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ORIGINAL PAPER

A non-invasive faecal survey for the study of spatial ecology and kinship of solitary felids in the Viruá National Park, Amazon Basin

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Abstract Jaguars and pumas are the largest felids in the Americas. Information about these two species is scarce, especially where both species are sympatric. We studied the use and selection of macrohabitats, spatial segregation and kinship in jaguars and pumas in the Viruá National Park (Amazonian lowlands) by non-invasive genetic analyses of faecal samples. Seven different jaguars (six males and one female) and nine different pumas (five males and four females) were identified. We found space use segregation between the two species, with pumas using mostly forested habitats and jaguars using open habitats slightly more than the forested ones. This result is unexpected, since previous studies have found that pumas favour more open habitats than jaguars. The results suggest that jaguars use the areas in a more random manner, corresponding to the habits of a dominant generalist species, whereas pumas use the area to reduce encounter rates with jaguars. Nevertheless, both species mainly used areas near upland forest-flooding habitats. Some kinship categories were supported with a $p < 0.05$ in 57 and 83% of the pair comparisons between the identified jaguars and the identified pumas, respectively. Non-invasive genetic analysis of faeces was useful to study the spatial ecology of solitary, rare and cryptic species in the Amazon.

Keywords Jaguar · Macrohabitat use and selection · Puma · Relatedness · Spatial segregation

Introduction

Jaguars (*Panthera onca*) and pumas (*Puma concolor*) are the largest felids in the Americas. The species coexist throughout the jaguar's distribution area, basically in the Neotropics, and share a similar life history and behavioural traits (Sunquist and Sunquist 2002). Although jaguars and pumas are respectively listed by the IUCN as Near Threatened and of Least Concern, and in the CITES Appendix I and II, in many areas of their distribution range are of conservation concern (Caso et al. 2008; Nielsen et al. 2015). Some of their populations have been extirpated, are at high risk of extirpation or are declining due to loss of habitat quality and fragmentation, poaching of their main prey and retaliatory killing due to livestock depredation (Nowell and Jackson 1996; Zanin et al. 2015; Petracca et al. 2014). This situation is particularly accentuated for the jaguar, which has lost more than 50% of its former distribution area (Sanderson et al. 2002). Much of the jaguar's remaining habitat is the rainforest of the Amazon Basin (88% of its remaining area of occupancy; Caso et al. 2008), which is also considered of relatively low suitability (Torres et al. 2007). Despite the importance of the Amazon Basin for jaguars, little information exists on the ecology of the species in that region and in the rest of Latin America, which is also true for pumas (Haines 2006; Caso et al. 2008; Laundré and Hernández 2010;

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Nielsen et al. 2015; Palomares et al. 2016). The only published information for the Amazon Basin relates to jaguar density estimations by camera trapping in the Colombian, Bolivian and Peruvian Amazon (Silver et al. 2004; Payan 2008; Tobler et al. 2013).

In this paper, we provide information on jaguar and puma spatial ecology and some population parameters of both felids in the Viruá National Park (northern Brazilian Amazon Basin) by non-invasive genetic analyses of faeces that is an increasingly efficient method for studying the use and selection of macrohabitats, and relationships among individuals in secretive mammals. Furthermore, we examined whether there was any spatial segregation between the species, as would be expected due to their ecologically similar requirements (Sunquist and Sunquist 2002; Haines 2006; Caso et al. 2008; Nielsen et al. 2015).

Materials and methods

Study area

The study was conducted in Viruá National Park (Caracaraí municipality, Roraima state, Brazil; 1° 29′ 9″ N, 61° 2′ 10″ W; 227.000 ha; Fig. 1), which is limited by the Branco River to the west, a national road to the northeast, an abandoned dirt road to the east and the Anauá River to the south. The climate is wet tropical, with a rainy period and a marked dry period from November to March (Marengo et al. 2001). The mean daily temperature and annual rainfall during the study years were 27 °C and 2300 mm, respectively. The vegetation is characterised by mosaics formed by transitions between savannahs and tropical upland forests, with the former being frequently flooded in some months of the year (Machado et al. 2004).

