PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

FILOGEOGRAFIA, HISTÓRIA DEMOGRÁFICA E DIVERSIDADE MOLECULAR DE DUAS ESPÉCIES NEOTROPICAIS DA FAMÍLIA PROCYONIDAE (MAMMALIA, CARNIVORA): *Nasua nasua* E *Procyon cancrivorus*

Mirian Tieko Nunes Tsuchiya Jerep

DISSERTAÇÃO DE MESTRADO

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Autor: Mirian Tieko Nunes Tsuchiya Jerep
Orientador: Prof. Dr. Eduardo Eizirik

DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE - RS - BRASIL

2009

DEDICATÓRIA

“As criaturas que habitam esta terra em que vivemos, sejam eles seres humanos ou animais, estão aqui para contribuir, cada uma com sua maneira peculiar, para a beleza e prosperidade do mundo.”

Sua Santidade, o Dalai Lama

Dedico a todos aqueles que acreditam e vivem no respeito por todas as formas de vida

AGRADECIMENTOS

Ao meu orientador-amigo-ouvinte, prof. Eduardo Eizirik, por todo apoio, paciência e confiança; obrigada por acreditar em mim.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico, pela bolsa de mestrado.

Aos professores do Programa de Pós-Graduação em Zoologia, pelos ensinamentos e pela troca de experiências.

Aos meus companheiros de pós-graduação, pelos bons momentos que passamos durante as aulas e pelas conversas.

À "família Genoma": vocês são os melhores companheiros de trabalho que alguém pode ter. Muito obrigada pelas conversas, pelas palavras de apoio, pelos momentos de descontração e em especial, por terem me acolhido tão bem.

À Cladinara Roberts Sarturi por inúmeros motivos: por cuidar do laboratório, por toda ajuda, apoio, carinho e amizade.

Ao grupo de "Carnivorólogos" por toda amizade e apoio em todos os momentos. Vocês são muito especiais para mim.

Às meninas laboratório: Taia, Ale, Fê Pedone, Catatau, Anne, Lisie, Marina, Rê, Flavinha, Talita, Gabi, Déa, Cris, Tati, Beta, Laura e Sofia, por tornarem tudo mais divertido e brilhante.

Aos meninos: Manoel, Marcelo, Ricardo, Thomaz, Lucas, Chris, Gabriel, Nelson, Henrique, Paulinho, André, Felipe... Por todas as conversas e toda ajuda sempre.

Aos meus amigos de todos os lugares: Gordinhas, Vivi e Carlinhos... Vocês estão sempre no meu coração.

Aos meus pais, Paulo e Dalva, meus exemplos, meus amigos, meus anjos... Obrigada por todo apoio e carinho, mesmo a distância.

Ao meu marido, Fernando, por me incentivar a sempre ser melhor, por acreditar em mim e me dar força em todos os momentos. Sem você eu não teria conseguido.

A Deus, em todas as suas manifestações.

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RESUMO

Estudos filogeográficos comparados são úteis na compreensão de processos históricos compartilhados que afetam faunas regionais, bem como na identificação de padrões espécie-específicos que podem influenciar suas atuais características genéticas. Neste estudo, foram realizadas análises filogeográficas de dois carnívoros Neotropicais de médio porte, o quati de focinho marrom (*Nasua nasua*) e o mão pelada (*Procyon* cancrivorus), usando marcadores mitocondriais e microssatélites, afim de caracterizar e comparar seus padrões de diversidade genética e compreender sua história evolutiva. Adicionalmente, descreve-se o isolamento e a caracterização de oito loci polimórficos de microssatélites para *Nasua nasua*. Ambas as espécies são bastante comuns na natureza e estão presentes em uma ampla variedade de habitats, sendo simpátricas ao longo da maior parte de sua distribuição. Análises de DNA mitocondrial mostram níveis de diversidade até dez vezes superiores para *N. nasua* com relação a *P. cancrivorus*. As análises de DNA mitocondrial revelaram a existência de seis filogrupos reciprocamente monofiléticos para *N. nasua*, os quais são também suportados como populações distintas através das análises de microssatélites. Por outro lado, dados de mtDNA de *Procyon cancrivorus* indicam a existência de três unidades populacionais; porém a magnitude desta diferenciação foi muito menos evidente do que a observada em *N. nasua*. Além disso, os dados de microssatélites não suportaram a existência de qualquer subdivisão genética para *P. cancrivorus*, sugerindo que persiste uma completa conectividade entre todas as áreas amostradas. Estes resultados demonstram que estas espécies apresentam uma historia evolutiva bastante distinta, a qual pelo menos em parte pode ser atribuída a diferenças na estrutura social e no padrão de dispersão das mesmas. Tais resultados destacam a complexidade evolutivaa da biota Neotropical e ressaltam a necessidade de análises multi-espécies empregando conjuntos de dados comparáveis, de forma que padrões comuns e contrastantes possam ser adequadamente investigados.

ABSTRACT

Phylogeography, demographic history and molecular diversity of two Neotropical species of family Procyonidae (Mammalia, Carnivora): *Nasua nasua* and *Procyon cancrivorus*.

Comparative phylogeographic analyses are useful to shed light on common historical processes affecting regional faunas, as well as to identify species-specific life history features that may influence their genetic legacy. Here we performed phylogeographic analysis of two medium-sized Neotropical carnivores, the brown-nosed coati (*Nasua nasua*) and the crab-eating raccoon (*Procyon cancrivorus*), using mitochondrial DNA and microsatellite markers, in order to characterize and compare their patterns of genetic diversity and underlying evolutionary history. We also describe the isolation and characterization of eight polymorphic microsatellite loci for brown-nosed coatis (*Nasua nasua*). Both species are farily common in the wild and present in a wide variety of habitats, being sympatric throughout most of their ranges. Mitochondrial DNA analyses showed levels of diversity that were up to ten-fold higher for *N. nasua* relative to to *P. cancrivorus*. Six reciprocally monophyletic mtDNA phylogroups were recognized for *N. nasua*, which were also supported as distinct populations by the microsatellite analyses. In contrast, the mtDNA data set for *Procyon cancrivorus* indicated the existence of three recognizable population units, but the magnitude of their differentiation was much less pronounced than that observed in *N. nasua*. Moreover, the microsatellite data did not support any genetic subdivision in this species, suggesting that full connectivity is maintain throughout all sampled areas. These results demonstrate that these species have very distinct evolutionary histories, which may at least in part be a consequence of differences in social structure and dispersal patterns. These results highlight the evolutionary complexity of the Neotropical biota, and underscore the need for multi-species analyses employing comparable data sets so that common and contrasting patterns can be adequately investigated.

APRESENTAÇÃO

O presente trabalho, intitulado "Filogeografia, história demográfica e diversidade molecular de duas espécies neotropicais da família Procyonidae (Mammalia, Carnivora): *Nasua nasua* e *Procyon cancrivorus* foi desenvolvido como parte dos requisitos necessários para a obtenção do título de Mestre junto ao Programa de Pós-Graduação em Zoologia da Pontifícia Universidade Católica do Rio Grande do Sul.

Este trabalho teve como principais objetivos (i) caracterizar a estrutura genética de duas espécies de procionídeos neotropicais, *Nasua nasua* (quati de focinho marrom) e *Procyon cancrivorus* (mão-pelada), (ii) inferir a história demográfica destas, comparando-a com outras espécies neotropicais a fim de investigar a ocorrência de padrões filogeográficos compartilhados (iii), além de integrar os dados moleculares obtidos com informações já disponíveis sobre estas espécies, a fim de contribuir para um melhor conhecimento de sua biologia, embasando estratégias para sua conservação a longo prazo na natureza.

Esta dissertação é apresentada no formato de dois artigos científicos: uma *Primer Note*, descrevendo o isolamento e caracterização de oito *loci* de microssatélites para *N. nasua* a ser submetido ao periódico *Molecular Ecology Resources* (apresentado no apêndice) e o artigo principal, tratando de filogeografia comparada, a ser submetido ao periódico *Molecular Ecology*.

1 **Comparative phylogeographic patterns reveal contrasting demographic**
2 **histories in two Neotropical procyonids (*Nasua nasua* and *Procyon cancrivorus*)**

3

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14

15 KEYWORDS: Procyonidae, brown-nosed coati, crab-eating raccoon, microsatellites, mitochondrial
16 DNA.

17

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21

22 Running title: Contrasting genetic patterns in procyonids

23 **Abstract**

24

25 Comparative phylogeographic analyses are useful to shed light on common historical
26 processes affecting regional faunas, as well as to identify species-specific life history features that
27 may influence their genetic legacy. Here we performed phylogeographic analysis of two medium-
28 sized Neotropical carnivores, the brown-nosed coati (*Nasua nasua*) and the crab-eating raccoon
29 (*Procyon cancrivorus*), using mitochondrial DNA and microsatellite markers, in order to
30 characterize and compare their patterns of genetic diversity and underlying evolutionary history.
31 Both species are farily common in the wild and present in a wide variety of habitats, being
32 sympatric throughout most of their ranges. Mitochondrial DNA analyses showed levels of diversity
33 that were up to ten-fold higher for *N. nasua* relative to to *P. cancrivorus*. Six reciprocally
34 monophyletic mtDNA phylogroups were recognized for *N. nasua*, which were also supported as
35 distinct populations by the microsatellite analyses. In contrast, the mtDNA data set for *Procyon*
36 *cancrivorus* indicated the existence of three recognizable population units, but the magnitude of
37 their differentiation was much less pronounced than that observed in *N. nasua*. Moreover, the
38 microsatellite data did not support any genetic subdivision in this species, suggesting that full
39 connectivity is maintain throughout all sampled areas. These results demonstrate that these species
40 have very distinct evolutionary histories, which may at least in part be a consequence of differences
41 in social structure and dispersal patterns. These results highlight the evolutionary complexity of the
42 Neotropical biota, and underscore the need for multi-species analyses employing comparable data
43 sets so that common and contrasting patterns can be adequately investigated.

44

45 **Introduction**

46

47 Several studies concerning different organisms attempted to understand the processes that
48 have shaped the current species distribution and genetic structure found in the Neotropical region
49 (Costa 2003; Hubert & Renno 2006; Carnaval & Bates 2007; Grazziotin *et al.* 2007; Martins *et al.*
50 2007). Although some common patterns can be identified among the studies, they all agreed that
51 there is no single model of vicariance or climatic change that could explain the Neotropical
52 complexity.

53

54 The use of common and widespread species in phylogeographic studies may be very useful
55 due to the possibility of going beyond species-specific biogeographic patterns to pursue
56 comparative analysis of regional or continental biotas. Some intra-specific studies concerning
57 common vertebrates have been conducted in the Neotropical region (e.g. Wüster *et al.* 2005;
58 Noonan & Wray 2006; Grazziotin *et al.* 2007), but still very have few have focused on carnivores
59 (e.g. Eizirik *et al.* 1998, 2001; Tchaicka *et al.* 2007; Trinca *et al.* 2007). Among the Carnivora,
60 some of the least studied species belong to the families Procyonidae, Mustelidae and Mephitidae
61 (Oliveira 2006), so that in many cases basic aspects of their biology, ecology and geographic
62 distributions remain to be clarified.

63

64 The family Procyonidae comprises six genera (Potos*, Procyon, Nasua, Nasuella,*
65 *Bassaricyon, Bassariscus*) and fourteen recognized species (Wozencraft 2005), distributed from
66 Canada to Argentina. The inter-generic relationships of the family have been subject of several
67 phylogenetic studies based on morphological and molecular characters (Decker & Wozencraft
68 1991; Baskin 2004; Fulton & Strobeck 2007; Koepfli *et al.* 2007). Contrasting patterns were

69 recovered depending on the type of information used. According to Koepfli *et al.* (2007), Potos is
70 the sister lineage to the clade containing the remaining genera (divergence time estimates: 21.6 – 24
71 mya [95% confidence intervals, CI = 12.1 – 36.0 mya]), which is divided into two subgroups:
72 *Nasua* plus *Bassarycion* and *Procyon* plus *Bassariscus* (divergence time estimates: 18.3 – 20.7 mya,
73 CI = 10.3 – 30.9 mya). Within *Nasua* and *Procyon*, the divergence time estimates are respectively 7
74 – 8 mya (CI = 3.7 – 12.9 mya) and 5 – 5.7 mya (CI = 2.6 – 9.2 mya), predating the closure of the
75 Panamanian land bridge.
76
77 The brown-nosed coati (*Nasua nasua*) is a diurnal, highly social mesocarnivore (3.5 – 6.0
78 kg) that is distributed in South America, from Colombia and Venezuela to Uruguay and northern
79 Argentina (Redford & Eisenberg 1992; Gompper & Decker 1998; Nowak 1999). It is found
80 primarily in forested habitats, ranging from tropical rainforest and gallery forest to chaco, cerrado
81 and dry scrub environments (Gompper & Decker 1998; Emmons 1990). Coatis forage both
82 arboreally and terrestrially, and their diet includes primarily fruits, invertebrates and occasionally
83 small vertebrates (Redford & Eisenberg 1992, Nowak 1999). Females and immature males form
84 permanent groups while males are solitary, joining the groups only during the mating season. After
85 this period, they seem to be excluded from the groups by adult females, apparently to avoid
86 aggression against the juveniles (Russel 1981; Redford & Eisenberg 1992). Females leave the
87 groups to give birth to young, which are born in an arboreal nest after seventy-seven days of
88 gestation (Nowak 1999).
89
90 The crab-eating raccoon (*Procyon cancrivorus*) is a nocturnal, medium-sized carnivore (3 –
91 8 kg), which is distributed from Central America (southern Costa Rica and eastern Panama)
92 throughout South America to northeastern Argentina and Uruguay. In Costa Rica and Panama, its

93 range overlaps with that of the northern raccoon (*Procyon lotor*), but the latter is mainly found in
94 mangrove swamps, and the crab-eating raccoon is found mostly near inland rivers (Eisenberg 1989;
95 Emmons 1990). Although this species occurs in diverse environments, it seems to be somewhat
96 restricted to waterside habitats (Redford & Eisenberg 1992, Emmons 1990). *Procyon cancrivorus* is
97 a generalized-omnivore, and its diet includes fruits, invertebrates and small vertebrates, depending
98 on resource availability (Bisbal 1986; Santos & Hartz 1999; Gatti *et al.* 2006). They tend to forage
99 alone, except for the female-offspring unit (Redford & Eisenberg 1992).

100

101 In this study, we aimed to characterize the genetic structure, phylogeographic patterns and
102 demographic history of *N. nasua* and *P. cancrivorus*, using both mitochondrial and microsatellite
103 markers. Our objective was to test if these two sympatric carnivores possess similar evolutionary
104 histories, or if their patterns are species-specific and may be correlated to known biological
105 differences between them. By comparing their phylogeographic structure and demographic history,
106 we aimed to look for common or contrasting patterns that may enhance our understanding of the
107 evolutionary dynamics of Neotropical mammals.

108

109 **Material and Methods**

110

111 ***Sample collection and laboratory techniques***

112

113 Biological samples (blood and tissue) were collected from 90 *Nasua nasua* and 44 *Procyon*
114 *cancrivorus* individuals (Tables 1 and 2, respectively) across the range of each species (Figure 1).
115 Blood samples were obtained from wild animals captured for ecological studies and captive

116 individuals of known origin, and preserved in a salt saturated solution (100mM Tris, 100mM
117 EDTA, 2% SDS). Tissue samples were collected from road-killed specimens and preserved in 96%
118 ethanol. Genomic DNA extraction was performed using a standard phenol-chloroform protocol
119 (Sambrook *et al.* 1989). All DNA samples were quantified in a 1% agarose gel stained with
120 GelRed® (Biotium Inc.) using the LowMass DNA Ladder (Invitrogen) as a concentration standard,
121 and diluted to a final working concentration of 10 ng/ μL.

