

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

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14 **Short running title:** Non-invasive survey of large American felids

15

16 **Abstract** Jaguars and pumas are the largest felids in the Americas. Information about
17 these two species is scarce, especially where both species are sympatric. We studied the
18 use and selection of macrohabitats, spatial segregation and kinship in jaguars and pumas
19 in the Viruá National Park (Amazonian lowlands) by non-invasive genetic analyses of
20 faecal samples. Seven different jaguars (six males and one female) and nine different
21 pumas (five males and four females) were identified. We found space use segregation
22 between the two species, with pumas using mostly forested habitats and jaguars using
23 open habitats slightly more than the forested ones. This result is unexpected since
24 previous studies have found that pumas favour more open habitats than jaguars. The
25 results suggest that jaguars use the areas in a more random manner, corresponding to the
26 habits of a dominant generalist species, whereas pumas use the area to reduce encounter
27 rates with jaguars. Nevertheless, both species mainly used areas near upland forest-
28 flooding habitats. Some kinship categories were supported with a $p < 0.05$ in 57% and
29 83% of the pair comparisons between the identified jaguars and identified pumas,
30 respectively. Non-invasive genetic analysis of faeces was useful to study the spatial
31 ecology of solitary, rare and cryptic species in the Amazon.

32 **Key words** Jaguar, macrohabitat use and selection, puma, relatedness, spatial
33 segregation

34 **Introduction**

35 Jaguars (*Panthera onca*) and pumas (*Puma concolor*) are the largest felids in the
36 Americas. The species coexist throughout the jaguar's distribution area, basically in the
37 Neotropics, and share a similar life history and behavioural traits (Sunquist and
38 Sunquist 2002). Although jaguars and pumas are respectively listed by IUCN as Near
39 Threatened and of Least Concern, and in CITES Appendix I and II, in many areas of

40 their distribution range are of conservation concern (Caso et al. 2008; Nielsen et al.
41 2015). Some of their populations have been extirpated, are at high risk of extirpation, or
42 are declining due to loss of habitat quality and fragmentation, poaching of their main
43 prey and retaliatory killing due to livestock depredation (Nowell and Jackson 1996;
44 Zanin et al. 2015; Petracca et al. 2014). This situation is particularly accentuated for the
45 jaguar, which has lost more than 50% of its former distribution area (Sanderson et
46 al. 2002). Much of the jaguar's remaining habitat is the rainforest of the Amazon Basin
47 (88% of its remaining area of occupancy; Caso et al. 2008), which is also considered of
48 relatively low suitability (Torres et al. 2007). Despite the importance of the Amazon
49 Basin for jaguars, little information exists on the ecology of the species in that region
50 and in the rest of Latin America, which is also true for pumas (Haines 2006; Caso et al.
51 2008; Laundré & Hernández 2010; Nielsen et al. 2015; Palomares et al. 2016). The only
52 published information for the Amazon Basin relates to jaguar density estimations by
53 camera-trapping in the Colombian, Bolivian and Peruvian Amazon (Silver et al. 2004;
54 Payan 2008; Tobler et al. 2013).

55 In this paper, we provide information on jaguar and puma spatial ecology and some
56 population parameters of both felids in the Viruá National Park (northern Brazilian
57 Amazon Basin) by non-invasive genetic analyses of faeces that is an increasingly
58 efficient method for studying the use and selection of macrohabitats, and relationships
59 among individuals in secretive mammals. Furthermore, we examined whether there was
60 any spatial segregation between the species, as would be expected due to their
61 ecologically similar requirements (Sunkist & Sunkist 2002; Haines 2006; Caso et al.
62 2008; Nielsen et al. 2015).

63 **Materials and methods**

64 **Study area**

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

74 The area has a sampling infrastructure that includes a trail system forming a 5x5
75 km grid (Magnusson et al. 2005). A description of the trail system and infrastructure
76 can be obtained from the Program for Biodiversity Research (PPBio) website
77 <<http://ppbio.inpa.gov.br>>. The grid consists of six parallel 5-km trails and six 5-km
78 trails perpendicular to those, totalling 60 km.