The area has a sampling infrastructure that includes a trail system forming a 5 × 5 km grid (Magnusson et al. 2005). A description of the trail system and infrastructure can be obtained from the Program for Biodiversity Research (PPBio) website (http://ppbio.inpa.gov.br). The grid consists of six parallel 5-km trails and six 5-km trails perpendicular to those, totalling 60 km.

Sample collection and preservation

Faecal sampling was carried out during the end of the dry season (February–March) in 2008, 2009 and 2011, by slowly walking along the trails of the 5 × 5 km grid system, the access trails to the park headquarters and the 56 km of the abandoned dirt road to the east. Additionally, in 2011, several cross-country transects were walked along the river border in the confluence area of the Branco and Anauá Rivers to the south of the park. In total, we walked 261 km on dirt roads and trails.

In 2009 and 2011, some faeces were collected with the help of a scat detector dog (Smith et al. 2001).

The samples were stored in 200-ml plastic containers with silica gel and their location georeferenced using a GPS. A few samples in 2008 were not georeferenced. Type of macrohabitat (upland forest, campinarana and campina; see below) and height of the vegetation in a circle of 25 m diameter around the faecal position were also recorded during the 2011 sampling.

### DNA extraction for species, sex and individual identification

DNA extraction of faecal samples was conducted according to protocols based on the GuSCN⁄silica method (Boom et al. 1990; Höss and Pääbo 1993; Frantz et al. 2003). Each batch of extractions ($n = 15$) included one PCR-negative extraction control to monitor for contamination by exogenous DNA. DNA extractions of faecal samples were performed in a UV-sterilised laminar flow hood in an isolated laboratory specially designated for the manipulation of non-invasive material.

Faecal samples were screened for species identity using species-specific primers as described by Roques et al. (2011). The method consists of a single-tube multiplex PCR yielding species-specific banding patterns on agarose gel, allowing the unambiguous identification of jaguars and pumas among other felid species. For sex identification, we used the method described by Pilgrim et al. (2005), based on the difference in size between the PCR products amplified from the male Y-chromosome copy (AMELY) and the X-chromosome gene (AMELX), optimised for faecal samples from Neotropical felid species such as jaguar, puma, ocelot and margay, as described by Palomares et al. (2012).

Individual genotyping for jaguars and pumas was conducted using an optimised set of 11 (Fca024, Fca026, Fca043, Fca077, Fca082b, Fca090, F115a, Fca126, Fca176, Fca547b, Fca566b; Menotti-Raymond et al. 1999) and 12 (Fca077, Fca82b, Fca126, Fca547b, PcoB003w, PcoB010w, PcoA208w, PcoB210w, PcoA216w, PcoC108w, PcoC112w and PcoA339w; Menotti-Raymond et al. 1999; Kurushima et al. 2006) microsatellite markers, respectively (Palomares et al. 2012; Roques et al. 2014; Zanin et al. 2016). Before genotyping the whole set of microsatellites, DNA extracts were evaluated for quality by direct amplification of the Fca82b locus, which was selected for its high amplification robustness. The samples that failed to amplify this locus would probably not amplify the remaining loci and were not genotyped.

A two-step strategy was used to improve genotyping success rate and accuracy through two sequential amplifications (Bellemain and Taberlet 2004; Piggott et al. 2004): a multiplex PCR that included the whole set of primer pairs and a reduced number of cycles (pre-PCR) at a moderate annealing