122

123 ***Mitochondrial sequencing***

124

125 Three different mtDNA fragments were amplified with the polymerase chain reaction
126 (PCR): (i) the 5' portion of the *NADH dehydrogenase subunit 5* (*ND5*) gene using primers
127 described by Trigo *et al.* (2008); (ii) the 5' portion of the control region using the forward primer
128 MTLPRO2 described by Tchaika *et al.* (2007) and the reverse primer LonCR-R2 described by
129 Trinca *et al.* (2007); and (iii) the complete cytochrome *b* (*cyt-b*) gene using primers described by
130 Irwin *et al.* (1991) [L14724, L15162 and H15915] and Koepfli & Wayne (1998) [H15494]. The cyt-
131 b segment was divided in two sub-segments of approximately 750 base pairs (bp) each, with an
132 overlap of nearly 300 bp. PCR reactions were carried out in a PTC-100 thermocycler (MJ Research)
133 in a 20 μL volume, including 1X PCR Buffer (Invitrogen), 0.2 mM of each dNTP, 2 mM $MgCl_2$,
134 0.2 μM of each primer, 0.25 U Platinum® *Taq* Polymerase (Invitrogen), and 10-20 ng of genomic
135 DNA. Thermocycling conditions for the *ND5* and control region segments consisted of an initial
136 denaturing step at 94ºC for 3' followed by 40 cycles of 45'' denaturing at 94ºC, 45'' annealing at
137 65ºC and 1'30'' extension at 72ºC, and a final extension step at 72ºC for 3'. PCR conditions for
138 cytochrome *b* started with an initial denaturing step for 3' at 94ºC, 10 touchdown cycles [45''
139 denaturing at 94ºC, 45'' annealing at 60-51ºC and 1'30'' extension at 72ºC], followed by 30

140 additional cycles with annealing at 50ºC and a final extension at 72ºC for 3'. PCR products were
141 checked in an agarose gel stained with GelRed, purified with PEG8000, and then quantified with a
142 second analysis in an agarose gel. Both strands of each PCR product were sequenced using the
143 DYEnamic ET Dye Terminator Sequencing Kit (GE Healthcare), and analyzed in a MegaBACE
144 1000 automated sequencer (GEHealthcare).
145

146 ***Microsatellite genotyping***

147

148 Eight microsatellite loci were employed for each species. For *N. nasua*, we used the primers
149 developed for this species, previously described by Tsuchiya-Jerep *et al.* (in preparation, see
150 Chapter 1); for *P. cancrivorus*, we employed primers previously described for *P. lotor* by
151 Cullingham *et al.* (2006) [PLO3-71, PLO3-86, PLO3-117, PLO-M3, PLO-M15 and PLO-M17] and
152 by Fike *et al.* (2007) [PLOT-08 and PLOT-10]. All forward primers contained an M13 tail on their
153 5' end (Boutin-Ganache 2001). PCR reactions were performed in a PTC-100 thermocycler (MJ
154 Research) in a 10µL volume, including 1X PCR Buffer (Invitrogen), 0.2 mM of each dNTP, 2 mM
155 $MgCl_2$, 0.2 µM of each the reverse and the fluorescent M13 primer (FAM, NED or HEX), 0.013
156 µM of the forward primer, 0.25 U Platinum® *Taq* Polymerase (Invitrogen), 0.3% Trehalose and 10-
157 20 ng of genomic DNA. Amplification conditions were as follows: initial denaturing step at 94ºC
158 for 3', 10 touchdown cycles [94ºC for 45'', annealing at 65-56ºC (-1ºC/cycle) for 45'' and 72ºC for
159 1.5'], 30 additional cycles with annealing at 55ºC, and a final extension at 72ºC for 30'. PCR
160 products were diluted 1:10, pooled in multiplex panels (Table 3), and genotyped in a
161 MegaBACE1000 automated sequencer (GE Healthcare), using the software Genetic Profiler 2.2
162 and the internal size standard ETRox-550.
163

164 ***mtDNA sequence analysis***

165

166 Forward and reverse sequences were assembled using the Phred/Phrap/Consed software
167 package (Ewing *et al.* 1998; Ewing & Green, 1998; Gordon *et al.* 1998) and consensus sequences
168 were inspected by eye using CHROMAS (Technelysium) and then aligned using the CLUSTAL W
169 algorithm implemented in MEGA 4.0 (Tamura *et al.* 2007). Alignments were manually checked and
170 edited, and only unambiguous sequences were used for analysis.

171

172 For both individual and concatenated mtDNA segments, basic statistics of DNA diversity,
173 including nucleotide (π) and haplotype (*H*d) diversities and neutrality tests (Tajima's *D* [Tajima
174 1989] and Fu's *Fs* [Fu 1997]), were estimated using DNASP (Rozas *et al.* 2003). We also
175 constructed a haplotype network using the median-joining method implemented in the program
176 NETWORK 4.5 (Bandelt *et al.* 1999). All subsequent analyses were performed using the only the
177 concatenated mtDNA dataset. To determine the appropriate model of sequence evolution, the
178 Akaike Information Criterion (AIC) implemented in MODELTEST ver. 3.7 (Posada & Buckley 2004)
179 was used and the selected model was employed in Bayesian inference (BI) and maximum likelihood
180 (ML) phylogenetic reconstruction. Neighbor-joining (NJ) and maximum likelihood trees were
181 estimated using PAUP*4.0b10 (Swofford 1998), maximum parsimony (MP) using WINCLADA
182 (Nixon 2002) and NONA (Goloboff 1999), and Bayesian inference was performed in MRBAYES
183 (Huelsenbeck & Ronquist 2001). For MP, we used the parsimony ratchet method (Nixon 1999) with
184 200 iterations, 5 sequential runs and random reweighting of 10% of characters; statistical
185 confidence was estimated by bootstrap resampling with 1000 replications, using a heuristic search
186 with TBR (tree-bisection-reconnection) branch-swapping. For the ML analyses, optimal
187 phylogenies were inferred with NNI branch-swapping starting from an NJ tree; nodal support was

188 assessed by 100 bootstrap replications using the NNI (nearest-neighbor-interchange) heuristic
189 search option. Bayesian inference was performed using 1,000,000 steps of the Markov Chain Monte
190 Carlo (MCMC) algorithm (with trees sampled every 100 generations), and the posterior
191 probabilities were calculated discarding the initial 100,000 iterations as burn-in, after the
192 stabilization of log-likelihood values. *Nasua narica* and *Procyon lotor* were used as outgroups for
193 all phylogenetic analyses of *N. nasua* and *P. cancrivorus* haplotypes, respectively.
194
195 An Analysis of Molecular Variance (AMOVA) was carried out using ARLEQUIN 3.11
196 (Excoffier *et al.* 2005). To define which geographic subdivision best reflects the genetic structure of
197 each species, several scenarios were tested. Initially, samples were divided into populations,
198 according to their geographical origin, main vegetational domains (based on Josse *et al.* 2003 and
199 www2.ibge.gov.br/downloads/mapa_murais/biomas_pdf.zip and) and phylogeographic information
200 (obtained from mtDNA phylogenies and microsatellite-based analyses performed in this study);
201 sampling points with only one individual were merged with the closest locality, based on
202 geographic and/or phylogenetic information. Using ARLEQUIN, we calculated the overall F_{ST} and
203 the pairwise F_{ST}'s among the populations incorporating a distance matrix (various distance models
204 were explored, including p-distances and the model of sequence evolution defined by MODELTEST,
205 when possible). If the pairwise comparisons found non-significant F_{ST} values for any pair of
206 populations, this specific pair was merged as a joint population. The procedure was then repeated
207 and if the overall F_{ST} value increased, this new configuration was accepted and we verified if there
208 was any other population pair that could be merged. We repeated these steps until all values of
209 pairwise difference became significant and the overall F_{ST} was maximized. If we found more than
210 one population pair with non-significant values of difference, we first united the pair with the
211 highest P value (and thus the smallest F_{ST}). We also performed a hierarchical AMOVA

212 incorporating two levels in the population structure, by testing different schemes based on the
213 single-level scenarios that had led to the highest F_{ST} values. Mismatch distribution analyses
214 (Harpending 1994) were also performed using ARLEQUIN. The correlation between genetic and
215 geographic distances among the sampling sites (and thus the occurrence of isolation by distance)
216 was assessed using a Mantel test (Mantel 1967) with 100,000 permutations in the program ALLELES
217 IN SPACE (AIS) (Miller 2005).
218

219 ***Microsatellite data set***

220

221 Diversity indices, including number of alleles per locus, observed and expected
222 heterozygosities, were calculated using both CERVUS 3.0.3 (Kalinowski *et al.* 2007) and ARLEQUIN;
223 CERVUS was also employed to test for departures from Hardy-Weinberg equilibrium and ARLEQUIN
224 was used to test for Linkage Disequilibrium. Possible genotyping errors and the presence of null
225 alleles were assessed with MICROCHECKER (Van Oosterhout *et al.* 2004).
226

227 To infer the number of populations and to assign individuals to these putative populations,
228 we employed the Bayesian approach implemented in the software STRUCTURE 2.2 (Pritchard *et al.*
229 2000): *K* values ranged from 1 to 10, and each run comprised 500,000 MCMC iterations, after an
230 initial burn-in of 200,000, using an ancestry model that allows for admixture and correlated allele
231 frequencies. Five independent analyses were performed for each *K* value; if the posterior probability
232 values did not show stability among the different runs, we increased two-fold the length of the burn-
233 in and the sampling portion of the MCMC, and ran five additional simulations for that specific *K*.
234 We analyzed the mean posterior probabilities [Ln(P|D)] for each *K*, and accepted the value that
235 provided the best fit to the data (Pritchard *et al.* 2000).

236

237 An AMOVA and related calculations of fixation indices (F_{ST} and R_{ST}) were performed using
238 ARLEQUIN. The population structure to be tested was defined based on the results obtained from the
239 program STRUCTURE and also using the best geographic division found for the *mt*DNA dataset. In
240 addition, Mantel tests were performed using AIS, to assess for the presence of an isolation-by-
241 distance pattern, indicated by a correlation between geographic and genetic distances.

242

243 **Results**

244

245 ***mtDNA dataset***

246

247 We obtained a total of 2,125 bp of sequence for *Nasua nasua* and 2,166 bp for *Procyon*
248 *cancrivorus*. Both species were sequenced for 697 bp of the *ND5* gene and 1140 bp of the *cyt-b*
249 gene; 288 bp and 329 bp of the mtDNA control region (CR) were sequenced for *N. nasua* and *P.*
250 *cancrivorus*, respectively. For *N. nasua*, nucleotide diversity (π) in individual segments varied from
251 0.0175 to 0.0195 and haplotype diversity (*H*d) from 0.783 to 0.868, while *P. cancrivorus* showed
252 nucleotide diversity values varying from 0.00187 to 0.00575 and haplotype diversity ranging from
253 0.762 to 0.832 (Table 4).

254

255 The haplotype networks for the three individual segments are shown in Figure 2, while
256 tables 5-10 provide a detailed description of these mtDNA regions for each species. Although the
257 absolute number of haplotypes for each segment was nearly equal for both *N. nasua* and *P.*
258 *cancrivorus*, there was a large difference in the number of mutational steps presented by each

259 species (Table 4, Figure 2). Another interesting difference was that while *P. cancrivorus* showed no
260 strong evidence of deep geographic structuring in its mtDNA networks, *N. nasua* exhibited a clear
261 genetic structure, with most haplotypes being exclusive to specific geographic regions and/or to
262 major vegetational domains. By comparing the three networks of each species, it was possible to
263 establish some general patterns: for *N. nasua*, individuals from Acre, Pará, Alagoas, Ceará, Goiás
264 and some from Mato Grosso do Sul states each possessed private haplotypes in all segments; MG
265 and ES2 individuals shared a common haplotype for CR, but for *ND5* and *cyt-b* each locality had its
266 own haplotype; and individuals from Rio Grande do Sul, Paraná, São Paulo and Mato Grosso do
267 Sul states (except those mentioned above) shared haplotypes in different combinations, depending
268 on the segment analyzed. The general pattern found for *P. cancrivorus* was the presence of one or
269 two more common haplotypes shared by individuals from different geographic regions. Although
270 some individuals presented haplotypes restricted to specific geographic regions, this pattern was not
271 consistently repeated among the three segments; the only exception were the individuals bPca024
272 and bPca 311 (from Maranhão and Alagoas states, respectively), which shared the same in all
273 segments. When the three mtDNA segments were concatenated (Tables 11 and 12; Figure 3), an
274 improved resolution of the relationships was achieved, and the overall patterns became more solid.
275 For *N. nasua*, as the general network shape was very consistent among the three segments, there
276 was a no significant change in the overall inference. However, for *P. cancrivorus*, some geographic
277 subdivision emerged: private haplotypes were found in the Cerrado and, although still sharing
278 haplotypes with other ecoregions, the Pantanal and Pampas domains seemed to be more
279 differentiated from the remaining populations.
280

281 The transversional model with allowance for a gamma distribution of rate variation among
282 sites and a proportion of invariable sites (TVM+Γ+I) was the selected model of sequence evolution

283 for *N. nasua* (I=0.4666 and α=0.7366). Using MP (parsimony ratchet), twelve best trees with 499
284 steps were found. In general, topology estimates from NJ, MP, ML and BI were very similar
285 (Figure 4): the most prominent patterns were maintained among the reconstruction methods, and the
286 differences were restricted to branches with shallow divergence. The deepest division was found
287 between the clade formed by individuals from Pantanal, Bolivia and Acre (haplotypes Nn-T2 and
288 Cb-12) and all the remaining specimens, followed by a North/ South subdivision. In the northern
289 clade, eastern Amazonia plus Caatinga (Nn-T14, T15, T16, T17 and T18) were separated from the
290 northern Atlantic forest (Nn-T11 and T12). The southern clade comprised three main subdivisions:
291 (i) Nn-T8 (São Paulo state) plus Nn-N7 (Espirito Santo state); (ii) Nn-T9 (Minas Gerais state) plus
292 Nn-T13 (Goiás state) and Nn-T10 (Espirito Santo state); (iii) and the remaining South Atlantic
293 forest and Pantanal haplotypes. The internal branches within this latter clade were weakly supported
294 and were collapsed in ML, MP and NJ analyses, reflecting the shallow divergence among these
295 haplotypes.

296

297 For *P. cancrivorus*, the Tamura-Nei model (Tamura & Nei, 1993) with gamma-distributed
298 (Γ) rates across sites (α=0.3873) provided the best fit for the data set. Using MP analyses, 63 best
299 trees (with 261 mutational steps) were recovered. The four different methods resulted in similar
300 relationships (Figure 5); the majority of clades did not receive strong support and when a strict
301 consensus rule was applied, almost all of them collapsed. Some geographic associations were found
302 but, except for the groupings of Pc-T13 (MA) plus Pc-T14 (AL), and Pc-T1 (RS1) plus Pc-N4
303 (bPca05, RS2), nodal support was weak.