79

80 **Sample collection and preservation**

81 Faecal sampling was carried out during the end of the dry season (February-
82 March) in 2008, 2009 and 2011, by slowly walking along the trails of the 5x5 km grid
83 system, the access trails to the park headquarters, and the 56 km of the abandoned dirt
84 road to the east. Additionally, in 2011, several cross-country transects were walked
85 along the river border in the confluence area of the Branco and Anauá Rivers to the
86 south of the Park. In total, we walked 261 km on dirt roads and trails. In 2009 and 2011,
87 some faeces were collected with the help of a scat detector dog (Smith et al. 2001).

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

93 **DNA extraction for species, sex and individual identification**

94 DNA extraction of faecal samples was conducted according to protocols based
95 on the GuSCN/silica method (Boom et al. 1990; Höss & Pääbo, 1993; Frantz et al.
96 2003). Each batch of extractions (n = 15) included one PCR negative extraction control
97 to monitor for contamination by exogenous DNA. DNA extractions of faecal samples
98 were performed in a UV-sterilized laminar flow hood in an isolated laboratory specially
99 designated for the manipulation of non-invasive material.

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

109 Individual genotyping for jaguars and pumas was conducted using an optimised
110 set of 11 (Fca024, Fca026, Fca043, Fca077, Fca082b, Fca090, F115a, Fca126, Fca176,
111 Fca547b, Fca566b; Menotti-Raymond et al. 1999) and 12 (Fca077, Fca82b, Fca126,

112 Fca547b, PcoB003w, PcoB010w, PcoA208w, PcoB210w, PcoA216w, PcoC108w,
113 PcoC112w and PcoA339w, Menotti-Raymond et al. 1999; Kurushima et al. 2006)
114 microsatellite markers, respectively (Palomares et al. 2012; Roques et al. 2014; Zanin et
115 al. 2016). Before genotyping the whole set of microsatellites, DNA extracts were
116 evaluated for quality by direct amplification of the Fca82b locus, which was selected for
117 its high amplification robustness. The samples that failed to amplify this locus would
118 probably not amplify the remaining loci and were not genotyped.

119 A two-step strategy was used to improve genotyping success rate and accuracy
120 through two sequential amplifications (Bellemain & Taberlet 2004; Piggott et al. 2004):
121 a multiplex PCR that included the whole set of primer pairs and a reduced number of
122 cycles (pre-PCR) at a moderate annealing temperature, followed by the amplification of
123 each locus separately, using the PCR products as templates (post-PCR). Pre-PCR was
124 performed in a multiplex reaction with 7 μ L of DNA extract in a 30 μ L reaction
125 including 67 mM Tris-HCl pH 8, 16mM $(\text{NH}_4)_2\text{SO}_4$, 1.5 mM MgCl_2 , 0.8 μ M dNTPs,
126 0.8 mg/ml BSA, 0.02 or 0.03 μ M each primer, and 0.6 U of Taq DNA polymerase
127 (Bioline). Pre-PCR conditions were as follows: initial denaturation at 94 $^\circ\text{C}$ for 2 min,
128 25 cycles of 30s at 92 $^\circ\text{C}$, 55 $^\circ\text{C}$ and 72 $^\circ\text{C}$ and a final extension of 5 min at 72 $^\circ\text{C}$.
129 Second-stage post-PCR amplifications were performed independently for each marker
130 using 4 μ l of PCR product in a final volume of 20 μ l reactions containing 67 mM Tris-
131 HCl pH 8, 16mM $(\text{NH}_4)_2\text{SO}_4$, 2mM MgCl_2 , 0.25mM dNTPs, 0.8 mg/ml BSA, 0.2 μ M
132 of each primer and 0.5 U of Taq polimerase. Post-PCR conditions were: initial
133 denaturation at 94 $^\circ\text{C}$ for 2 min, 40 cycles of 30s at 92 $^\circ\text{C}$, specific annealing
134 temperature (see Roques et al. 2014; Zanin et al. 2016) and 72 $^\circ\text{C}$ and a final extension
135 of 5 min at 72 $^\circ\text{C}$. Up to 6 PCR products of jaguar samples from the second
136 amplification step, with fluorescently labelled primers, or 12 PCR products in the case

