	226	131	133	113	113	167	169	196	196	155	161	231	231	147	147	198	198	108	110	211	225	
PNEM1	PNEM	PNEM	CERRADO	faeces	228	230	133	139	117	117	167	167	200	218	159	163	224	226	146	157	196	204	102	110	0	0	
PNEM2	PNEM	PNEM	CERRADO	faeces	0	226	133	133	111	115	162	169	209	211	155	157	230	233	145	145	194	198	102	120	215	225	
PNEM3	PNEM	PNEM	CERRADO	faeces	224	228	135	139	117	121	165	171	209	211	143	155	0	0	119	146	198	208	106	114	217	225	
PNEMSG15	PNEM	PNEM	CERRADO	blood	220	220	129	131	113	113	167	169	194	209	143	155	227	231	145	147	0	0	106	110	0	0	
PNEHSG29	PNEM	PNEM	CERRADO	blood	224	224	139	139	121	121	165	165	19														