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Genetic variability in maned wolf based on heterologous short-tandem repeat markers from domestic dog

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ABSTRACT. The maned wolf (*Chrysocyon brachyurus*) is the largest South American canid. Habitat loss and fragmentation, due to agricultural expansion and predatory hunting, are the main threats to this species. It is included in the official list of threatened wildlife species in Brazil, and is also protected by IUCN and CITES. Highly variable genetic markers such as microsatellites have the potential to resolve genetic relationships at all levels of the population structure (among individuals, demes or metapopulations) and also to identify the evolutionary unit for strategies for the conservation of the species. Tests were carried out to verify whether a class of highly polymorphic tetranucleotide repeats described for the domestic dog effectively amplifies DNA in the maned wolf. All five loci studied were amplified; however, one of these, was shown to be monomorphic in 69 maned wolf samples. The average allele number and estimated heterozygosity per polymorphic locus were

4.3 and 67%, respectively. The genetic variability found for this species, which is considered threatened with extinction, showed similar results when compared to studies of other canids.

Key words: *Chrysocyon brachyurus*, Canidae, Domestic dog, Cross-amplification, Microsatellite, Population genetics

INTRODUCTION

The maned wolf, *Chrysocyon brachyurus*, listed as near threatened with extinction by IUCN and vulnerable by IBAMA, is the largest South America canid (Dietz, 1984). Its geographic range covers the natural savannahs of central Brazil (the Cerrado), the north of Bolivia, Paraguay, the north of Argentina, the northwest of Uruguay, and a small area in the east of Peru (Langguth, 1975; Dietz, 1984; Moreira et al., 1998).

The main threats to maned wolf conservation are habitat loss and fragmentation due to agricultural expansion, road kills, susceptibility to disease transferred from domestic dogs, and the belief that maned wolves prey on domestic stock, resulting in their persecution (Dietz, 1984; Vieira, 1996; Rodrigues, 2002).

Microsatellites are widely regarded as the most versatile genetic markers yet discovered, and their applicability for identifying relationships in natural populations is well documented (Bruford and Wayne, 1993; Queller et al., 1993). However, some of the obstacles in the use of microsatellites are the time required, costs and difficulty in isolating these short-tandem repeats and their flanking regions from the genome of target organisms. An alternative approach to *de novo* development is to exploit the available information by cross-species amplifications among a range of phylogenetically related species (Moore et al., 1991; Blanquer-Maumont and Crouau-Roy, 1995; Pepin et al., 1995; FitzSimmons et al., 1995; Wintero and Fredholm, 1995; Coltman et al., 1996; Scribner et al.,1996; Gemmell et al., 1997; Hille, 2002).

The objective of the present study was to investigate the genetic diversity of the maned wolf based on microsatellite markers transferred from dog (*Canis familiaris*).

MATERIAL AND METHODS

Blood samples were collected from a hundred captive wolves from 11 Brazilian zoos (Brasília, Goiânia, Uberlândia, Uberaba, Araxá, Belo Horizonte, Varginha, Bauru, Sorocaba, São Paulo, and Curitiba) over two different periods (1994 and 1996) and also from 12 free-living individuals from the "Águas Emendadas Ecological Station" (ESECAE), in 1997.

Only the animals of known origin, born in the wild and not closely related, were used for genetic variability analysis, totaling 69 individuals. These individuals were gathered into four groups according to their capture region (not their zoo residence) and also taking into account existing landscape barriers such as rivers and mountain ranges. The four groups formed were considered here as the Brazilian region populations to be compared (Figure 1).

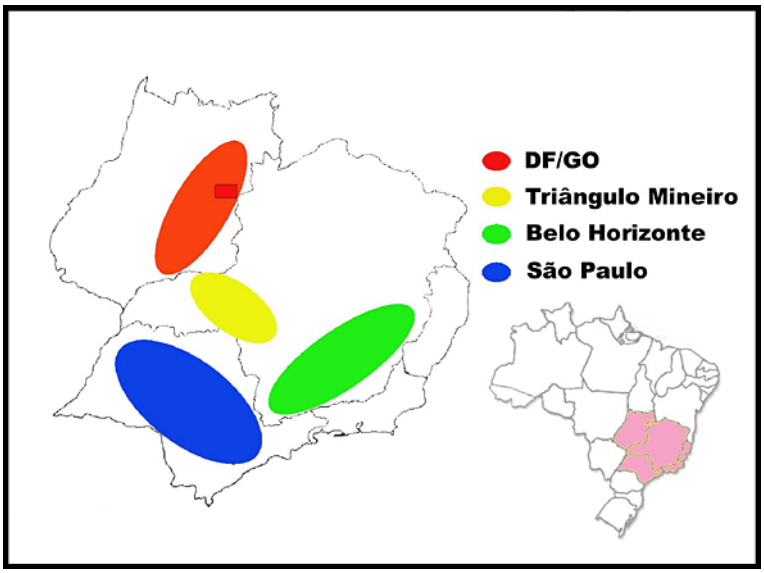

Figure 1. Range of the Brazilian region populations of maned wolf considered in this study.

Approximately 10 mL of whole blood (with EDTA) was collected from each animal and transported to the genetics laboratory at the University of Brasília for appropriate processing. DNA extraction was performed with GFXTM "Genomic Blood DNA Purification Kit" from Amersham Pharmacia Biotech Inc. The procedures adopted were those described in the product's manual.

Five tetranucleotide loci, originally isolated from a domestic dog genomic library (Francisco et al., 1996), were tested against the maned wolf in a cross-species amplification experiment. Table 1 shows the main characteristics of these markers for the species for which they were originally developed.