137 of puma samples, were combined on an ABI PRISM 3130 XL Genetic Analyzer.
138 Alleles were sized using GeneMapper Software version 3.7 (Applied Biosystems).
139 Samples were genotyped using a multi-tube approach (Navidi et al 1992;
140 Taberlet et al 1996, 1999; Taberlet & Luikart, 1999; Goossens et al. 2000) with four
141 replicates per locus per individual. For a locus to be considered homozygous, only the
142 same allele could be observed in at least three independent replicates, without observing
143 an additional allele in the fourth replicate. The heterozygous loci were those with the
144 same two different alleles in at least two replicates. A quality index (QI) similar to the
145 one described by Miquel et al. (2006) was calculated for each sample, referred to
146 individual alleles instead of the genotype of a given locus. We calculated the percentage
147 of replicates that were equal to the consensus for each given allele, and then we
148 averaged the values across individuals and loci. Samples with QI below 0.5 or with less
149 than seven loci genotyped were discarded from further analyses.

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

152 **Data analysis**

153 *Spatial segregation*

154 We tested for spatial overlap between jaguars and pumas with a null model (Gotelli and
155 Graves 1996), which randomised the spatial distributions of jaguar and puma samples.
156 Given the apparent stability of jaguar and puma detection over time (see Fig. 1), we
157 pooled the data from all years. For the randomisation process, we first assigned each
158 faecal sample to a 1 km² cell, and then computed the observed spatial overlap between
159 the species (i.e., number of 1 km² cells with detection of both species in relation to the
160 total number of cells with detections). In a second step, we built the randomisation

161 procedure based on the mean percentage of overlapping jaguar and puma occurrence
162 cells, to test whether the puma or jaguar cells were distributed randomly relative to the
163 other species' cells. At each step of the process, we randomly distributed the samples of
164 each species over the total number of occurrence cells containing any sample, and
165 computed the percentage again. We repeated this process 1000 times and then compared
166 the observed percentages with the distribution of simulated percentages, to compute a p-
167 value. This algorithm was built in R software, and it is available on request.

168 Macrohabitat selection

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

176 For macrohabitat characterisation using ArcGIS® we used a detailed landcover
177 map elaborated for the management plan of Viruá National Park and surrounding areas
178 (ICMBio 2014), with a resolution of 1m² grid cells. Nine different landcover types are
179 defined in the map, which were re-classified in the three macrohabitats described above
180 (upland forest, campina and campinarana; Supporting Information Table S1). Since
181 there were few samples for the flooding macrohabitats, for analyses we only considered
182 two general types of macrohabitat: upland forest and flooding habitats (comprising
183 campina and campinarana).

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

194

195 *Relatedness and categories of relationship among individuals*

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

204 Relationship categories and relatedness are more confidently estimated when allele
205 frequencies of the population are well sampled, so to calculate allele frequencies we
206 used a total of 24 genotypes of jaguars and 20 genotypes of pumas from the Amazon
207 Basin that we had in our data bases from samples of both species. Both jaguars and

208 pumas from the Amazon Basin (including those of the Virua area) belong to the same
209 genetic populations, respectively (Roques et al. 2016; authors unpublished), thus, the
210 inclusion of these samples increased robustness of analyses. These genotypes were
211 obtained using the same molecular techniques previously described and in the same
212 laboratory, and were collected between 2005 and 2011 in a total of four study areas in
213 the Amazon basin (see Roques et al. 2016 for a description of the study areas).