Fig. 1 Location of Viruá National Park in Brazil and of the study area (the *grey square within the dashed rectangle* of the study area indicates the location of the 25-km² trail grid; *left-hand panels*). The *right-hand panels* show the locations of identified jaguar and puma samples in each study year (*black points* = females; *white squares* = males; *white circles with black point* = samples with no sex identification; *numbers* close to samples indicate the ID of individual when genotyping was possible). Number of identified jaguars and pumas in the years 2008, 2009 and 2011 were 6, 4 and 5 and 20, 14 and 11 in case of faecal samples, and 1, 1 and 2 and 5, 5 and 5 in case of individuals, respectively
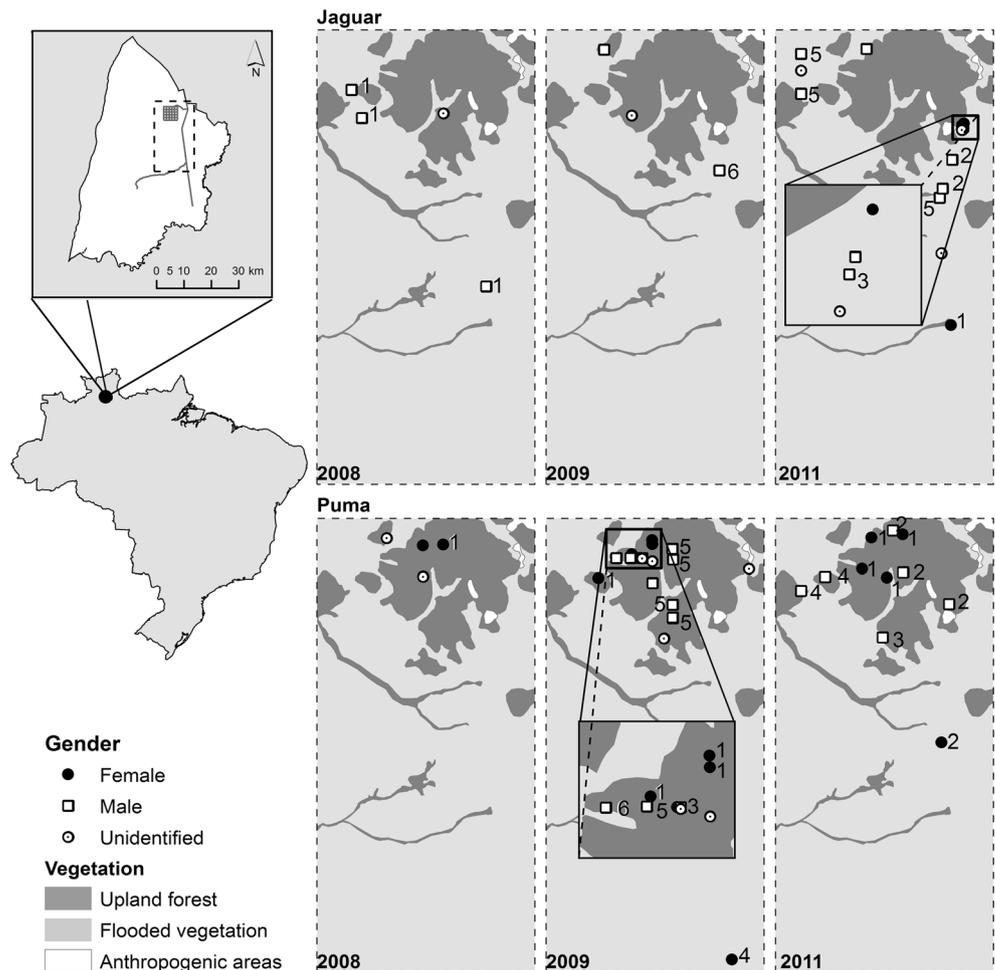
temperature, followed by the amplification of each locus separately, using the PCR products as templates (post-PCR). Pre-PCR was performed in a multiplex reaction with 7 μl of DNA extract in a 30-μl reaction including 67 mM Tris-HCl pH 8, 16 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.8 μM dNTPs, 0.8 mg/ml BSA, 0.02 or 0.03 μM each primer and 0.6 U of Taq DNA polymerase (Bioline). Pre-PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 25 cycles for 30 s at 92, 55 and 72 °C and a final extension of 5 min at 72 °C. Second-stage post-PCR amplifications were performed independently for each marker using 4 μl of PCR product in a final volume of 20-μl reactions containing 67 mM Tris-HCl pH 8, 16 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.8 mg/ml BSA, 0.2 μM of each primer and 0.5 U of Taq polymerase. Post-PCR conditions were initial denaturation at 94 °C for 2 min, 40 cycles for 30 s at 92 °C, specific annealing temperature (see Roques et al. (2014); Zanin et al. (2016)) and 72 °C and a final extension of 5 min at 72 °C. Up to 6 PCR products of jaguar samples from the second amplification step, with fluorescently labelled primers, or 12 PCR products, in the case of puma samples, were combined on an ABI

PRISM 3130xl Genetic Analyzer. Alleles were sized using the GeneMapper Software version 3.7 (Applied Biosystems).