304

305 The AMOVA results are shown in Tables 13 and 14 for *N. nasua* and *P. cancrivorus*,
306 respectively. For *N. nasua*, the results indicated that most of the genetic variability can be explained

307 by the presence of five populations: (i) Brazilian South (RS and PR states) plus São Paulo and Mato
308 Grosso do Sul states, comprising the southern part of the Atlantic Forest and Pantanal domains; (ii)
309 Espírito Santo, Minas Gerais and Goiás states, comprising the central Atlantic Forest and Cerrado
310 domains; (iii) Alagoas state, comprising the northern portion of the Atlantic forest; (iv) Pará and
311 Ceará States, comprising the Eastern Amazonian forest and Caatinga biomes; and (v) Bolivia plus
312 Acre state (Brazil), also composed primarily by Amazonian forest domains. Espirito Santo state can
313 also be separated from Minas Gerais and Goiás states, resulting in six significantly differentiated
314 populations. When only geographic information was considered, even under the highest overall F_{ST}
315 value (0.618) there were still pairs of populations with non-significant pairwise P values; if these
316 populations were merged, the overall F_{ST} decreased (0.582). By coupling geographic and genetic
317 information, the maximum observed F_{ST} value was higher (0.645), and the number of population
318 was set to five. The results of the AMOVA incorporating two levels showed that six populations
319 divided into four groups is the scenario that best reflects the *N. nasua* genetic structure. For *P.*
320 *cancrivorus*, the AMOVA results indicated that the scheme that could best explain its genetic
321 variability was the presence of three populations (Table 14): (i) Mato Grosso and Mato Grosso do
322 Sul states, comprising the Pantanal and Cerrado domains; (ii) São Paulo state, comprising the
323 Cerrado domain; and (iii) the Brazilian southern region plus Espirito Santo State and the northern
324 and northeastern regions (Maranhão, Alagoas, Paraíba and Pará states), comprising Atlantic Forest
325 and Amazonian domains. The AMOVA with two levels was calculated using three different
326 schemes: six populations grouped into four and five groups, and five populations grouped into four
327 groups (Table 13). For all these scenarios, there was an increase in the F_{ST} values; however, the
328 only combination that resulted in significant values of differentiation among groups was the one
329 comprising six populations and four groups (F_{CT} = 0.560). The Mantel test results (Figure 6)
330 indicated the presence of an isolation-by-distance component in the genetic variability of *N. nasua*

331 (r = 0.388, P = 0.000), but not for *P. cancrivorus* (r = -0.002, P = 0.476). The mismatch distribution
332 of pairwise differences (Figure 7) showed contrasting patterns between *N. nasua* and *P.*
333 *cancrivorus*. The former presented a random distribution of pairwise differences, non-significant
334 values of Tajima's *D* (*D* = 0.1358, p = 0.648) but significant negative values of Fu's *Fs*, (Fs = -
335 203675, p = 0.002) while the latter depicted a mismatch distribution pattern associated with
336 populations that have suffered a sudden expansion, which is corroborated by significantly negative
337 values for both Tajima's *D* and Fu's *Fs* tests (*D* = -1.624, p = 0.027; *Fs* = -25.233, p = 0.000).
338

339 ***Microsatellite dataset***

340

341 Tables 15 and 16 show the microsatellite diversity indices for *Nasua nasua* and *Procyon*
342 *cancrivorus*, respectively. The populations were defined based on the Bayesian clustering
343 performed with STRUCTURE (see text below). Although we were not able to employ the same set of
344 microsatellite loci for both species, some comparative observations could be made based on these
345 results. The mean number of alleles per locus was very similar between *N. nasua* and *P.*
346 *cancrivorus* (12 and 10.25, respectively) and the observed heterozygosity was slight higher for *P.*
347 *cancrivorus*, even though the number of individuals sampled for this species was half that used for
348 *N. nasua*. Null alleles were detected in three loci for each species (PLO3-71, PLO-M17 and PLO3-
349 117 for *P. cancrivorus*; for *N. nasua*, each locus was detected in a different population [NnSTR-
350 A08 for the southern population, NnSTR-H07 for Center-west population and NnSTR-D03 for that
351 of the Northeast]). Departures from Hardy-Weinberg Equilibrium (after Bonferroni correction, p =
352 0.00625) were found for two loci (PLO3-117 and PLO3-71) in *P. cancrivorus* and for one locus
353 (NnSTR-D03) for the Center-west population of *N. nasua*. Linkage disequilibrium was found for
354 six pairs of loci in *P. cancrivorus* (PLO3-71 and PLOT-10, PLOT-08 and PLO3-117; PLOT-10 and

355 PLOT-08; PLOT-08 and PLO3-86; PLO-M17 and PLO3-117) and in three locus-population
356 combinations of *N. nasua*: Center-west population (NnSTR-A08 and NnSTR-F02) and northeastern
357 population (NnSTR-B09 and NnSTR-D03; NnSTR-E05 and NnSTR-H07).
358

359 For *Nasua* nasua*, t*he Bayesian clustering analysis performed with STRUCTURE showed that
360 the number of clusters that best explains the data was five [(Ln (P|D) = -1855] (Figures 4 and 8a
361 and b), corresponding to the following subdivision: (i) Rio Grande do Sul, Paraná and São Paulo
362 states representing the southern Atlantic Forest domain, hereafter called “South” (pink); (ii) Minas
363 Gerais, Espirito Santo and Goiás states, corresponding to the central part of the Atlantic forest plus
364 Cerrado and designated “Southeast” (yellow); (iii) Mato Grosso do Sul state, corresponding to the
365 Pantanal domain, named here “Center-west” (blue); (iv) Pará state, comprising the Amazonian
366 forest domain, designated the “North” population (red); (v) and Ceará and Alagoas states,
367 designated the “Northeast” population (green), and comprising the northern part of the Atlantic
368 forest and Caatinga domains. The Mantel test (r = 0.680) (Figure 9) showed a strong and significant
369 (P = 0.000) correlation between geographic and genetic distances. For *P. cancrivorus*, the Bayesian
370 clustering analysis showed no evidence of subdivisions and the best-fitting value of K was one
371 (Figure 8c and d). Remarkably, the result of the Mantel test (r = 0.0387; P = 0.214) further
372 indicated the absence of even an isolation-by-distance pattern of population structure, given the
373 absence of correlation between genetic and geographic distances (Figure 9).
374

375 The AMOVA results (Table 17) for *N. nasua* were very similar in the three schemes tested,
376 with the overall F_{ST} being a little higher when the population subdivision suggested by the
377 STRUCTURE software was applied. Differently, the highest R_{ST} value was found following the
378 population structure recommended by mtDNA analysis. The pairwise comparisons among

379 populations (R_{ST}) (Table 18) showed significant results for all comparisons. The absence of
380 subdivision for *P. cancrivorus* was corroborated by the low and non-significant F_{ST} value (0.035)
381 found when individuals were divided into the three populations defined with the mtDNA dataset,
382 and by the contained pairwise comparisons (R_{ST}, data not shown) which yielded only non-
383 significant P values.

384

385 **Discussion**

386

387 ***Genetic diversity***

388

389 The mtDNA nucleotide diversity indices (π) estimated for *Nasua nasua* was about ten times
390 higher than those found for *Procyon cancrivorus* (except for the control region, which was
391 approximately three times greater for *N. nasua* than for *P. cancrivorus*). The haplotype diversity,
392 meanwhile, was slightly higher for *P. cancrivorus*, considering the three concatenated segments
393 (Table 4). Comparing both species with other carnivores, we found that haplotype diversity was
394 very similar among species, but the levels of nucleotide diversity presented some differences:
395 considering the mtDNA control region, *N. nasua* have diversity indices similar to *Cerdocyon thous*
396 (Tchaika *et al.* 2007), a Neotropical canid, higher than the Neotropical jaguar *Panthera onca*
397 (Eizirik *et al.* 2001) and smaller than *L. pardalis* and *L. wiedii* (Eizirik *et al.* 1998). On the other
398 hand, *P. cancrivorus* have diversity indices more similar to the *cytochrome*-b of *Gulo gulo*
399 (Tomasik & Cook 2005) and smaller than the mtDNA control region of the Neotropical otter *Lontra*
400 *longicaudis* (Trinca *et al.* 2007). Comparing the levels of diversity of *N. nasua* and *N. narica* (17
401 individuals from Belize, Panama, Mexico and United States) for the mtDNA control region, we

402 found that *N. narica* presented higher haplotype diversity (*H*d = 0.888), but smaller nucleotide
403 diversity (π = 0.0092) than *N. nasua* (MacFadden 2004). Comparing *P. cancrivorus and P. lotor*
404 (308 individuals, across the United States) also for the mtDNA control region, we found that both
405 diversity indices were higher for *P. lotor* (π = 0.013 and *H*d = 0.945) (Cullingham 2007).
406 Comparing the three segments at the intra-specific level, the most diverse segment for *N. nasua* was
407 *ND5*, providing the better resolution in the network trees. For *P. cancrivorus*, the CR was the
408 segment with the highest diversity; however, the resolution provided by it was not better than that
409 of the other fragments.
410
411 According to Grant & Bowen (1998), a high haplotype diversity coupled with low levels of
412 nucleotide diversity, such as the results found for *P. cancrivorus*, indicate a signal of demographic
413 expansion following a period of low population size. In the case of *P. cancrivorus*, this inference is
414 also supported by the significantly negative results of Tajima's *D* and Fu's *Fs* tests, along with the
415 shape of its mtDNA mismatch distribution (Figure 7). In contrast, high levels of both nucleotide and
416 haplotype diversities may indicate (i) secondary contact between previously differentiated lineages
417 or (ii) a history of large stable population size (Grant & Bowen 1998). In the case of *N. nasua*, both
418 causes are compatible with our data, since (i) there was strong evidence for population
419 differentiation more than one population – actually, the AMOVA results indicated five populations
420 significantly differentiated; (ii) there was no evidence that these populations have suffered from
421 population decline. However, to assure these statements, we have to increase our sampling
422
423 The observed heterozygosity (*H*o) found using microsatellite markers were very similar for
424 both species, although we have used a different set of loci for each of them. *Procyon cancrivorus*
425 showed heterozygosity levels similar to those found for *P. lotor* (Cullingham 2007), comparing six

426 loci used in common, and both *N. nasua* and *P. cancrivorus* exhibited levels of *H*o that were higher
427 than those of *Potos flavus* (Kays *et al.* 2000) and *L. longicaudis* (Trinca 2007).
428
429 Comparing the Mantel tests results between *N. nasua* and *P. cancrivorus* (Figures 6 and 9),
430 it became clear that there was a strong correlation between geographic and genetic distances for *N.*
431 *nasua* but not for *P. cancrivorus*. Comparing the two *N. nasua* graphs, the one depicting the
432 correlation for microsatellites showed a more homogeneous pattern, while we can note the presence
433 of three "classes" of correlated distances for mtDNA: (i) individuals with geographic distances
434 ranging from zero up to 2000 km and low genetic differentiation (bottom); (ii) intermediate levels
435 of genetic difference and geographic distance ranging from 500 up to 3200 km (which is the
436 maximum) (middle); (iii) highest levels of genetic differentiation and geographic distances ranging
437 from zero up to 2500 km. This latter class comprised comparisons involving some individuals from
438 the Pantanal and Bolivian Chaco domains (haplotypes Nn-Cb9, N2, CR2 and T2), which also
439 formed the most basal clade found in BI, MP, ML and NJ trees (Figure 4). It means that, in the
440 same area (geographic distance nearly equals to zero), we can found very divergent mtDNA
441 haplotypes, what resulted in a less pronounced r-value ofr Mantel test. When these individuals were
442 excluded from the mtDNA analysis, the differences among the populations become more
443 noticeable: the *r*-value increases from 0.388 to 0.757 and the F_{ST} goes from 0.645 to 0.760 (data not
444 shown).
445

446 ***Nasua nasua* versus *Procyon cancrivorus***

447

448 *Nasua nasua* exhibited a highly structured pattern of genetic diversity for both types of
449 markers. This species seems to have maintained an overall large population size for a long time, and

450 in general, the relationships among populations were well-supported by the phylogenetic analyses
451 (Figure 4). On the other hand, *P. cancrivorus* presented low levels of population differentiation (or
452 even none, considering the microsatellites); it showed signs of a recent expansion in population size
453 and the phylogenetic relationships among clades were shallow and weakly supported (Figure 5).
454
455 The overall phylogeographic partitions found for *N. nasua* (considering both markers) were
456 the following: (i) Eastern Amazonia; (ii) Northern Atlantic forest (iii) Central Atlantic forest (iv)
457 Southern Atlantic forest (v) Pantanal; (vi) Bolivian Chaco plus Western Amazonia (only for
458 mtDNA). However, depending on marker considered, there were some changes in this general
459 pattern: the Caatinga population seemed to be more closely related to eastern Amazonia based on
460 the mtDNA data, but was more associated with the Northern Atlantic forest with microsatellites.
461 The Pantanal domain was inferred to be a distinct population with the microsatellite analyses, but it
462 was not distinguishable from the southern Atlantic forest considering only the mtDNA. On the other
463 hand, the Central Atlantic forest, which was subdivided into two populations based on the mtDNA,
464 was considered to be a single population with the microsatellite data (Figure 4). The Bolivian Chaco
465 plus Acre clade (which also includes some individuals from the Pantanal domain) was the most
466 basal mtDNA lineage found for *N. nasua* in this study. A similar result was found by Eizirik *et al.*
467 (1998) for the Neotropical cat *Leopardus wiedii*, in which the most basal lineage for this species in
468 South America was also found in Bolivia. Trinca (2006) found a distinct mtDNA lineage for the
469 Neotropical otter, *L. longicaudis*, in Bolivia, and Costa *et al.* (2000) recognized in this region a
470 center of endemism. The paleoenvironmental changes that took place in this area since the
471 Oligocene might be the cause of its distinctiveness (Sempere *et al.* 1990; Delsuc *et al.* 2004), and
472 warrant additional efforts in terms of further characterization of phylogeographic patterns in
473 multiple species.

474

475 The second major partition found for *N. nasua* was between northern (including the "North"
476 and "Northeast" regions) and southern (including the "Center-West", "Southeast" and "South"
477 regions) Brazil, although these regions did not correspond to a single population each (Figure 4).
478 This North-South subdivision is in agreement with the pattern found by Tchaicka *et al.* (2007) for
479 the crab-eating fox (*Cerdocyon thous*), a Neotropical canid sympatric with *N. nasua* in most of its
480 range. The Northern Brazilian clade is subdivided into eastern Amazonia and northern Atlantic
481 forest, with the Caatinga domain being more related to the former with mtDNA and to the latter
482 with microsatellites. The origin of the Caatinga vegetation is still the subject of much debate, and
483 this domain is considered to be highly related to both the Amazonian and Atlantic forest biomes
484 (Borges-Nojosa & Caramaschi 2003; Prado 2003). The observed pattern may possibly indicate a
485 more effective historical connection with eastern Amazonia, but a more recent one with the Atlantic
486 forest.

487

488 The southern clade is divided into Central Atlantic forest/Cerrado and Southern Atlantic
489 forest/Pantanal clades based on the mtDNA; for microsatellites, the Pantanal domain was inferred to
490 be a distinct population from the southern Atlantic forest. Considering that microsatellite markers
491 are able to recover more recent events due to their higher mutation rates in comparison to the
492 mtDNA (Goldstein & Schlötterer 1999; Brown *et al.* 1979), this subdivision may possibly reflect a
493 more recent fragmentation of the Atlantic forest, which interrupted the gene flow between these two
494 once contiguous domains. Another point relative to the Pantanal domain is that it seems to be an
495 area of secondary contact between two very divergent mtDNA lineages (Figure 4, circles blue and
496 green). The microsatellite results also supported this hypothesis, once this set of markers recognized
497 only one population in that area.

498

499 For *Procyon* cancrivorus, the mtDNA and microsatellite markers showed different patterns:
500 the mtDNA analyses identified three significantly differentiated populations, but the Bayesian
501 clustering approach applied to the microsatellite data indicated only one panmictic population. The
502 three phylogroups identified with mtDNA (Table 14) correspond to different habitats: (i) forests
503 [Amazonian and Atlantic forests]; (ii) Cerrado; (iii) Pantanal + Cerrado. To explain the contrasting
504 patterns between mtDNA and microsatellites, we can infer that females tend to be more philopatric
505 (leading to more structured patterns of mtDNA diversity) and the males are responsible for
506 mediation of gene flow among populations, explaining the absence of subdivision observed with the
507 biparentally inherited nuclear markers.

508

509 *Nasua nasua* and *Procyon cancrivorus* are sympatric species distributed along a broad
510 range; each has its own sister-species in the northern hemisphere, and both intrageneric splits were
511 dated to before the complete closure of the Panamanian land bridge (Koepfli *et al.* 2007). However,
512 their recent evolutionary history in South America seems to be very contrasting given the marked
513 differences in their genetic structure, which cannot not be explained only by their current range,
514 habitat and food preferences. An interesting avenue for future research is an investigation of
515 whether these contrasting histories may be caused by differences in social structure and dispersal
516 patterns in these species, which in turn might influence their response to common climatic and
517 vegetational shifts in their pasts. Future analyses targeting this question would be important to shed
518 light on the underlying processes shaping these different genetic structures.

519

520 ***Implications for conservation***

521

522 Considering Moritz`s genetic criterion for recognizing 'Evolutionarily Significant Units'
523 (ESUs), which assumes that "ESUs should be reciprocally monophyletic for mtDNA alleles and
524 show significant divergence of allele frequencies at nuclear loci" (Moritz 1994), we advocate that
525 each of the six mtDNA lineages found for *Nasua nasua* should be treated as a distinct ESU, being
526 conserved and managed as a separate entity. Moreover, five of these phylogroups are also correlated
527 to *N. nasua* subspecies previously described: *N. n. nasua* (in northeastern Brazil – maybe Caatinga
528 and northern Atlatic forest); *N. n. solitaria* (in central Atlantic forest); *N. n. spadicea* (in southern
529 Atlantic forest); *N. n. dorsalis* (in eastern Amazonia); *N. n. boliviensis* (In Bolivian Chaco).
530 However, further work is required to understand the magnitude and causes of this marked genetic
531 partitioning, including an assessment of morphological and ecological features, as well as an effort
532 to map the boundaries of these identified units. For *P. cancrivorus*, we did not identify major
533 evolutionary lineages, and according to this, it may be treated as a single population throughout the
534 sampled areas. However, additional work is still required to assess whether adaptive differences
535 might occur among biomes or regional populations, even though a recent history of expansion and
536 recurrent gene flow seem to homogenizing the genetic composition of this species across broad
537 geographic regions.
538

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714

715 **Acknowledgements**

716

717 The authors would like to thank all the institutions and people listed in Tables 1 and 2, who
718 generously provided the biological samples. We also thank Cladinara Sarturi for all laboratory
719 assistance and all laboratory colleagues for support at various stages of this research and data
720 analysis. We are also grateful to Centro Nacional de Pesquisas para a Conservação de Predadores
721 Naturais – CENAP/IBAMA, Instituto Pró-Carnívoros and CNPq for having supported this project.