214 **Results**

215 **Scat analyses**

216 We collected 175 scats, of which 51.4% were identified: 25 from jaguars and 35
217 from pumas. Overall, we collected 0.10 and 0.13 jaguar and puma scats/km,
218 respectively. Eighty four percent of jaguar scats and 55% of puma scats were from
219 males. The number of samples genotyped after positive amplification of the Fca82b
220 locus were 19 for jaguars and 29 for pumas. Ten out of 11 microsatellite loci used for
221 jaguars had an amplification success higher than 77%, except one (locus Fca176) with
222 48% success. For pumas, three microsatellite markers (locus PcoB3, locus Fca82, and
223 locus Fca547) had an amplification success of 66-67%, and the nine remaining between
224 71-94%. The probability of identity estimated for the 11 and 12 analysed loci indicated
225 that our microsatellite panel was sufficient to discriminate individuals within the entire
226 dataset ($P_{(ID)_{sib}} = 4.23 \times 10^{-5}$, and 1.01×10^{-4} , for jaguars and pumas, respectively).
227 Fourteen jaguar genotypes and 26 puma genotypes obtained a QI value greater than 0.5
228 and reached more than 7 loci genotyped, thus, meeting our quality requirements. Seven
229 different jaguars (six males and one female) and nine different pumas (five males and
230 four females) were identified using these high quality genotypes, with a mean number
231 of 2.0 (SE= 0.49, range=1-4) and 2.8 (SE= 1.92, range= 1-9) scats collected per

232 individual for jaguars and pumas, respectively. Only one male jaguar was resampled in
233 2009 and 2011, and only one female puma was resampled in all surveyed years (Fig. 1).

234

235 **Spatial distribution and macrohabitat selection**

236 We recorded the location of 56 scats (21 of jaguar and 35 of puma; four scats were not
237 georeferenced), which were distributed within 32 1 km² cells, of which 12.5% contained
238 samples of both species. The null model clearly showed that both species segregated in
239 space more than expected by random (simulated percentage of simultaneous occurrence
240 was 31.3%, significantly higher than the 12.5% observed with a $p < 0.001$).

241 Eighty-six percent of jaguar faeces ($n=21$) were found in flooding habitats,
242 whereas only 11% of puma scats ($n=35$) were found in this type of vegetation (Table 1;
243 Fig. 1; $\chi^2 = 27.3$, $df = 1$, $p < 0.001$). Data obtained directly in the field during sampling,
244 greatly coincided with map-derived data (Table 1). The height of the vegetation canopy
245 was 2-12 m for flooding habitats and 15-25 m for upland forests. Comparing the use
246 with the availability of these macrohabitats, pumas clearly avoided flooding areas
247 (Jacobs index = -0.94) and selected upland forests (Jacobs index = 0.94), whereas jaguars
248 seemed to use close to available the two types of macrohabitats (Jacobs indexes = 0.17
249 and -0.16, respectively; Table 1). The logistic regression showed that the probability of
250 a scat belonging to a jaguar or puma increased with distance from the forest edge
251 (jaguar to flooding habitats and puma to upland forest interior; $p < 0.001$). Nevertheless,
252 both species preferred to be near the edge (86% and 89% jaguar and puma detections,
253 respectively, < 1 km from the edge; Figs. 1 and 2).

254

255 **Relatedness and categories of relationship among individuals**

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

267 Differences in percentage of pairs with a possible relationship between jaguars and
268 pumas approached significance ($Z = 1.854$, $p = 0.064$; Z test), and the number of pair
269 comparisons with higher values of the maximum likelihood estimates of relatedness
270 (i.e., a closer relationship) was clearly higher in pumas than in jaguars (Supplementary
271 Information Table S2). This result did not seem to be biased due to the fact that we
272 detected mainly males in jaguars, and a similar number of both sexes in pumas.