Samples were genotyped using a multi-tube approach (Navidi et al. 1992; Taberlet et al. 1996; Taberlet and Luikart 1999; Goossens et al. 2000) with four replicates per locus per individual. For a locus to be considered homozygous, only the same allele could be observed in at least three independent replicates, without observing an additional allele in the fourth replicate. The heterozygous loci were those with the same two different alleles in at least two replicates. A quality index (QI) similar to the one described by Miquel et al. (2006) was calculated for each sample, referred to individual alleles instead of the genotype of a given locus. We calculated the percentages of replicates that were equal to the consensus for each given allele, and then we averaged the values across individuals and loci. Samples with a QI below 0.5 or with less than seven loci genotyped were discarded from further analyses.

All molecular analyses were carried out in the Laboratory of Molecular Ecology of the Doñana Biological Station (Seville, Spain).

Data analysis

Spatial segregation

We tested for spatial overlap between jaguars and pumas with a null model (Gotelli and Graves 1996), which randomised the spatial distributions of jaguar and puma samples. Given the apparent stability of jaguar and puma detection over time (see Fig. 1), we pooled the data from all years. For the randomisation process, we first assigned each faecal sample to a 1-km^2 cell, and then computed the observed spatial overlap between the species (i.e., number of 1-km^2 cells with detection of both species in relation to the total number of cells with detections). In the second step, we built the randomisation procedure based on the mean percentage of overlapping jaguar and puma occurrence cells, to test whether the puma or jaguar cells were distributed randomly relative to the other species' cells. At each step of the process, we randomly distributed the samples of each species over the total number of occurrence cells containing any sample, and computed the percentage again. We repeated this process 1000 times and then compared the observed percentages with the distribution of simulated percentages, to compute a *p* value. This algorithm was built in the R software, and it is available on request.

Macrohabitat selection

Macrohabitats were determined directly in the field only for a few samples and for all samples with GPS location using ArcGIS®. Three different types of macrohabitats were distinguished: upland forest (forest areas with 15–30 m canopy height, which are not flooded during the rainy period), campina (savannah-like open areas normally flooded during the rainy period, presenting scrub areas up to 6–8 m high) and campinarana (more open forest, with 8–12 m canopy height, in transition areas between upland forest and campina, normally flooded during the rainy period).

For macrohabitat characterisation using ArcGIS®, we used a detailed land cover map elaborated for the management plan of Viruá National Park and surrounding areas (ICMBio 2014), with a resolution of 1-m^2 grid cells. Nine different land cover types are defined in the map, which were reclassified in the three macrohabitats described previously (upland forest, campina and campinarana; Supplementary Information Table S1). Since there were few samples for the flooding macrohabitats, for the analyses, we only considered two general types of macrohabitat: upland forest and flooding habitats (comprising campina and campinarana).

Differences between jaguars and pumas in the number of scats located within each macrohabitat were examined by a chi-squared test. We counted the number of scats in each type of macrohabitat and related it to their availability in the study area using the Jacobs index (Jacobs 1974), which varies from +1 for maximum preference to −1 for maximum avoidance. Availability of macrohabitats was measured within the minimum convex polygon traced around all the scat samples collected. From the actual location of scat samples of jaguars and pumas (Fig. 1), we examined the relationship between the probability of finding a scat from a jaguar or puma and the distance to the upland forest-flooding habitat edge using a logistic regression. The logistic regression was conducted in the R software with the "stats" package.