722 **Figure Legends**

723

724 **Figure 1** Map depicting currently the recognized range for *Nasua nasua* (according to Patterson *et*
725 *al.* 2007), identification of sampling locales (see Table 1 for details) and main vegetational domains
726 (see legend).

727

728 **Figure 2** Map depicting currently the recognized range for *Procyon cancrivorus* (according to
729 Patterson *et al.* 2007), identification of sampling locales (see Table 2 for details) and main
730 vegetational domains (see legend).

731

732 **Figure 3** Haplotype network for *Nasua nasua* and *Procyon cancrivorus* mitochondrial segments. A,
733 *N. nasua cytochrome b* gene; B, *N. nasua ND5* gene; C, *N. nasua* control region; D, *P. cancrivorus*
734 *cytochrome b* gene; E, *P. cancrivorus ND5* gene; F, *P. cancrivorus* control region. The number of
735 differences among the haplotypes is represented by lines across branches, each one corresponding
736 to one mutation.

737

738 **Figure 4** Haplotype network for *N. nasua* (left) and *P. cancrivorus* (right) based on the
739 concatenated mitochondrial data set.

740

741 **Figure 5** Bayesian phylogram depicting the intra-specific relationships for *Nasua nasua*. Values
742 above branches indicate, from top to bottom, NJ, MP, ML and BI support for the adjacent node (see
743 text for details). Labels are haplotype identification numbers (see Tables 5, 6, 7 and 11). Dotted
744 lines indicate the populations identified using microsatellite markers. For mtDNA, Mato Grosso do
745 Sul (Pantanal) and São Paulo (south Atlantic forest) states possesses individuals which belongs to

746 different haplotype groups. For microsatellites, only one individual from São Paulo state is more
747 related to central Atlantic forest group, instead of the south Atlantic forest one.
748

749 **Figure 6** Bayesian phylogram depicting the intra-specific relationships for *P. cancrivorus*. Values
750 above branches indicate, from top to bottom, NJ, MP, ML and BI support for the adjacent node (see
751 text for details). Labels indicate haplotype identification numbers (see Tables 8, 9, 10 and 12).
752

753 **Figure 7** Graphs depicting the correlation between genetic and geographic distances for *N. nasua*
754 (top) and *P. cancrivorus* (bottom), using the concatenated mitochondrial data set.
755

756 **Figure 8** Observed and expected mismatch distributions for *N. nasua* (top) and *P. cancrivorus*
757 (bottom) using the concatenated mtDNA data set.
758

759 **Figure 9** Barplots (proportion of individual assignment to each population cluster) and graphs
760 depicting the variation in likelihood as a function of the number of assumed populations (k) based
761 on the Bayesian analysis performed with STRUCTURE for *N. nasua* (A and B) *P. cancrivorus* (C and
762 D). In **A**, each color represents one geographic region (see text for details): red = "North"; green =
763 "Northeast"; yellow = "Southeast"; blue = "Center-west"; pink = "South". In C, a barplot assuming
764 two population units is shown, so as to demonstrate the even allocation of all individuals to both
765 populations.
766

767 **Figure 10** Graphs depicting the correlation between genetic and geographic distances for *N. nasua*
768 (top) and *P. cancrivorus* (bottom), using the microsatellite data set.
769

Table 1 Brown-nosed coati *(Nasua nasua)* samples analyzed in this study.

Ecoregion	Geographic Origin (Sampling site)	Samples	Institution/ contact
Southern Atlantic Forest	P. N. Iguaçu, Paraná State (PR) S Brazil	bNna02*	Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS1) S Brazil	bNna03*	Vanessa Fortes, Everton Behr and Marilise Krügel
	Rio Grande do Sul State (RS2) S Brazil	bNna04*, bNna05*, bNna06*	Júlio César Menezes de Sá
	Rio Grande do Sul State (RS3) S Brazil	bNna61†,‡,§	Felipe Peters
	São Paulo State (SP) S Brazil	bNnaSPA*, bNnaSPB*, bNnaSPC*, bNnaSPD*, bNnaSPE* bNnaSPF†,¶,‡,§	Ligia Motta
	P. N. Iguazu, Argentina (AR)	bNnaARG1‡, bNnaARG2†,¶,‡, bNnaARG3†,¶,‡, bNnaARG4‡, bNnaARG5†,¶,‡, bNnaARG6†,¶,‡, bNnaARG7†,¶,‡, bNnaARG8†,¶,‡	Ben Hirsch
Central Atlantic Forest	Espírito Santo State (ES1) SE Brazil	bNna14£,†,‡,§	Rodosol/ Andreas Kierbusch
	Espírito Santo State (ES2) SE Brazil	bNna302*, bNna303*, bNna304*, bNna305*	CENAP-IBAMA
	Minas Gerais State (MG) SE Brazil	bNna51*, bNna52*, bNna53*, bNna54*, bNna55*, bNna56*, bNna57*, bNna58*, bNna59*, bNna60*	Nadja Hemétrio and Fabrício Rodrigues dos Santos
Northern Atlantic Forest	Alagoas State (AL1) NE Brazil	bNna307*, bNna308*, bNna309*, bNna311*	CENAP - IBAMA
	Alagoas State (AL2) NE Brazil	bNna310*	CENAP – IBAMA
Cerrado	P. N. Emas Goiás State (GO) Central Brazil	bNna01*	Museu Nacional
Pantanal	Mato Grosso do Sul State (MS) SW Brasil	bNna07*, bNna08*, bNna09£,¶,‡,§, bNna10*, bNna11*, bNna12*, bNna13†,¶,‡, bNna16*, bNna17*, bNna18*, bNna19£,†,‡,§, bNna20‡,§, bNna22£,†,‡,§, Nna023£,†,¶,§, bNna24*, bNna25£,†,‡,§, bNna26£,†,¶,§, bNna27£,¶,‡,§, bNna28£,¶,§, bNna29*, bNna30£,†,¶,§, bNna31*, bNna32£,†,¶,§, bNna33*, bNna34£,†,¶,§, bNna35£,†,¶,§, bNna36†,¶,‡,§, bNna37£,†,¶,§, bNna38£,†,¶,§, bNna39£,†,¶,§, bNna40£,†,¶,§, bNna41£,†,¶,§, bNna42*, bNna43*, bNna44£,†,¶,§, bNna45£,†,¶,§, bNna46£,†,¶,§, bNna47£,†,¶,§, bNna48£,†,¶,§, bNna49£,†,¶,§,	Guilherme Mourão, Rita de Cássia Bianchi, Fabiana Rocha and Natalie Olifers
Caatinga	Ceará State (CE) NE Brazil	bNna21*	Marco Renato Mattos
Eastern Amazônia	Pará State (PA) N Brazil	bNnaPAA*, bNnaPAB*, bNnaPAC*, bNnaPAD*, bNnaPAE*, bNnaPAF*, bNnaPAG*, bNnaPAH*	Ligia Motta
Western Amazônia	Acre State (AC) N Brazil	bNnaAC †,¶,‡	Museum of Vertebrate Zoology (MVZ195089)
Bolivian Chaco	San Rámon, Santa Cruz Bolivia	bNnaBol †,¶,‡	Museum of Southwestern Biology (MSB12987)
Outgroup *Nasua narica*	Barro Colorado Island Panama	bNnr07 †,¶,‡	UCLA

* samples typed for the three mtDNA segments and microsatellites

£ samples typed for the mtDNA control region

† samples typed for the first segment of the cytochrome *b* gene

¶ samples typed for the second segment of the cytochrome *b* gene

‡ samples typed for the *ND5* gene

§ samples typed for microsatellites

Table 2 Crab-eating raccoon (*Procyon cancrivorus)* samples analyzed in the present study.

Ecoregion	Geographic Origin (Sampling site)	Samples	Institution/ contact
Pampas (Southern Grasslands)	Rio Grande do Sul State (RS1) S Brazil	bPca07*	Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS2) S Brazil	bPca09*	Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS3) S Brazil	bPca15*	Paulo Chaves Barcelos
	Rio Grande do Sul State (RS4) S Brazil	bPca16*	Marcus Lisenfield and Rodrigo Magalhães
	Rio Grande do Sul State (RS5) S Brazil	bPca17*	Fundação Zoobotânica
	Rio Grande do Sul State (RS6) S Brazil	bPca29*	Carlos Benhur Kasper
	Rio Grande do Sul State (RS7) S Brazil	bPca33£,‡,§	Felipe Peters
Southern Atlantic Forest	Rio Grande do Sul State (RS8) S Brazil	bPca01£,‡,§	Joceleia Koenemann
	Rio Grande do Sul State (RS9) S Brazil	bPca02*	Thales Freitas and Juliana Silva
	Rio Grande do Sul State (RS10) S Brazil	bPca03*, bPca05*	Carla Kotzian, Alberto Senra and Diego Hoffmann; Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS11) S Brazil	bPca04*	Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS12) S Brazil	bPca06*	Instituto Pró-Carnívoros
	Rio Grande do Sul State (RS13) S Brazil	bPca31*	Carlos Benhur Kasper and Marina Piccoli
	Rio Grande do Sul State (RS14) S Brazil	bPca34*	Felipe Peters
	Santa Catarina State (SC1) S Brazil	bPca22§	Fernanda Trierveiler
	Santa Catarina State (SC2) S Brazil	bPca25*	Felipe Grazziotin, Adrian Garda
	Paraná State (PR) S Brazil	bPca26*	Felipe Grazziotin and Adrian Garda
Central Atlantic Forest	Minas Gerais State (MG) SE Brazil	bPca32£,§	Fernando Jerep, Tiago Carvalho and Christian Cramer
	Espirito Santo State (ES1) SE Brazil	bPca19*	Rodosol/ Andreas Kierbusch
	Espirito Santo State (ES2) SE Brazil	bPca18*	Rodosol/ Andreas Kierbusch

Table 2 *Continued.*

Central Atlantic Forest (continued)	Espirito Santo State (ES3) SE Brazil	bPca20*	Rodosol/ Andreas Kierbusch
	Espirito Santo State (ES4) SE Brazil	bPca21*	Rodosol/ Andreas Kierbusch
Northern Atlantic Forest	Paraíba State (PB) NE Brazil	bPca308*	CENAP-IBAMA
	Alagoas State (AL) NE Brazil	bPca311*, bPca312*	CENAP-IBAMA
Cerrado	São Paulo State (SP1) SE Brazil	bPca301*, bPca302*	CENAP-IBAMA
	São Paulo State (SP2) SE Brazil	bPca303*	CENAP-IBAMA
	São Paulo State (SP3) SE Brazil	bPca14*	Juliana Griese
	Mato Grosso do Sul State (MS1) SW Brazil	bPca27*	Felipe Grazziotin and Adrian Garda
Pantanal	Mato Grosso do Sul State (MS2) SW Brazil	bPca28*	Rita Bianchi
	Mato Grosso do Sul State (MS3) Central Brazil	bPca35£,‡,§	Guilherme Mourão and Fabiana Rocha
	Mato Grosso State (MT1) SW Brazil	bPca10*, bPca12*	Instituto Pró-Carnívoros
	Mato Grosso State (MT2) SW Brazil	bPca13*	Instituto Pró-Carnívoros
	Mato Grosso State (MT3) SW Brazil	bPca11*, bPca307£,¶,‡,§	Instituto Pró-Carnívoros
	Mato Grosso State (MT4) SW Brazil	304*, bPca305*, bPca306*, bPca307£,¶,‡,§, bPca309£,¶,‡,§	Instituto Pró-Carnívoros
Eastern Amazônia	Maranhão State (MA) NE Brazil	bPca24*	Tadeu Gomes de Oliveira
	Para State (PA) N Brazil	bPca23*	Tadeu Gomes de Oliveira
Outgroup *Procyon lotor*	Genbank	Plo9126 Plo7804	Accession numbers: NC009126 AB297804

* samples typed for the three mtDNA segments and microsatellites

£ samples typed for the mtDNA control region

† samples typed for the first segment of the cytochrome *b* gene

¶ samples typed for the second segment of the cytochrome *b* gene

‡ samples typed for the *ND5* gene

§ samples typed for microsatellites.

Table 3 Microsatellite loci used in this study, including the genotyping multiplex panels employed for *Nasua nasua* and *Procyon cancrivorus*.

	Multiplex panel	Microsatellite Loci	Dye	Repeat size (jn bp)
Nasua nasua				
	N1	NnSTR-D03	FAM	2
		NnSTR-E05	FAM	2
		NnSTR-H03	HEX	2
		NnSTR-H07	NED	2
	N2	NnSTR-A08	FAM	2
		NnSTR-B09	HEX	2
		NnSTR-F02	NED	2
		NnSTR-F03	NED	2
Procyon cancrivorus				
	P1	PLO3-71	NED	4
		PLO-M15	FAM	4
		PLOT-10	HEX	4
		PLO-M3	NED	4
		PLOT-08	HEX	4
	P2	PLO-M17	FAM	4
		PLO3-86*	HEX	2
		PLO3-117*	NED	2

* Loci originally described as containing tetranucleotide repeats.

Table 4 Mitochondrial DNA diversity estimates for *Nasua nasua* and *Procyon cancrivorus* using segments of *ND5* and *cytochrome b* genes and the control region.

Species	Segments	*L*	*N*	*h*	*V*	*S*	*P*	π (SD)	k	*H*d (SD)
Nasua nasua	*ND5*	697 (679)	71	16	86	84	60	0.01949 (± 0.00001)	13.2350	0.864 (± 0.0005)
	Cytochrome b	1140 (1090)	80	13	119	106	96	0.01771 (± 0.00253)	19.3082	0.783 (± 0.00153)
	CR	288 (287)	77	15	24	24	20	0.01748 (± 0.00182)	5.0161	0.790 (± 0.00133)
	Concatenated*	2125 (2107)	50	18	197	189	174	0.02080 (± 0.00288)	43.8351	0.909 (± 0.00044)
Procyon cancrivorus	*ND5*	697 (686)	42	12	14	14	9	0.00239 (± 0.00060)	1.6376	0.829 (± 0.00212)
	Cytochrome b	1140 (1138)	34	10	10	10	8	0.00187 (± 0.00014)	2.1244	0.832 (± 0.00161)
	CR	329 (323)	43	11	13	13	8	0.00575 (± 0.00035)	1.8560	0.762 (± 0.00181)
	Concatenated*	2166 (2148)	34	20	34	39	19	0.00272 (± 0.00026)	5.8396	0.938 (± 0.00071)

* Only samples without missing data were used for this analysis.

L, sequence length; numbers in parentheses indicate the segments lengths after exclusion of all sites containing gaps or missing information

N, number of sequences

h, number of haplotypes

V, variable sites

S, segregating sites

P, parsimony-informative sites

π, nucleotide diversity

k, average number of nucleotide differences

*H*d, haplotype diversity

SD, standard deviation

Table 5 List of haplotypes of the *cytochrome b* gene for *Nasua nasua*, including the individuals that bear each haplotype, the absolute frequency in total sample (Fr) and geographic distribution of each haplotype.

Haplotype	Individuals	Fr	Ecoregion
Nn-Cb1	bNna02,03,06,07,08,10,011,012,16,23,26,29,30,31,32,34,35,36, 37,39,40,41,43,44,45,46,47,48,49,SPA,SPB,SPD,SPE,SPF	34	Southern Atlantic Forest, Pantanal
Nn-Cb2	bNna04,05	2	Southern Atlantic Forest
Nn-Cb3	bNnaSPC	1	Southern Atlantic Forest
Nn-Cb4	bNnaARG2,ARG3,ARG5,ARG6,ARG7,ARG8	6	Southern Atlantic Forest
Nn-Cb5	bNna302,303,304,305	4	Central Atlantic Forest
Nn-Cb6	bNna01,51,52,53,54,55,56,57,58,59,60	11	Central Atlantic Forest, Cerrado
Nn-Cb7	bNna307,308,309,311	5	Northern Atlantic Forest
Nn-Cb8	bNna310	1	Northern Atlantic Forest
Nn-Cb9	bNnaBol,17,18,24,33,38,42	7	Pantanal, Bolivian Chaco
Nn-Cb10	bNnaPAA,PAB,PAC,PAE,PAF,PAG,PAH	7	Eastern Amazônia
Nn-Cb11	bNnaPAD	1	Eastern Amazônia
Nn-Cb12	bNnaAC	1	Western Atlantic Forest
Nn-Cb13	bNna21	1	Caatinga
Nn-Cb14*	bNna014		

* Samples that possess distinct haplotypes when we consider only the second part of the cytochrome b gene (not show in the network).