273 Considering only comparisons between males, percentages of pairs with a possible
274 relationship was 46.7% and 90.0%, for jaguars and pumas, respectively ($Z = 1.771$, $p =$
275 0.077). However, the indices of relatedness were often higher for pair comparisons
276 involving a female (Supplementary Information Table S2), although the few available
277 samples prevented statistical testing of this trend.

278

279 **Discussion**

280 Both jaguars and pumas are considered as habitat generalists, found from arid areas to
281 rain forests. In sympatric areas, the species can be found in the same areas and
282 macrohabitats (Núñez et al. 2002; Scognamillo et al. 2003; Noss et al. 2006, Estrada
283 Hernández 2008; Monroy-Vilchis et al. 2009; Harmsen et al. 2009, Di Bitetti et al.
284 2010; Palomares et al. 2016). In a few cases, very small differences in macrohabitat use
285 were recorded within local scale studies (Schaller & Crawshaw 1980; Emmons 1987;
286 Chávez 2010; Sollman et al. 2012), which pointed to pumas favouring more open
287 habitats, although also using forest areas, and jaguars using both open and forested
288 habitats in proportion to availability (Farrell and Sunquist 2000; Scognamillo et al.
289 2003; Silveira 2004). Pumas have also been described as more tolerant to human-
290 influenced landscapes than jaguars (De Angelo et al. 2011; Sollman et al. 2012; but also
291 see Foster et al. (2010) for a contrary result).

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

301 Under a potential competition scenario, jaguars should be dominant over pumas (Ruth
302 & Murphy, 2010; Oliveira & Pereira, 2014), and if so, both theoretical and empirical
303 studies predict that pumas should avoid habitats or areas used by jaguars to decrease the
304 risk of interspecific encounters (Case and Gilpin 1974; Palomares and Caro 1999,

305 Linnell and Strand 2000). The threat of aggression can create a ‘landscape of fear’
306 (Laundré, Hernández & Altendorf 2001) that excludes prey or subordinate species from
307 suitable habitats, normally in core areas of the dominant species (e.g. Palomares et al.
308 1996; Swanson et al. 2014). This was partially the case in our study, as both species
309 segregated in the use of space, and pumas mainly selected upland forests and avoided
310 flooding habitats, but jaguars did not avoid upland forests. Therefore, our results
311 suggest that jaguars use the study area in a more random manner, probably triggered by
312 prey availability, as would be expected from a dominant generalist species. Pumas
313 behave spatially in a way to diminish encounter rates with jaguars, preferring forest
314 areas, where visibility is lower and escape possibilities higher if a jaguar is close. In a
315 larger scale study, Palomares et al. (2016) found microhabitat segregation between
316 jaguars and pumas, which was explained well by a scenario of interference competition
317 between the two species with pumas being subordinate. Furthermore, both species
318 mainly used areas close to upland forest-flooding habitat transitions, where prey-species
319 richness is expected to be higher (Schluter & Ricklefs 1993; Brown 1995).

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

327 Our data showed that pumas presented higher levels of relatedness than jaguars. Three
328 non-exclusive facts might explain these differences: 1) Puma home ranges may be
329 smaller than jaguar home ranges in Viruá. If puma home ranges are smaller, and

330 accepting female philopatry, the number of potentially related individuals in pumas
331 would be higher than in jaguars. There is no information on home range size for jaguars
332 or pumas in the Amazon Basin, but in the savannah habitat of Emas National Park,
333 where both species have been radio-tracked, pumas had home ranges of 124-763 km²,
334 and jaguars of 401-1102 km² (Silveira 2004). 2) Jaguars might have higher mortality
335 rates than pumas in the area, promoting a higher exchange of unrelated individuals in
336 the population. Jaguars are usually more persecuted by retaliatory hunting due to
337 livestock depredation than pumas (e.g. Conforti & Cascelli de Azevedo 2003; Michalski
338 et al. 2006). 3) Differential reproductive parameters might allow, for a given time
339 period, for more related individuals of pumas than jaguars to be present in the area. For
340 example, litter size is usually larger in pumas than in jaguars (Shaw 2010; Desbiez et al.
341 2012).