Relatedness and categories of relationship among individuals

We calculated maximum likelihood estimates of relatedness and relationship (see Blouin (2003) for definitions) between dyads of jaguars and dyads of pumas with the program ML-Relate (Kalinowski et al. 2006) for all the different individuals identified in the study area. This program uses microsatellite data and can accommodate null alleles. Thus, we determined the probability ($p < 0.05$) among four common categories of relationships between the individuals of each species (i.e., parent-offspring, full sibs, half-sibs and unrelated) and the index r of relatedness between each dyad using 999 simulated genotypes.

Relationship categories and relatedness are more confidently estimated when allele frequencies of the population are well sampled, so to calculate the allele frequencies, we used a total of 24 genotypes of jaguars and 20 genotypes of pumas from the Amazon Basin that we had in our data bases from samples of both species. Both jaguars and pumas from the Amazon Basin (including those of the Viruá area) belong to the same genetic populations, respectively (Roques et al. 2016; authors unpublished); thus, the inclusion of these samples increased the robustness of analyses. These genotypes were obtained using the same molecular techniques previously described and in the same laboratory, and were collected between 2005 and 2011 in a total of four study areas in the Amazon Basin (see Roques et al. (2016) for a description of the study areas).

Results

Scat analyses

We collected 175 scats, of which 51.4% were identified: 25 from jaguars and 35 from pumas. Overall, we collected 0.10 and 0.13 jaguar and puma scats per kilometre, respectively. Eighty-four percent of the jaguar scats and 55% of the puma scats were from males. The number of samples genotyped after positive amplification of the Fca82b locus was 19 for jaguars and 29 for pumas. Ten out of 11 microsatellite loci used for jaguars had an amplification success higher than

77%, except one (locus Fca176) with 48% success. For pumas, three microsatellite markers (locus PcoB3, locus Fca82 and locus Fca547) had an amplification success of 66–67%, and the nine remaining between 71 and 94%. The probability of identity estimated for the 11 and 12 analysed loci indicated that our microsatellite panel was sufficient to discriminate individuals within the entire dataset ($P_{(ID)sib}$ = 4.23 × 10^{-5} and 1.01 × 10^{-4}, for jaguars and pumas, respectively). Fourteen jaguar genotypes and 26 puma genotypes obtained a QI value greater than 0.5 and reached more than 7 loci genotyped, thus, meeting our quality requirements. Seven different jaguars (six males and one female) and nine different pumas (five males and four females) were identified using these high-quality genotypes, with a mean number of 2.0 (SE = 0.49, range = 1–4) and 2.8 (SE = 1.92, range = 1–9) scats collected per individual for jaguars and pumas, respectively. Only one male jaguar was resampled in 2009 and 2011, and only one female puma was resampled in all surveyed years (Fig. 1).

Spatial distribution and macrohabitat selection

We recorded the location of the 56 scats (21 of jaguar and 35 of puma; four scats were not georeferenced), which were distributed within 32 1-km^2 cells, of which 12.5% contained samples of both species. The null model clearly showed that both species were segregated in space more than expected by random (simulated percentage of simultaneous occurrence was 31.3%, significantly higher than the 12.5% observed with a $p < 0.001$).

Eighty-six percent of jaguar faeces (n = 21) were found in flooding habitats, whereas only 11% of the puma scats (n = 35) were found in this type of vegetation (Table 1; Fig. 1; χ^2 = 27.3, df = 1, $p < 0.001$). Data obtained directly in the field during sampling greatly coincided with map-derived data (Table 1). The height of the vegetation canopy was 2–12 m for flooding habitats and 15–25 m for upland forests. Comparing the use with the availability of these macrohabitats, pumas clearly avoided the flooding areas (Jacobs index = −0.94) and selected upland forests (Jacobs index = 0.94), whereas jaguars seemed to use close to

available the two types of macrohabitats (Jacobs indexes = 0.17 and −0.16, respectively; Table 1). The logistic regression showed that the probability of a scat belonging to a jaguar or puma increased with distance from the forest edge (jaguar to flooding habitats and puma to upland forest interior; $p < 0.001$). Nevertheless, both species preferred to be near the edge (86 and 89% jaguar and puma detections, respectively, <1 km from the edge; Figs. 1 and 2).