Table 6 List of the *ND5* haplotypes for *Nasua nasua*, including the individuals that bear each haplotype, the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype.

Haplotype	Individuals	Fr	Ecoregion
Nn-N1	bNna07,08,09,10,11,12,13,16,19,20,22,25,27,29,31,36,43	17	Pantanal
Nn-N2	bNna17,18,24,33,42,Bol	6	Pantanal, Bolivian Chaco
Nn-N3	bNna02,03,04,06,SPA,SPD,SPE,SPF,ARG1,ARG2,ARG3,ARG4,ARG5, ARG6,ARG7,ARG8	16	Southern Atlantic Forest
Nn-N4	bNna05	1	Southern Atlantic Forest
Nn-N5	bNna61	1	Southern Atlantic Forest
Nn-N6	bNnaSPC	1	Southern Atlantic Forest
Nn-N7	bNna14	1	Central Atlantic Forest
Nn-N8	bNna51,52,53,54,55,56,57,58,59,60	10	Central Atlantic Forest
Nn-N9	bNna302,303,304,305	4	Central Atlantic Forest
Nn-N10	bNna01	1	Cerrado
Nn-N11	bNna307 ,308,309,310,311	5	Northern Atlantic Forest
Nn-N12	bNnaPAB ,PAC,PAG,PAH	4	Eastern Amazonia
Nn-N13	bNnaPAD	1	Eastern Amazonia
Nn-N14	bNnaPAE	1	Eastern Amazonia
Nn-N15	bNna21	1	Caatinga
Nn-N16	bNnaAC	1	Western Amazonia

Table 7 List of haplotypes of the mitochondrial DNA control region for *Nasua nasua*, including the individuals that bear each haplotype, the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype

Haplotype	Individuals	Fr	Ecorregion
Nn-CR1	bNna04,05,07,08,09,10,11,12,16,19,22,23,25,26,27,29,30,31,32, 34,35,37,39,40,43,44,45,46,47,48,49	21	Southern Atlantic Forest, Pantanal
Nn-CR2	bNna17,18,24,28,33,38,42	7	Pantanal
Nn-CR3	bNna41	1	Pantanal
Nn-CR4	bNna02,SPA ,SPB,SPC,SPD,SPE	6	Southern Atlantic Forest
Nn-CR5	bNna03	1	Southern Atlantic Forest
Nn-CR6	bNna06	1	Southern Atlantic Forest
Nn-CR7	bNna14	1	Central Atlantic Forest
Nn-CR8	bNna51,52,53,54,55,56,57,58,59,60,302,303,304,305	14	Central Atlantic Forest
Nn-CR9	bNna307,308,309,311	4	Northern Atlantic Forest
Nn-CR10	bNna310	1	Northern Atlantic Forest
Nn-CR11	bNnaPAA,PAC,PAE,PAF,PAG,PAH	6	Eastern Amazonia
Nn-CR12	bNnaPAB	1	Eastern Amazônia
Nn-CR13	bNnaPAD	1	Eastern Amazônia
Nn-CR14	bNna01	1	Cerrado
Nn-CR15	bNna21	1	Caatinga

Table 8 List of *cytochrome b* haplotypes for *Procyon cancrivorus*, including the individuals that bear each haplotype, along with the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype

Haplotype	Individuals	Fr	Ecoregion
Pc-Cb1	bPca04,07,09,15,16,17,23,29,34	10	Eastern Amazonia, Southern Atlantic Forest, Pampas
Pc-Cb2	bPca02,03,06,10,25,28,304,306,307,308	10	Southern Atlantic Forest, Northern Atlantic Forest, Pantanal
Pc-Cb3	bPca05,26	1	Southern Atlantic Forest
Pc-Cb4	bPca12,13	2	Pantanal
Pc-Cb5	bPca305	1	Pantanal
Pc-Cb6	bPca14,21,301,312	4	Central Atlantic Forest, Northern Atlantic Forest, Cerrado
Pc-Cb7	bPca19	1	Central Atlantic Forest
Pc-Cb8	bPca24,311	2	Northern Atlantic Forest, Eastern Amazonia
Pc-Cb9	bPca31	1	Southern Atlantic Forest
Pc-Cb10	bPca302,303	2	Cerrado
Pc-Cb11*	bPca27	1	Cerrado
Pc-Cb12*	bPca309	1	Pantanal

* Samples that possess distinct haplotypes when only the second segment of cytochrome b were considered (not show in the network).

Table 9 List of the *ND5* haplotypes for *Procyon cancrivorus*, including the individuals that bear each haplotype, the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype

Haplotype	Individuals	Fr	Ecorregion
Pc-N1	bPca02,03,06,11,12,13,18,25,27,28,33,304,305,309,308	15	Southern Atlantic Forest, Central Atlantic Forest, Northern Atlantic Forest, Pantanal, Pampas
Pc-N2	bPca04,07,15,16,23,29,34	7	Southern Atlantic Forest, Pampas, Eastern Amazonia
Pc-N3	bPca05	1	Southern Atlantic Forest
Pc-N4	bPca01	1	Southern Atlantic Forest
Pc-N5	bPca09	1	Pampas
Pc-N6	bPca26	1	Southern Atlantic Forest
Pc-N7	bPca17,35	2	Pampas, Pantanal
Pc-N8	bPca14,21,301,302,303,312	6	Central Atlantic Forest, Northern Atlantic Forest, Cerrado
Pc-N9	bPca19,306,307	3	Central Atlantic Forest, Pantanal
Pc-N10	bPca20,31	2	Central Atlantic Forest, Southern Atlantic Forest
Pc-N11	bPca10	1	Pantanal
Pc-N12	bPca24,311	2	Eastern Amazonia, Northern Atlantic Forest

Table 10 List of haplotypes of the mtDNA control region for *Procyon cancrivorus*, including the individuals that bear each haplotype, the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype

Haplotype	Individuals	Fr	Ecorregion
Pc-CR1	bPca04,07,09,15,16,17,18,19,21,23,34,302,303,312	14	Southern Atlantic Forest, Pampas, Central Atlantic Forest, Northern Atlantic Forest, Eastern Amazonia, Cerrado
Pc-CR2	bPca03,06,10,11,12,13,25,27,28,302,304,305,306, 307,308,309	16	Southern Atlantic Forest, Northern Atlantic Forest, Pantanal, Cerrado
Pc-CR3	bPca01,05	2	Southern Atlantic Forest
Pc-CR4	bPca26	1	Southern Atlantic Forest
Pc-CR5	bPca29	1	Pampas
Pc-CR6	bPca33	1	Pampas
Pc-CR7	bPca20,31	2	Central Atlantic Forest, Southern Atlantic Forest
Pc-CR8	bPca32	1	Central Atlantic Forest
Pc-CR9	bPca24,311	2	Northern Atlantic Forest, Eastern Amazonia
Pc-CR10	bPca35	1	Pantanal
Pc-CR11	bPca14,301	2	Cerrado

Table 11 List of haplotypes identified in the concatenated mtDNA data set for *Nasua nasua*, including the individuals that bear each haplotype, the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype

Haplotype	Individuals	Fr	Ecorregion
Nn-T1	bNna07,08,10,11,12,16,29,31,43	9	Pantanal
Nn-T2	bNna17,18,24,33,42	5	Pantanal
Nn-T3	bNna02,SPA ,SPD,SPE	4	Southern Atlantic Forest
Nn-T4	bNna03	1	Southern Atlantic Forest
Nn-T5	bNna04	1	Southern Atlantic Forest
Nn-T6	bNna05	1	Southern Atlantic Forest
Nn-T7	bNna06	1	Southern Atlantic Forest
Nn-T8	bNnaSPC	1	Southern Atlantic Forest
Nn-T9	bNna51,52,53,54,55,56,57,58,59,60	10	Central Atlantic Forest
Nn-T10	bNna302,303,304,305	4	Central Atlantic Forest
Nn-T11	bNna307,308,309,311	4	Northern Atlantic Forest
Nn-T12	bNna310	1	Northern Atlantic Forest
Nn-T13	bNna01	1	Cerrado
Nn-T14	bNnaPAB	1	Eastern Amazônia
Nn-T15	bNnaPAC ,PAG,PAH	3	Eastern Amazônia
Nn-T16	bNnaPAD	1	Eastern Amazônia
Nn-T17	bNnaPAE	1	Eastern Amazônia
Nn-T18	bNna21	1	Caatinga

Table 12 List of haplotypes identified in the concatenated mtDNA data set for *Procyon cancrivorus*, including the individuals that bear each haplotype, along with the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype.

Haplotype	Individuals	Fr	Ecorregion
Pc-T1	bPca05	1	Southern Atlantic Forest
Pc-T2	bPca09	1	Pampas
Pc-T3	bPca16	1	Pampas
Pc-T4	bPca17	1	Pampas
Pc-T5	bPca26	1	Southern Atlantic Forest
Pc-T6	bPca29	1	Pampas
Pc-T7	bPca31	1	Southern Atlantic Forest
Pc-T8	bPca04,07,15,23,34	5	Pampas, Southern Atlantic Forest, Eastern Amazonia
Pc-T9	bPca02,03,06,25,28,304,308	7	Southern Atlantic Forest, Northern Atlantic Forest, Pantanal
Pc-T10	bPca014	1	Cerrado
Pc-T11	bPca019	1	Central Atlantic Forest
Pc-T12	bPca21,312	2	Central Atlantic Forest, Northern Atlantic Forest
Pc-T13	bPca24	1	Eastern Amazonia
Pc-T14	bPca311	1	Northern Atlantic Forest
Pc-T15	bPca301	1	Cerrado
Pc-T16	bPca302 ,303	2	Cerrado
Pc-T17	bPca10	1	Pantanal
Pc-T18	bPca12,13	2	Pantanal
Pc-T19	bPca305	1	Pantanal
Pc-T20	bPca306,307	2	Pantanal

Table 13 F_{ST} values calculated for different population groupings of *N. nasua*, calculated using the mtDNA concatenated data set. Sample sites are labeled according to the abbreviations defined in Table 1 and Figure 1. Values in bold indicate the highest F_{ST} values found for each specific scenario.

Scenario		Populations	F_{ST}
One-level AMOVA (Only geographic information)	1. Nine geographic groups (initial definition)	(RS) (PR+Arg) (MS) (SP) (ES) (GO+MG) (AL+CE) (PA) (Bol+AC)	0.574*
	2. Eight populations based on pairwise F_{ST} results [a]	(RS) (PR+Arg+MS) (SP) (ES) (GO+MG) (AL+CE) (PA) (Bol+AC)	0.590*
	3. Seven populations based on pairwise F_{ST} results [a]	(RS+PR+Arg+MS) (SP) (ES) (GO+MG) (AL+CE) (PA) (Bol+AC)	0.602*
	4. Six populations based on pairwise F_{ST} results [a]	(RS+PR+Arg+MS+SP) (ES) (GO+MG) (AL+CE) (PA) (Bol+AC)	**0.618***
	5. Five populations based on pairwise F_{ST} results [a]	(RS+PR+Arg+MS+SP) (ES+GO+MG) (AL+CE) (PA) (Bol+AC)	**0.618***
	6. Five populations based on pairwise F_{ST} results	(RS+PR+Arg+MS+SP) (ES) (GO+MG) (AL+CE+Bol+AC) (PA)	**0.582***
One-level AMOVA (Including phylogenetic information)	1. Nine geographic and phylogenetic groups (initial definition)	(RS) (PR+Arg) (MS) (SP) (ES) (GO+MG) (AL) (CE+PA) (Bol+AC)	0.602*
	2. Eight populations based on pairwise F_{ST} results	(RS) (PR+Arg+MS) (SP) (ES) (GO+MG) (AL) (CE+PA) (Bol+AC)	0.618*
	3. Seven populations based on pairwise F_{ST} results	(RS+PR+Arg+MS) (SP) (ES) (GO+MG) (AL) (CE+PA) (Bol+AC)	0.630*
	5. Five populations suggested by STRUCTURE	(RS+PR+Arg + SP) (MS+Bol) (ES+GO+MG) (AL+CE) (PA+AC)	0.546*
	6. Six populations based on pairwise F_{ST} results	(RS+PR+Arg+MS+SP) (ES) (GO+MG) (AL) (CE+PA) (Bol+AC)	**0.644***
	7. Five populations based on pairwise F_{ST} results	(RS+PR+Arg+MS+SP) (ES+GO+MG) (AL) (CE+PA) (Bol+AC)	**0.645***
Two-level AMOVA	1. Six populations divided into five groups, according to phylogenetic information.	[(RS+PR+Arg+MS+SP)] [(ES) (GO+MG)] [(AL)] [(CE+PA)] (Bol+AC)]	0.650* (F_{CT} = 0.602)
	2. Six populations divided into four groups, according to phylogenetic information.	[(RS+PR+Arg+MS+SP) (ES) (GO+MG)] [(AL)] [(CE+PA)] [(Bol+AC)]	**0.732*** **(F_{CT} = 0.560*)**
	3. Five populations divided into four groups, according to phylogenetic information	[(RS+PR+Arg+MS+SP) (ES+GO+MG)] [(AL)] [(CE+PA)] [(Bol+AC)]	0.729* (F_{CT} = 0.557)

[a] Despite the higher values of F_{ST}, the differences among the putative populations were not significant.

* Significant value.

Table 14 F_{ST} values calculated for different population groupings of *P. cancrivorus*, calculated using the mtDNA concatenated data set. Sample sites are labeled according to the abbreviations defined in Table 2 and Figure 1. Values in bold indicate the highest F_{ST} values found for each specific scenario.

Scenario		Populations/ Groups	F_{ST}
One-level AMOVA	1. Seven populations based on geographic and main vegetational domains information	(MT+MS) (RS1*) (RS2*+SC+PR) (SP) (ES) (PA+MA) (AL+PB)	0.235*
	2. Six populations based on pairwise F_{ST}	(MT+MS) (RS1) (RS2+SC+PR) (SP) (ES) (PA+MA+AL+PB)	0.248*
	3. Five populations based on pairwise F_{ST}	(MT+MS) (RS+SC+PR) (SP) (ES) (PA+MA+AL+PB)	0.273*
	4. Four populations based on pairwise F_{ST}	(MT+MS) (RS+SC+PR) (SP) (ES+PA+MA+AL+PB)	0.255*
	5. Four populations based on pairwise F_{ST}	(MT+MS) (SP) (ES) (RS+SC+PR+PA+MA+AL+PB)	0.274*
	6. Three populations based on pairwise F_{ST}	(MT+MS) (SP) (RS+SC+PR+ES+PA+MA+AL+PB)	**0.285***
Two-level AMOVA	1. Five populations divided in three groups, corresponding to general vegetational patterns.	[(MT+MS)] [(RS+SC+PR) (ES) (PA+MA+AL+PB)] [(SP)]	**0.314*** (F_{CT} = 0.208)
	2. Five populations divided in four groups, corresponding to general vegetational patterns.	[(MT+MS)] [(RS+SC+PR) (ES)] [(PA+MA+AL+PB)] [(SP)]	0.287* (F_{CT} = 0.183)
	3. Four populations divided in three groups, corresponding to general vegetational patterns.	[(MT+MS)] [(SP)] [(RS+SC+PR+PA+MA+AL+PB) (ES)]	0.298* (F_{CT} = 0.206)

* RS1 and RS2 refer to two vegetational domains: RS1, Pampas and RS2, Southern Atlantic Forest (See Figure 1 and Table 2 for details)

Table 15 Summary of genetic variation at eight microsatellite loci scored for *N. nasua* populations.