342 Our results show that non-invasive faecal surveys of solitary and elusive felids may be a
343 suitable methodology to provide information on space use and spatial relationships
344 between species, in addition to several genetic population parameters (see Roques et al.
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346

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363

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577

578 **Table 1** Macrohabitats where jaguar and puma faeces were found according to data
 579 directly gathered during fieldwork and from vegetation maps of Viruá National Park.
 580 For data recorded during fieldwork, height of the vegetation is also shown.

Macrohabitats	Availability (220.4 km ²)	Percentage of use					
		Jaguar			Puma		
		Field (n=9)	Height range (m)	Map (n=21)	Field (n=10)	Height range (m)	Map (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

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584 **Figure captions**

585 **Fig. 1** Location of Viruá National Park in Brazil and of the study area (the grey square
586 within the dashed rectangle of the study area indicates the location of the 25-km² trail
587 grid; left hand panels). The right-hand panels show the locations of identified jaguar and
588 puma samples in each study year (black points= females; white squares= males; white
589 circles with black point= samples with no sex identification; numbers close to samples
590 indicate the ID of individual when genotyping was possible). Number of identified
591 jaguar and pumas in the years 2008, 2009 and 2011 were 6, 4, 5 and 20, 14, 11 in case
592 of faecal samples, and 1, 1, 2 and 5, 5, 5 in case of individuals, respectively.