Relatedness and categories of relationship among individuals

Some level of relatedness was supported under a $p < 0.05$ in 57.1% of the 21 pair comparisons between the seven identified jaguars (Supplementary Information Table S2). However, all of them also included "unrelated" as a possibility, and in only one case (JVIRH1-JVIRM6) a half-sib relationship was clearly ranked first (Supplementary Information Table S2) according to its maximum likelihood estimate. On the other hand, 83.3% out of the 36 pair comparisons in pumas may be from related individuals according to a $p < 0.05$, although in only 8.3% (three cases) that the lack of relatedness was totally discarded (Supplementary Information Table S2). According to the maximum likelihood estimates, the parent-offspring relationships between PVIRH1-PVIRH4 and PVIRH3-PVIRH4, and full sibs between PVIRM2-PVIRH4 and PVIRM6-PVIRM5, were ranked as the most probable (Supplementary Information Table S2).

Differences in percentage of pairs with a possible relationship between jaguars and pumas approached significance (Z = 1.854, p = 0.064; Z test), and the number of pair comparisons with higher values of the maximum likelihood estimates of relatedness (i.e., a closer relationship) was clearly higher in pumas than in jaguars (Supplementary Information Table S2). This result did not seem to be biased due to the fact that we detected mainly males in jaguars, and a similar number of both sexes in pumas. Considering only comparisons between males, percentages of pairs with a possible relationship was 46.7 and 90.0%, for jaguars and pumas, respectively (Z = 1.771, p = 0.077). However, the indices of relatedness were often higher for pair comparisons involving a female

Table 1 Macrohabitats where jaguar and puma faeces were found according to data directly gathered during fieldwork and from vegetation maps of Viruá National Park

Macrohabitats	Availability (220.4 km^2)	Percentage of use					
		Jaguar			Puma		
		Field (n = 9)	Height range (m)	(n = 21)	Field (n = 10)	Height range (m)	(N = 35)
Upland forest	18.6%	33.3	15–20	14.3	80.0	20–25	88.6
Flooding habitats	80.9%	66.7	3–12	85.7	20.0	4–6	11.4

For data recorded during fieldwork, height of the vegetation is also shown
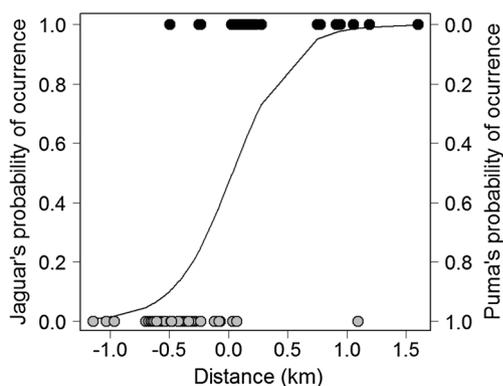
Fig. 2 Logistic regression testing the probability of finding a scat from a jaguar or a puma in relation to the distance to the upland forest-flooding habitat edge. Logit $p = -0.0137 + (0.00411 \times distance)$; likelihood ratio test statistic $= 28.14$, $p < 0.001$ (one puma sample located 6.8 km from the edge in flooding habitats was removed from the analysis; the next sample was located 1.1 km from the edge). *Points* represent raw data from jaguars (*black*) and pumas (*grey*)

(Supplementary Information Table S2), although the few available samples prevented statistical testing of this trend.

Discussion

Both jaguars and pumas are considered as habitat generalists, found from arid areas to rain forests. In sympatric areas, the species can be found in the same areas and macrohabitats (Núñez et al. 2002; Scognamillo et al. 2003; Noss et al. 2006; Estrada Hernández 2008; Monroy-Vilchis et al. 2009; Harmsen et al. 2009; Di Bitetti et al. 2010; Palomares et al. 2016). In a few cases, very small differences in macrohabitat use were recorded within local-scale studies (Schaller and Crawshaw 1980; Emmons 1987; Chávez 2010; Sollmann et al. 2012), which pointed to pumas favouring more open habitats, although also using forest areas, and jaguars using both open and forested habitats in proportion to availability (Farrell et al. 2000; Scognamillo et al. 2003; Silveira 2004). Pumas have also been described as more tolerant to human-influenced landscapes than jaguars (De Angelo et al. 2011; Sollmann et al. 2012; but also see Foster et al. (2010) for a contrary result).