	NnSTR-A08					NnSTR-B09					NnSTR-D03					NnSTR-E05				
	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}
South	12	5	0.417	0.710	0.097	12	3 (1)	0.417	0.518	-0.044	12	5	0.667	0.656	0.229	12	3	0.667	0.507	-0.001
Southeastern	16	6 (1)	0.812	0.774	-0.336	14	5 (1)	0.500	0.655	0.324	14	6 (1)	0.857	0.783	0.239	15	4	0.857	0.751	0.120
Center-west	38	8 (3)	0.846	0.728	-0.099	39	3	0.316	0.382	0.267	39	7	0.718	0.763	-0.045	39	6 (1)	0.282	0.281	-0.096
North	8	6 (2)	0.875	0.717	-0.263	8	5 (4)	0.750	0.667	0.044	8	4 (2)	0.750	0.742	-0.533	8	4 (2)	0.750	0.642	0.087
Northeastern	5	4 (1)	0.333	0.454	0.443	6	4 (1)	0.600	0.778	-0.367	6	6 (1)	0.333	0.818	0.037	6	4 (1)	0.667	0.757	0.283
Overall	79	14	0.741	0.864	-0.115	79	11	0.430	0.660	0.026	79	12	0.709	0.840	0.005	80	9	0.519	0.604	0.006

Table 15 (*continued*)

	NnSTR-F02					NnSTR-F03					NnSTR-H03					NnSTR-H07					Averaged over all loci				
	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}	N	A	H_O	H_E	F_{iS}	N	a	H_O	H_E	F_{iS}
S	11	5	0.583	0.659	-0.148	12	6	0.545	0.783	0.127	11	4 (1)	0.500	0.764	0.643	12	3	0.454	0.480	-0.066	12	4.250	0.531	0.635	0.082
SE	16	6	0.533	0.616	-0.172	16	8 (1)	0.688	0.774	-0.063	16	8 (3)	0.688	0.802	0.585	15	6	0.625	0.673	0.225	16	6.130	0.695	0.729	0.288
CW	37	6 (1)	0.744	0.790	0.240	39	5	0.676	0.746	-0.239	38	8 (3)	0.641	0.686	0.480	38	9 (2)	0.500	0.715	0.227	39	6.500	0.590	0.636	0.121
N	7	4 (2)	0.875	0.692	-0.190	8	5 (1)	0.714	0.670	-0.097	8	6 (1)	0.875	0.800	-0.201	8	3	1.000	0.667	0.067	8	4.630	0.823	0.699	-0.162
NE	6	4 (1)	0.667	0.773	-0.312	6	5	0.500	0.576	-0.245	6	3 (1)	0.667	0.590	-0.500	6	5 (1)	0.833	0.788	0.054	6	4.380	0.575	0.691	0.033
Overall	77	11	0.688	0.802	0.036	81	11	0.649	0.837	-0.119	79	17	0.654	0.859	0.498	79	11	0.595	0.776	0.218	81	12	0.623	0.780	

N, number of individuals;

a, average number of alleles

H_O, observed heterozygosity

H_E, expected heterozygosity

A, number of alleles per locus; number between parentheses indicates the number of privates alleles.

F_{iS}, Weir and Cockerham's (1984) analog of Wright's fixation index.

Table 16 Summary of genetic variation at eight microsatellite loci scored from *P. cancrivorus*.

	PLO3-86					PLO3-71					PLO3-117					PLO-M3				
	N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}
Only one population considered	30	17	0.889	0.905	0.108	39	8	0.533	0.847	0.475	37	18	0.410	0.932	0.024	41	8	0.649	0.771	0.060

Table 16 (*continued*)

PLO-M15					PLO-M17					PLOT-08					PLOT-10					Averaged over all loci				
N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}	N	A	H_O	H_E	F_{IS}	N	a	H_O	H_E	F_{IS}
39	6	0.805	0.789	0.294	37	7	0.538	0.734	0.016	38	8	0.784	0.828	0.074	37	10	0.789	0.847	0.569	36	10.250	0.675	0.832	0.253

N, number of individuals

a, average number of alleles

H_O, observed heterozygosity

H_E, expected heterozygosity

A, number of alleles per locus.

F_{iS}, Weir and Cockerham's (1984) analog of Wright's fixation index.

Table 17 F_{ST} and R_{ST} values calculated for different population groupings of *N. nasua* using eight microsatellite loci. Sample sites are labeled according to the abbreviations defined in Table 1 and Figure 1. Values in bold indicate the highest F_{ST} values found.

Scenario	Populations	F_{ST}	R_{ST}
1. Four populations suggested by mtDNA results	(RS+PR+ SP+MS) (ES+GO+MG) (AL+CE) (PA)	0.195*	0.332*
2. Five populations suggested by mtDNA results	(RS+PR+ SP+MS) (ES) (GO+MG) (AL+CE) (PA)	0.197*	**0.336***
3. Five populations suggested by STRUCTURE	(RS+PR+ SP) (MS) (ES+GO+MG) (AL+CE) (PA)	**0.202***	0.306*

*Significant values

Table 18 Pairwise R_{ST} estimates for *Nasua nasua* populations (below the diagonal) and corresponding significance level (above the diagonal). All values were statistically significant.

	South	Southeastern	Center-west	North	Northeastern
South	*	0.00020	0.00307	0.00000	0.00000
Southeastern	0.29670	*	0.00663	0.00000	0.01317
Center-west	0.09093	0.07813	*	0.00000	0.00040
North	0.70886	0.42420	0.52476	*	0.00040
Northeastern	0.58954	0.18573	0.25024	0.37379	*

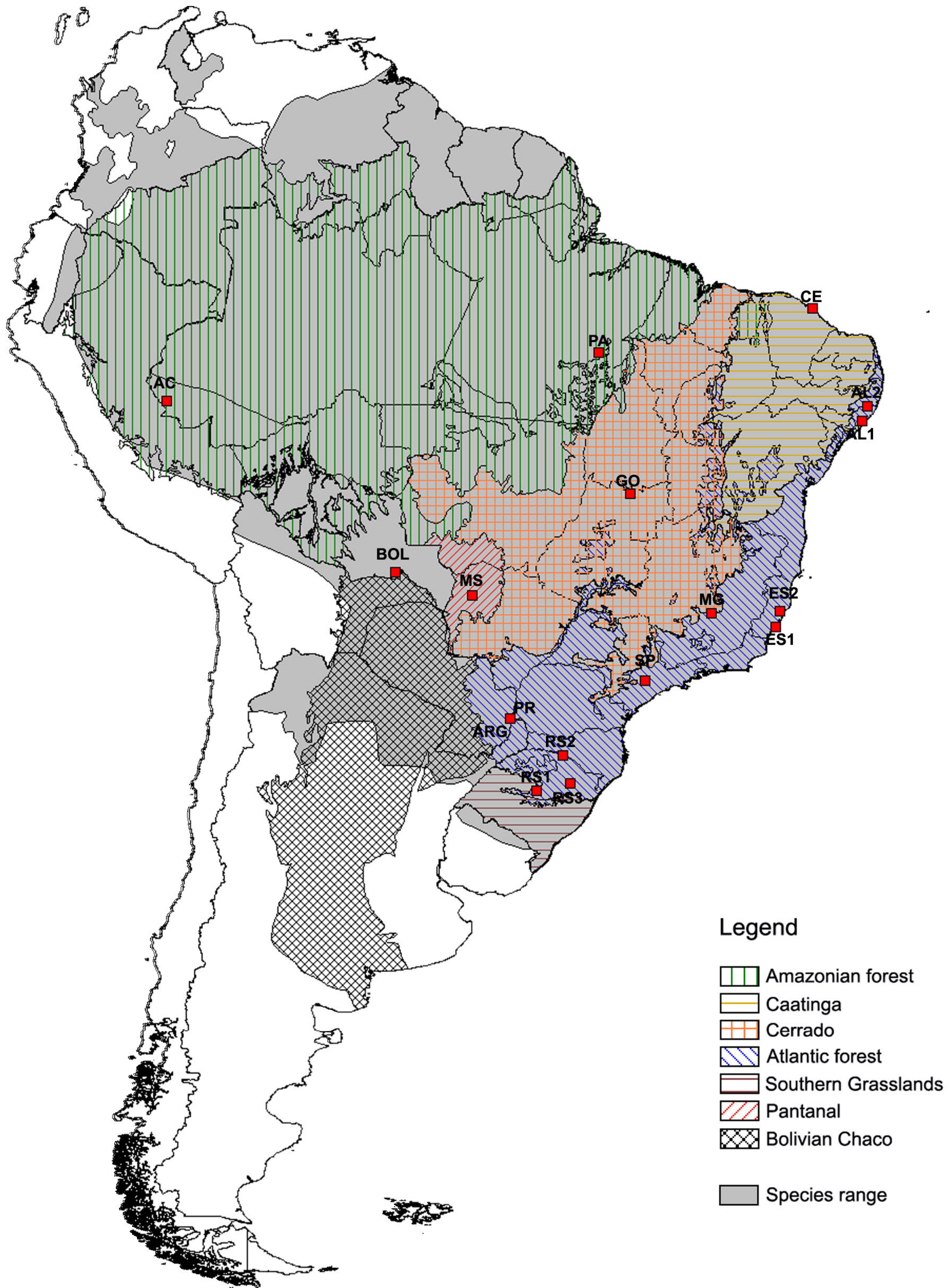


Figure 1

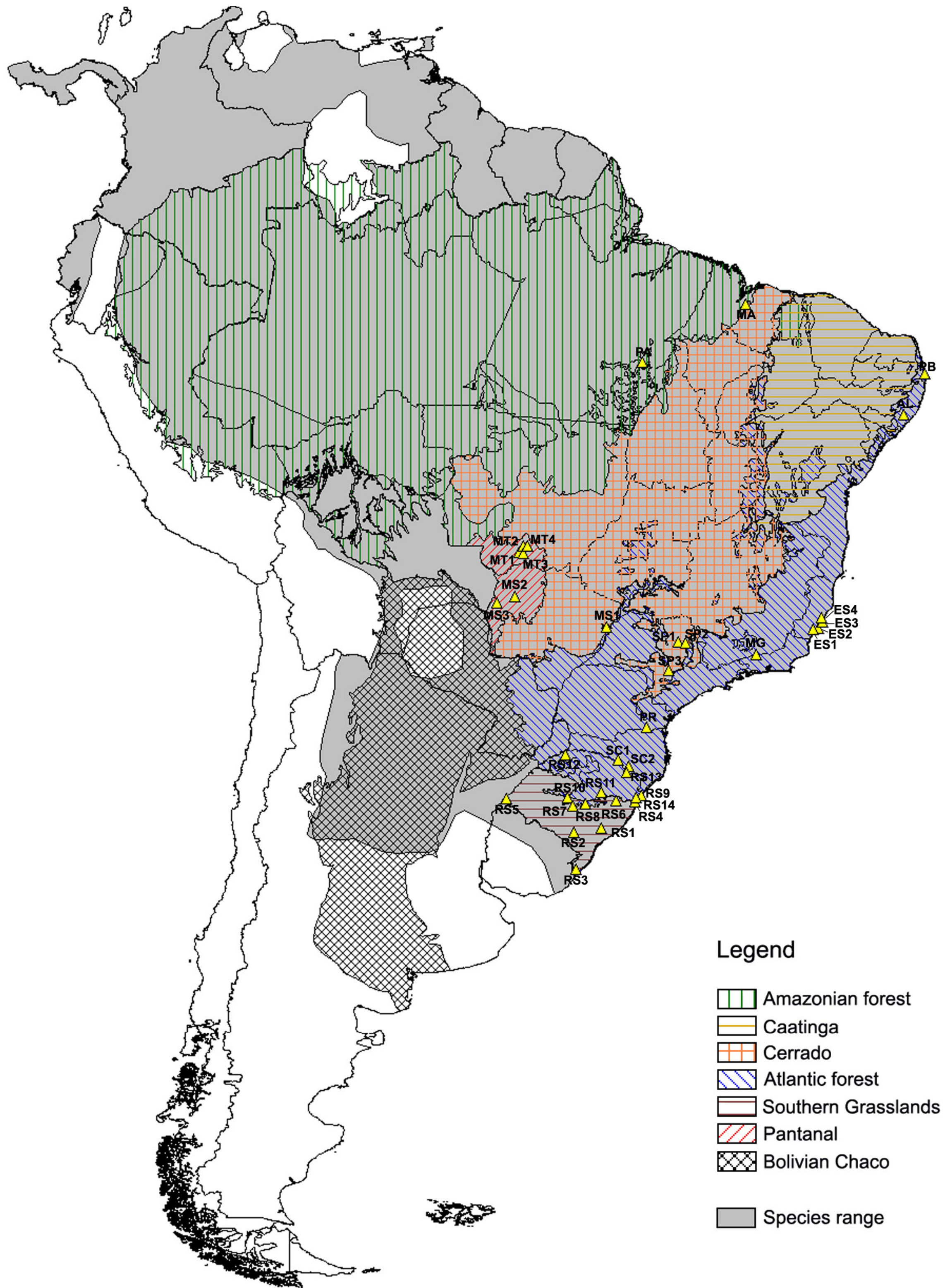
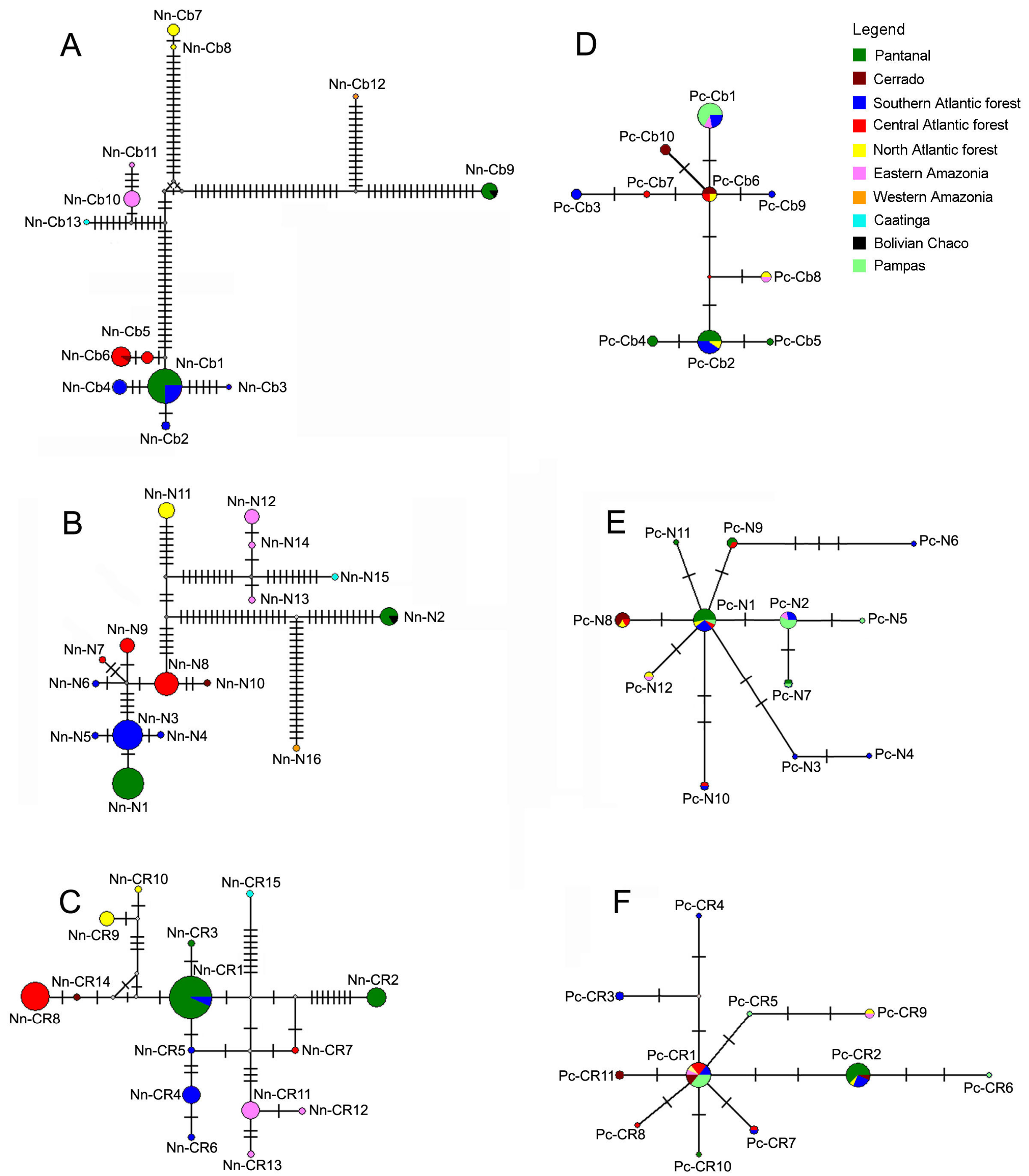