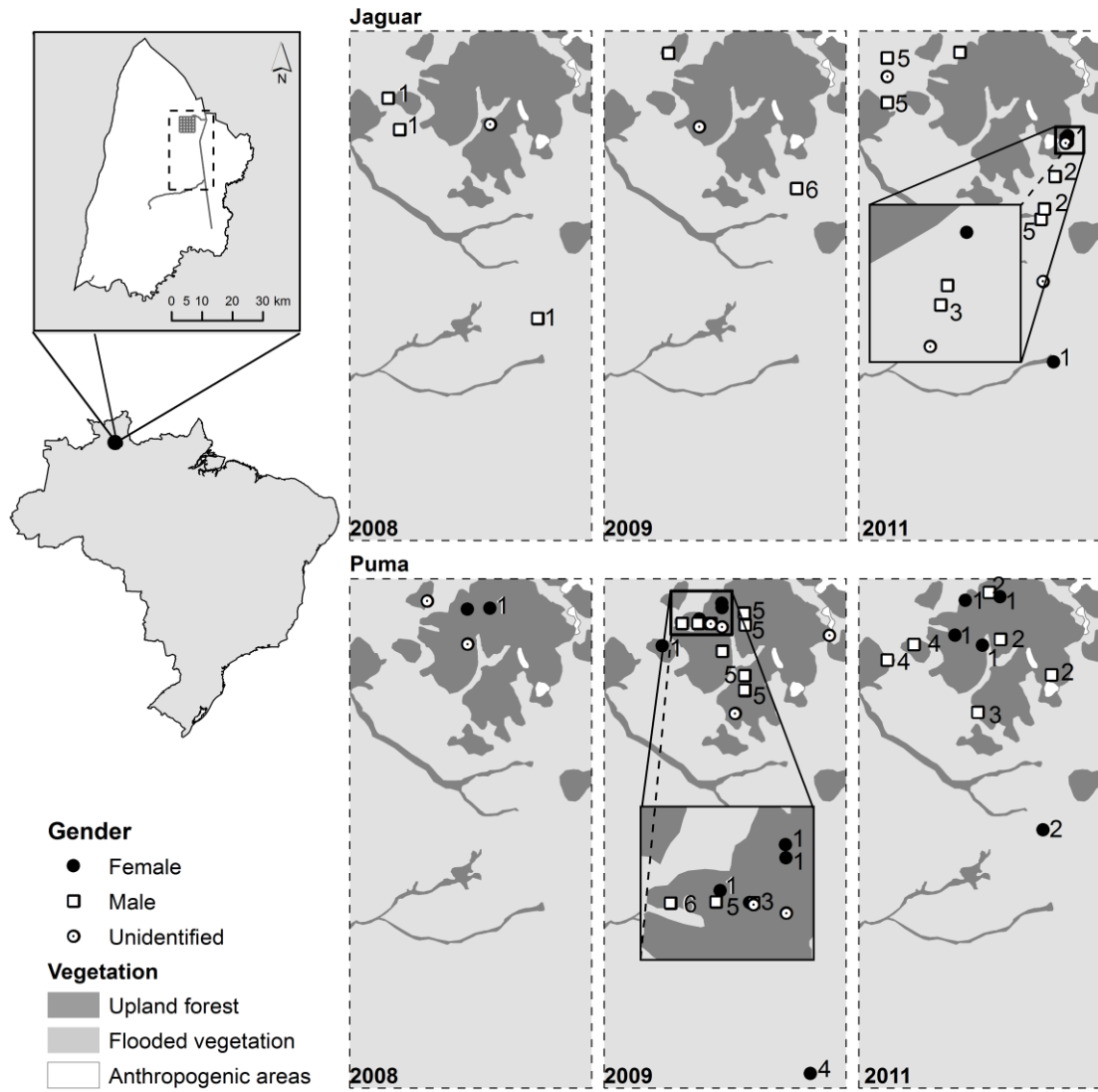
593

594 **Fig. 2** Logistic regression testing the probability of finding a scat from a jaguar or a
595 puma in relation to the distance to the upland forest-flooding habitat edge. Logit P= -
596 $0.0137 + (0.00411 * \text{Distance})$; likelihood ratio test statistic= 28.14, $p < 0.001$ (one puma
597 sample located 6.8 km from the edge in flooding habitats was removed from the
598 analysis; the next sample was located 1.1 km from the edge). Points represent raw data
599 from jaguars (black) and puma (grey).

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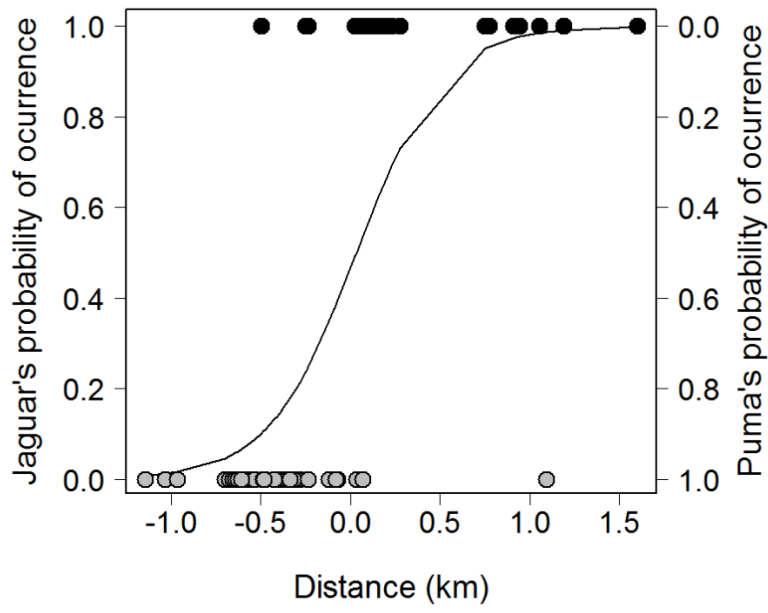
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606 FIGURE 2



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