We found an apparently clear segregation in space use between the species, as well as a differential use of macrohabitats, with pumas mostly using the forested habitats and jaguars slightly favouring the open areas. The latter result is unexpected, since previous studies have found that the pumas favour more open habitats than jaguars. The reasons for this discrepancy are not clear, but might be related to food availability and competition interactions between the species. We believe that the low sample size obtained for the two species or the sampling procedure to collect faecal samples did not affect this result, because the sampling procedure and effort were identical for both species, and the statistical analyses clearly confirmed the observed spatial patterns.

Under a potential competition scenario, jaguars should be dominant over pumas (Ruth and Murphy 2010; Oliveira and Pereira 2014), and if so, both theoretical and empirical studies predict that pumas should avoid habitats or areas used by jaguars to decrease the risk of interspecific encounters (Case and Gilpin 1974; Palomares and Caro 1999; Linnell and Strand 2000). The threat of aggression can create a "landscape of fear" that excludes prey or subordinate species from suitable habitats, normally in core areas of the dominant species (e.g., Palomares et al. 1996; Swanson et al. 2014). This was partially the case in our study, as both species were segregated in the use of space, and pumas mainly selected upland forests and avoided flooding habitats, but jaguars did not avoid upland forests. Therefore, our results suggest that the jaguars use the study area in a more random manner, probably triggered by prey availability, as would be expected from a dominant generalist species. Pumas behave spatially in a way to diminish encounter rates with jaguars, preferring forest areas, where visibility is lower and escape possibilities are higher if a jaguar is close. In a larger scale study, Palomares et al. (2016) found a microhabitat segregation between jaguars and pumas, which was explained well by a scenario of interference competition between the two species with pumas being subordinate. Furthermore, both species mainly used areas close to upland forest-flooding habitat transitions, where prey-species richness is expected to be higher (Schluter and Ricklefs 1993; Brown 1995).

Most solitary carnivore species exhibit female philopatry and male-biased dispersal (Logan and Sweanor 2001; Støen et al. 2005). Our results seem to support this, since relatedness was higher when females were included. Nevertheless, our data did not allow for testing this hypothesis. In other studies with pumas, contrasting results have been found. Biek et al. (2006) and Miotto et al. (2012) found that females were closely related among them than males with other males, while Onorato et al. (2011) found that males were closely related among them than females with other females.

Our data showed that pumas presented higher levels of relatedness than jaguars. Three non-exclusive facts might explain these differences: (1) puma home ranges may be smaller than jaguar home ranges in Viruá. If puma home ranges are smaller, and accepting female philopatry, the number of potentially related individuals in pumas would be higher than in jaguars. There is no information on home range size for jaguars or pumas in the Amazon Basin, but in the savannah habitat of Emas National Park, where both species have been radio tracked, pumas had home ranges of 124–763 km^2, and jaguars of 401–1102 km^2 (Silveira 2004). (2) Jaguars might have higher mortality rates than pumas in the area, promoting a higher exchange of unrelated individuals in the population. Jaguars are usually more persecuted by retaliatory hunting due

to livestock depredation than pumas (e.g., Conforti and Cascellide Azevedo 2003; Michalski et al. 2006). (3) Differential reproductive parameters might allow, for a given time period, for more related individuals of pumas than jaguars to be present in the area. For example, litter size is usually larger in pumas than in jaguars (Shaw 2010; Desbiez et al. 2012).

Our results show that non-invasive faecal surveys of solitary and elusive felids may be a suitable methodology to provide information on space use and spatial relationships between species, in addition to several genetic population parameters (see Roques et al. (2016)).

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