Figure 2



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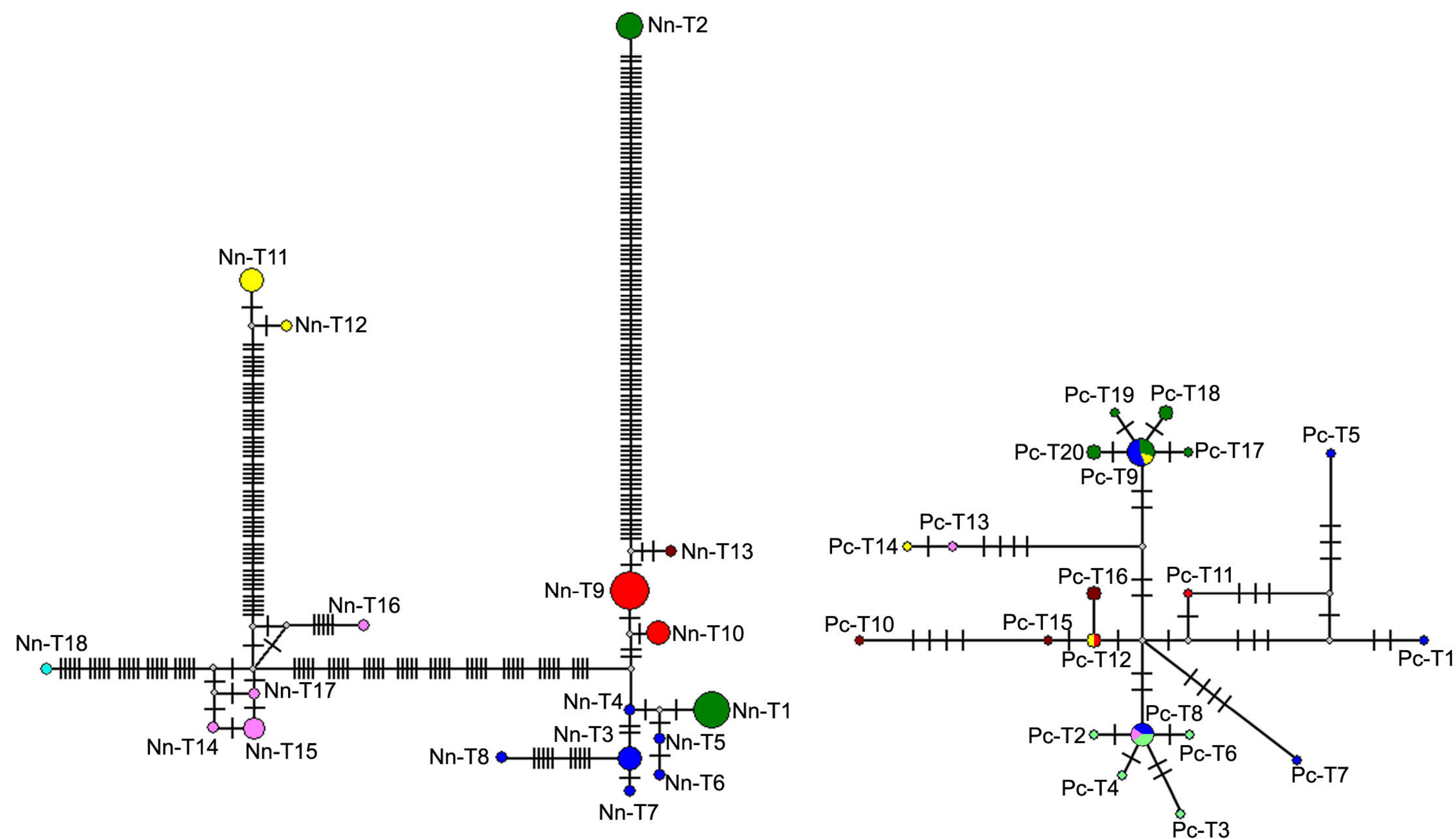

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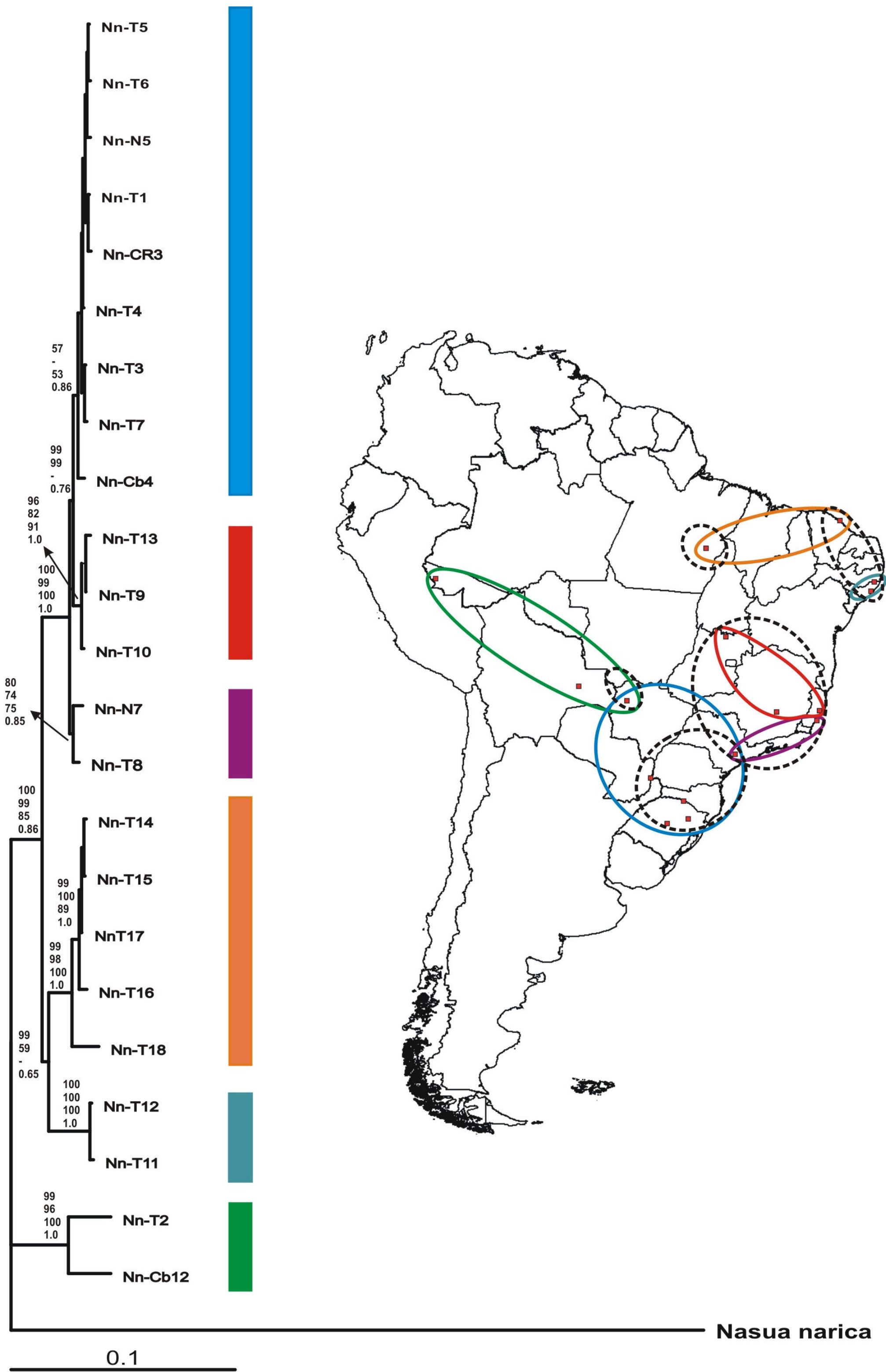

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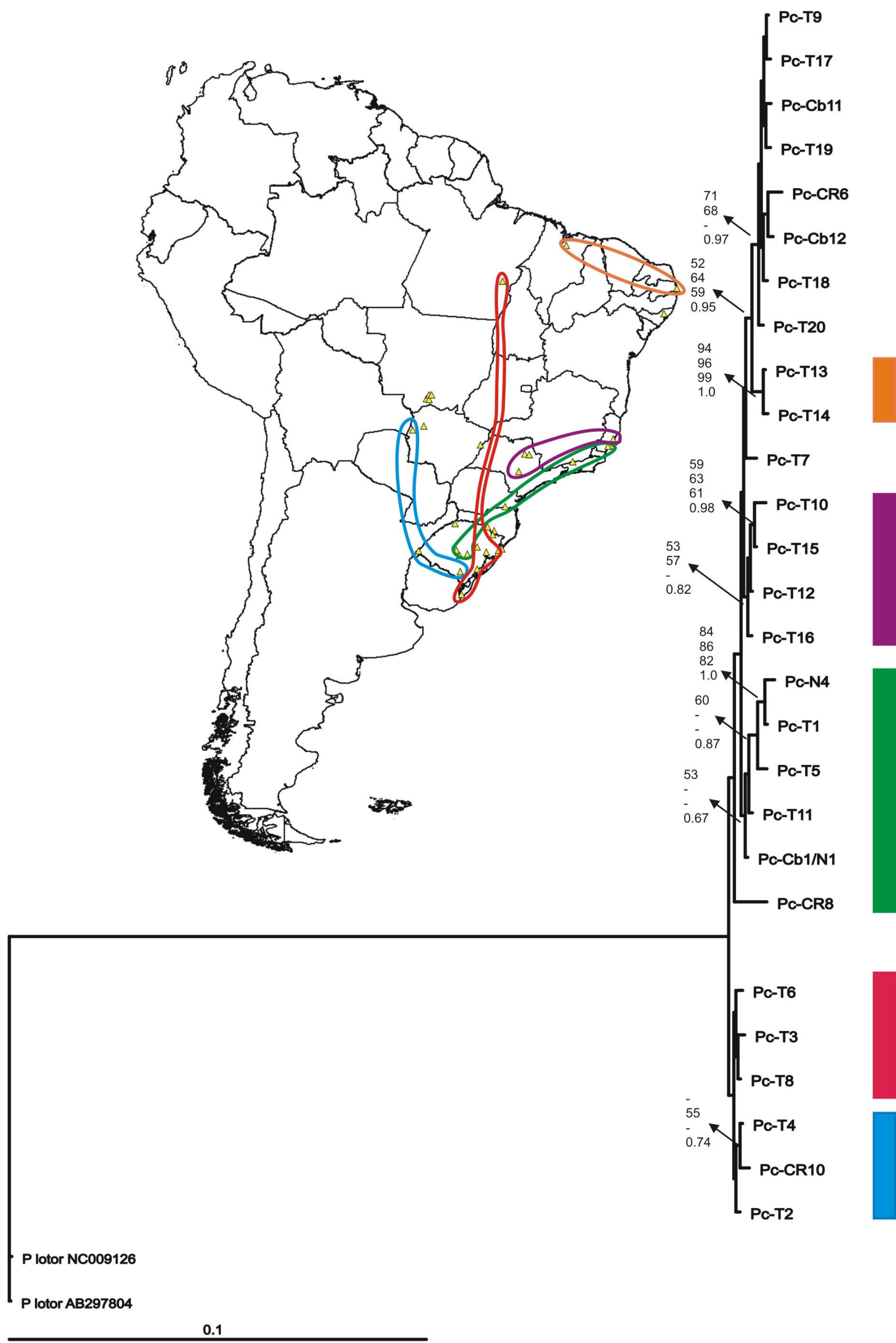

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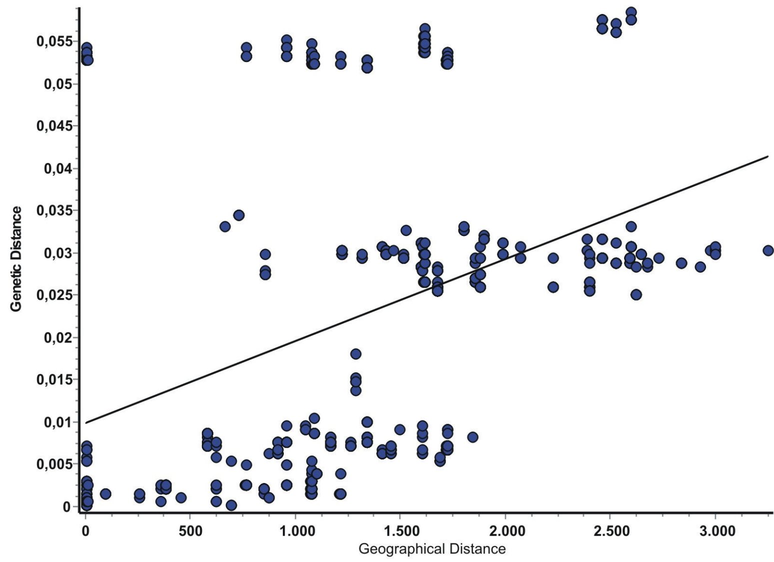


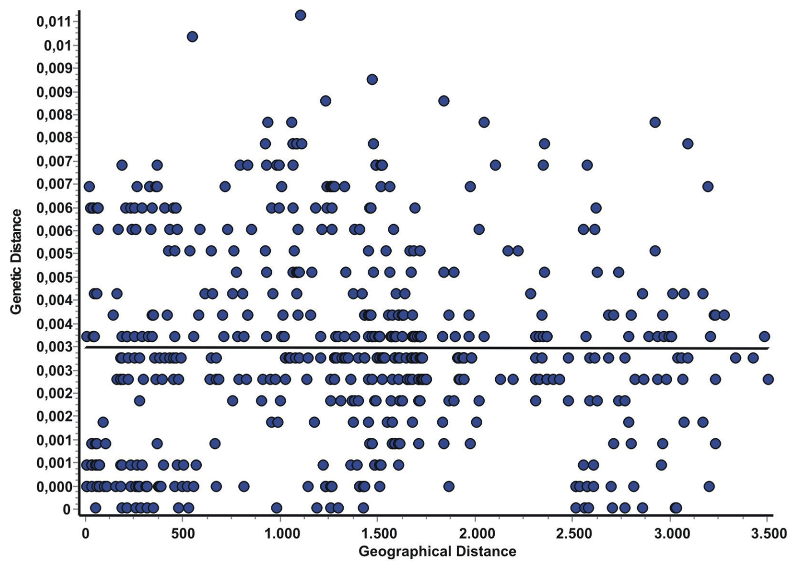
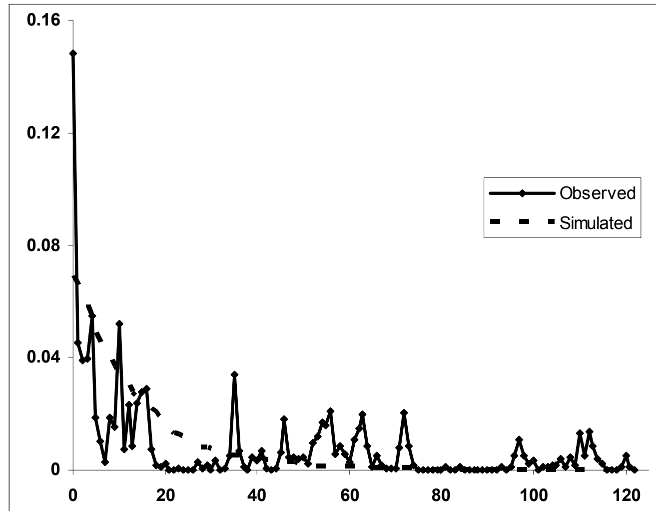


Figure 7



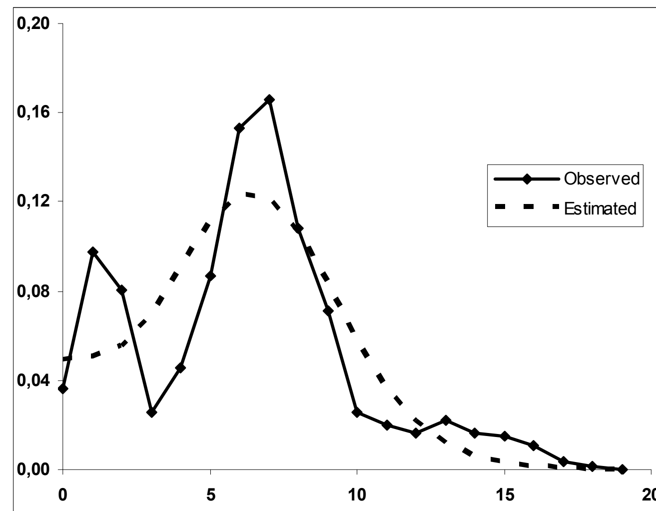


Figure 8

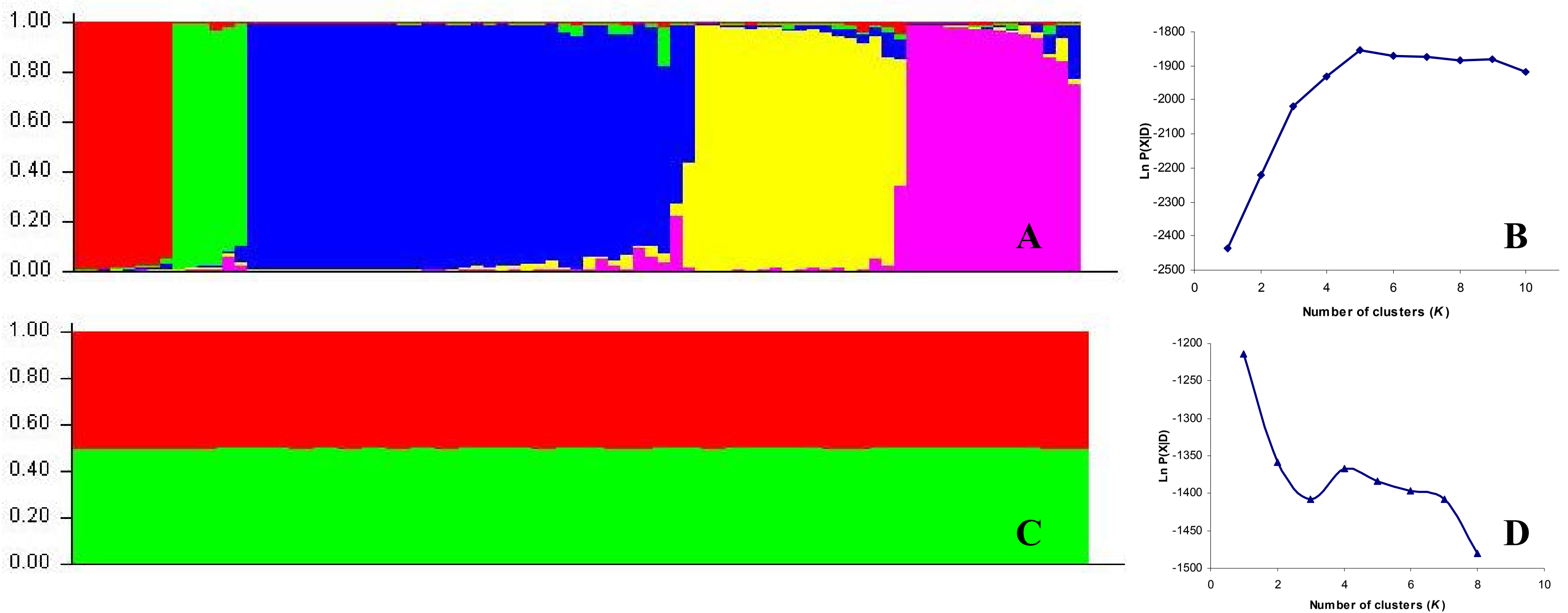
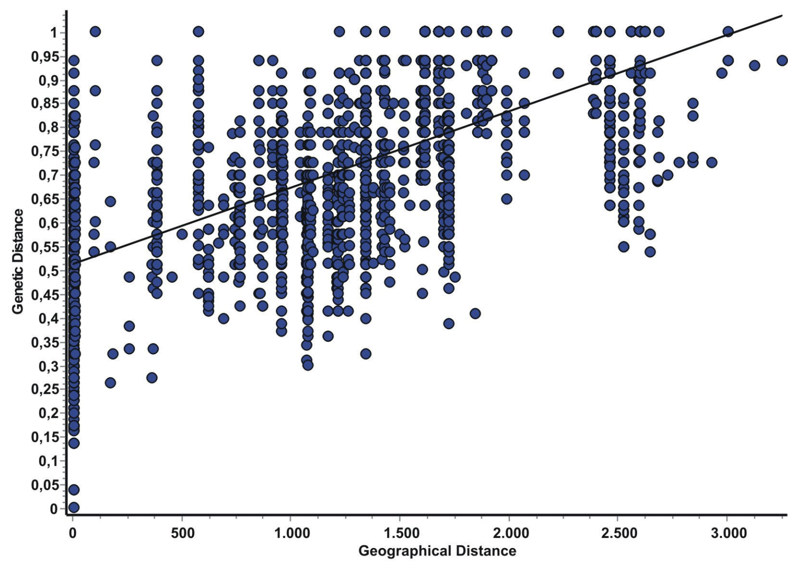


Figure 9



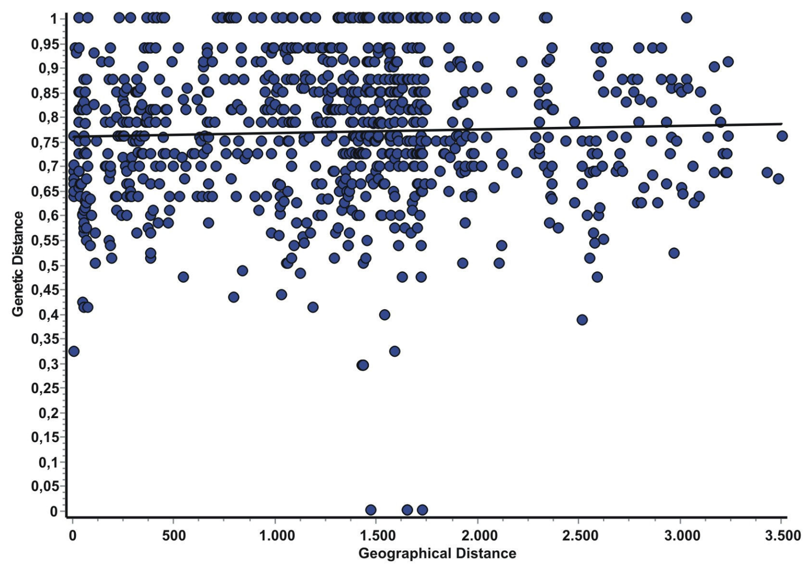


Figure 10

APÊNDICE

Isolation and characterization of eight microsatellite loci in the Brown-nosed Coati, *Nasua nasua* (Mammalia, Carnivora, Procyonidae)

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Keywords: microsatellite, *Nasua nasua*, procyonid, Neotropical

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Running title: Microsatellites in Brown-nosed Coati.

23 **Abstract**

24

25 We describe the isolation and characterization of eight polymorphic microsatellite loci
26 for brown-nosed coatis (*Nasua nasua*). Two multiplexed panels were designed and employed
27 to genotype 24 individuals from a single population in the southern Pantanal biome, Brazil.
28 The allelic diversity ranged from two to seven alleles per locus, and the observed
29 heterozygosity ranged from 0.250 to 0.792. One locus showed a departure from Hardy-
30 Weinberg equilibrium due to an excess of heterozygotes, and no evidence of linkage
31 disequilibrium was found. These markers should be useful for studies addressing population
32 genetics, ecology, and social structure of this poorly known species as well as related
33 procyonids.

34 The brown-nosed or South American coati (*Nasua nasua*) is a diurnal, highly social
35 mesocarnivore belonging to the family Procyonidae (Eisenberg 1989; Gompper & Decker
36 1998). The species is distributed from Colombia and Venezuela to Uruguay and Northern
37 Argentina, and is found in many vegetation types, although it prefers wooded areas (Gompper
38 & Decker 1998; Nowak 1999; Redford & Eisenberg 1992). Coatis are omnivorous and forage
39 on trees as well as on the ground. Their diet is composed predominantly of fruits and
40 invertebrates (and occasionally small vertebrates), depending on local availability. A recent
41 study in an Atlantic forest fragment showed that coatis are effective seed dispersers (Alves-
42 Costa & Eterovick 2007). The social organization is thought to be similar to that of its Central
43 American congener, *N. narica*: groups are formed by females and immature males, while
44 adult males are solitary, joining the groups during the mating season (Beisiegel & Mantovani
45 2005; Gompper & Decker 1998).

46 Despite being a common, broadly distributed species, *Nasua nasua* remains among the
47 least studied Neotropical carnivores (Oliveira 2006). The existing studies focus mainly on diet
48 and behavioral ecology (Alves-Costa & Eterovick 2007; Alves-Costa *et al.* 2004; Beisiegel &
49 Mantovani 2005; Blanco & Hirsch 2006), and until now there is no study addressing genetic
50 aspects of this species. Since microsatellites are useful markers for most applications in
51 population genetics and molecular ecology (Vali *et al.* 2008), the objective of this study was
52 to identify and characterize multiple such loci for *N. nasua*, in order to provide new molecular
53 tools that allow the development of in-depth studies targeting this species.

54 We constructed a microsatellite-enriched genomic library using a protocol modified
55 from the one described by Billotte *et al.* (1999), starting from genomic DNA extracted using
56 a standard phenol-chloroform method (Sambrook *et al.* 1989). Five μg of DNA were digested
57 with *Rsa*I (Invitrogen), and *Rsa*21 (5′-CTCTTGCTTACGCGTGGACTA-3′) and *Rsa*25 (5′-

58 TAGTCCACGCGTAAGCAAGAGCACA-3′) linkers were ligated to the digested fragments.
59 The library was enriched for dinucleotide repeats using $(CT)_8$ and $(GT)_8$ biotin-labeled probes
60 and streptavidin-coated paramagnetic beads (Streptavidine MagneSphere Paramagnetic
61 Particles, Promega). The selected fragments were amplified by PCR using *Rsa*21 primers, and
62 the products were cloned into pGEM-T vectors (Promega). These plasmids were introduced
63 into *Escherichia coli* XL-1 Blue strains, and transformed cells were grown onto agar plates
64 containing 100 µg.ml–1 ampicilin and 50 µg.ml–1 X-galactosidase. We selected 95 positive
65 colonies which were grown for 22 hours in a 96-well plate containing 100 µg/uL amplicilin
66 and 1 mL of Circle Grow medium (QBio-Gene), followed by plasmid isolation as described
67 by Sambrook *et al.* (1989). All positive clones were sequenced using SP6 primers, the
68 DYEnamic ET Dye Terminator Sequencing Kit (GE Healthcare), and a MegaBACE 1000
69 (GE Healthcare) automated sequencer. We also employed T7 primers for sequencing the
70 reverse strand of selected clones to increase the reliability of primer design.

71 Microsatellite repeats were found in 55 clones (ca. 60% of the total), of which 27 were
72 perfect (STRs without interruptions): 25 bore simple repeats (only one motif) and two
73 contained compound repeats (more than one motif). We selected 9 clones (based on repeat
74 number and availability of reliable flank sequences) for primer design, which was performed
75 using the program PRIMER 3 (Rozen & Skaletsky 2000) was employed for this purpose. All
76 forward primers received an M13 tail at their 5' end for flexible dye-labeling (Boutin-
77 Ganache *et al.* 2001). PCR reactions were carried out in a PTC-100 thermocycler (MJ
78 Research) in 10µL volume, including 1X PCR Buffer (Invitrogen), 0.2 mM of each dNTP, 2
79 mM MgCl2, 0.2 µM each of the reverse and the fluorescent M13 primer (FAM, NED or
80 HEX), 0.013 µM of the forward primer, 0.25 U Platinum® *Taq* Polymerase (Invitrogen),
81 0.3% Trehalose and 10-20 ng of genomic DNA. The amplification profile consisted of an

82 initial denaturing step at 94ºC for 3 min, 10 touchdown cycles [94ºC for 45 s, annealing at 65-
83 56ºC (-1ºC/cycle) for 45 s and 72ºC for 1 min 30 s], 30 additional cycles with annealing at
84 55ºC, and a final extension at 72ºC for 30 min. PCR products were diluted 1:10 and then
85 genotyped with a MegaBACE1000 (GE Healthcare) automated sequencer, using the software
86 Genetic Profiler 2.2 and the internal size standard ETRox-550.

87 Initially, we genotyped five specimens from different geographic regions (data not
88 shown) to assess amplification success, product size range and overall polymorphism. Eight
89 loci showed positive amplification and some level of polymorphism. Based on the allelic size
90 range, two multiplex panels were designed (Table 1). Twenty-four coati samples from a
91 single population in the Pantanal biome (160 km east of Corumbá, MS, Brazil) were
92 genotyped following the same PCR and genotyping conditions described above. The observed
93 and expected heterozygosities (*H*o and *H*e, respectively) were calculated using CERVUS
94 3.0.3 (Kalinowski *et al.* 2007) and the presence of null alleles was assessed with
95 MICROCHECKER (Oosterhout *et al.* 2004). GENEPOP 3.4 (Raymond & Rousset 1995) and
96 ARLEQUIN 3.11 (Excoffier *et al.* 2005) were used to test for departures from Hardy-
97 Weinberg equilibrium Linkage equilibrium (a Bonferroni correction was used for both tests).

98 Allelic diversity ranged from two to seven alleles per locus, and the observed
99 heterozygosity varied from 0.250 to 0.792 (Table 1). There was no evidence of null alleles at
100 any of the loci. We found no deviation from Hardy-Weinberg equilibrium using ARLEQUIN
101 3.11 (Excoffier *et al.* 2005), but GENEPOP 3.4 (Raymond & Rousset 1995) detected HW
102 disequilibrium due to excess of heterozygotes at locus STRNn-F02. We tested 28
103 combinations of loci for linkage disequilibrium and found no significant value. These results
104 indicate that the markers described here are informative and reliable for population-level
105 studies, and will likely be very useful to investigate the genetic structure, behavioral ecology

106 and evolutionary history of coatis, opening up new research avenues aimed at understanding

107 this poorly known species.

108

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155 **Acknowledgements**

156

157 We thank Anete de Souza Pereira, Adna Souza, Fernanda Cidade, Manoel Ludwig da

158 Fontoura-Rodrigues and for assisting with study design and implementation and CNPq and

159 for financial support.

160

Table 1 Main features and primer sequences for eight polymorphic microsatellite loci identified in the brown-nosed coati. See text for PCR conditions. M13 tails added to the 5' end of forward primers are indicated in bold types. Number of alleles (A), expected (H_E) and observed (H_O) heterozygosities were assessed based on 24 individuals from the Pantanal biome, Brazil. Occurrence of significant heterozygote excess is denoted by an asterisk adjacent to the locus ID.

Multiplex	Locus		Primer Sequences	Repeat Motif	Dye	Size range	*A*	H_O	H_E
1	NnSTR-D03	F:	**CACGACGTTGTAAAACGAC** AGG CTT GAA TTT GTC CAG CTA	$(CA)_{14}$	FAM	275-293	7	0.792	0.735
		R:	CCA AGA ATC CTG TGG CAA A						
	NnSTR-E05	F:	**CACGACGTTGTAAAACGAC** CCC AAT CCT GAT AGC CCT TC	$(CA)_{18}$	FAM	134-174	4	0.292	0.301
		R:	TAT TTT TGT TGG GCC CGA GT						
	NnSTR-H03	F:	**CACGACGTTGTAAAACGAC** GCC CCT GAG CCA ATT CTT	$(TC)_{17}$_$(AC)_{12}$	HEX	137-167	7	0.750	0.723
		R:	TTC TCC TGT ATT AGG GTT CTC CA						
	NnSTR-H07	F:	**CACGACGTTGTAAAACGAC** GAA GTC AAT AAG GCA GCC AAA	$(TG)_{18}$	NED	179-197	7	0.542	0.683
		R:	TGC CTG ACT GAT CCT TGT CA						
2	NnSTR-A08	F:	**CACGACGTTGTAAAACGAC** CCT TCA TTC CAA CTG TAA ATG ACT	$(TG)_{17}$	FAM	223-245	5	0.792	0.704
		R:	TCC CTA CAA ATG GAA AAA GGA A						
	NnSTR-B09	F:	**CACGACGTTGTAAAACGAC** GCT TTT GCT GGC CAT AGT TT	$(TG)_{19}$	HEX	232-234	2	0.250	0.337
		R:	TCA CTA ATT ACA ACT AAA AAC CCT GA						
	NnSTR-F02*	F:	**CACGACGTTGTAAAACGAC** CAT TTG AGT GAA AAT CCA GTG A	$(TG)_{15}$	NED	220-234	6	0.792	0.772
		R:	GCT CTT GAT AAA GCA AGC ACA A						
	NnSTR-F03	F:	**CACGACGTTGTAAAACGAC** TTG TGT CTG AAA TGG CCG TA	$(CG)_{9}$_$(CA)_{16}$	NED	132-140	5	0.708	0.735
		R:	GCG TCT ATG TTG ATT TGA GGT G						

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