Amplification reactions were performed for each marker separately. The PCR reactions were carried out in a 25-µL final reaction mixture containing PCR buffer (1X), 250 ng of target DNA, 0.25 mM of each dNTP, 2 mM $MgCl_2$, and 1 unit *Taq* DNA polymerase (Biotools). Primer concentration was optimized for each marker: 0.8 µM (FH2088 and FH2054) and 0.96 µM (FH2006, FH2010 and FH2144). Samples were amplified in a PT-100 MJ thermocycler (Research, Inc.) by denaturing at 94°C for 1 min, followed by 29 cycles of denaturation at 94°C for 1 min, annealing at 56°, 58° or 60°C for 45 s and extension at 74°C for 1 min. The final extension step was for 5 min at 74°C.

For polymorphism analysis, 4 µL PCR reaction product was briefly mixed with 4 µL formamide loading dye and denatured at 95°C for 10 min, placed immediately in an ice-water bath, and then electrophoresed through 10%, 29:1 (acrylamide:bis-acrylamide) denaturing gels containing urea. Gel electrophoresis was performed at room temperature, in an appropriate vertical cube. A constant voltage of 150 V was used. The electrophoresis time was specific for each system, varying according to the size of the amplified fragments: 4 h for FH2088 and FH2054, 4 h and 30 min for FH2006, 6 h and 30 min for FH2010, and 7 h for FH2144. Bands were visualized by specific-staining procedures (Sanguinetti et al., 1994).

Table 1. Genetic markers used and their main characteristics for the original species - *Canis familiaris*.

Markers	Primers [a]	Annealing temperature[a]	PCR product size[a]	Sequence of clone repeat[a]	PIC value[a]	Chromosome
FH2088	F: CCCTCTGCCTACATCTCTGC R: TAGGGCATGCATATAACCAGC	58°C	117 bp	$(TTTA)_{10}(TTCA)_4$	0.59	cfa 15[b]
FH2010	F: AAATGGAACAGTTGAGCATGC R: CCCCTTACAGCTTCATTTTCC	59°C	228 bp	$(ATGA)_{10}$	0.69	cfa 24[b]
FH2006	F: TGGGGGCGTTAAGAGTAATG R: CTAGGCCTAAACCCCTGAGC	58°C	191 bp	$(GAAT)_7$	0.48	cfa 25[b]
FH2054	F: GCCTTATTCATTGCAGTTAGGG R: ATGCTGAGTTTTGAACTTTCCC	58°C	151 bp	$(GATA)_{16}$	0.66	?
FH2144	F: GTGGCTCTTTTTGATGAGGG R: CCTGGGTGGTTCAGTCAGTT	58°C	289 bp	$(GAAA)_{14}$	0.63	cfa 08[c]

[a]Francisco et al. (1996); [b]Breen et al. (2001); [c]Guyon et al. (2003). PIC = polymorphism information content.

A 25-bp molecular weight marker (Invitrogen) was run adjacent to the control and wolf samples on a 10%, 29:1 (acrylamide:bis-acrylamide) non-denaturing gel to provide an estimated allele size of the product amplification.

Dog control samples were utilized in order to detect successful homologous loci. The majority of the amplifications displayed "stutter" bands typical of microsatellite loci.

Allele frequencies and heterozygosity values were calculated with Biosys-1 version 1.7 software package (Swofford and Selander, 1981). Polymorphism information content was estimated using the Cervus 2.0 program (Marshall et al., 1998).

RESULTS AND DISCUSSION

All five microsatellite loci amplified for the maned wolf samples. Despite the amplification success (100%), one locus (FH2144) was monomorphic (20%). All maned wolf allele sizes were smaller than the ones observed for the dog control sample. The sizes of the amplification products were 100-125 bp for the locus FH2088; 175 bp for FH2054; 225-250 bp for FH2006; 250-275 bp for FH2010, and 350-375 bp for FH2144.

The chance of a successful cross-species (heterologous) amplification of any DNA sequence is inversely related to the evolutionary distance between the two species as has been demonstrated in several other papers. Indeed, several studies have shown that microsatellites isolated from various species amplify the corresponding and polymorphic loci in closely related species but not in more distant ones (Moore et al., 1991; Primmer et al., 1996; Gemmell et al., 1997; Galbusera et al., 2000; Hille et al., 2002; Williamson et al., 2002; Gonela, 2003; Nguyen et al., 2005). In the present study, highly polymorphic tetranucleotide microsatellites, characterized and developed for *Canis familiaris*, were successfully transferred to *Chrysocyon brachyurus* (Table 1). Microsatellite transferability was also obtained for other carnivore species evolutionarily related to (Primmer et al., 1996; Gemmell et al.,1997; Koskinen and Bredbacka, 2000; Altet et al., 2001; Williamson et al., 2002) and very recently to maned wolf for different markers than the ones discussed here (Rodrigues et al., 2006). Genetic variability measures and allele frequencies for each microsatellite locus for the total population and for the different Brazilian region population samples are shown in Table 2. The whole population genotypic frequencies, as well as that of each regional population, were in Hardy-Weinberg equilibrium.

The number of alleles per locus for each regional population ranged from 3.50 (Triângulo Mineiro) to 4.25 (DF/GO), H_E from 66% (DF/GO and Belo Horizonte) to 68% (Triângulo Mineiro) and polymorphism information content from 56% (Belo Horizonte) to 59% (São Paulo) (Table 2). There were no genetic differences among regional population samples except for DF/GO and São Paulo ($\chi^2 = 22.779$; P = 0.04; Table 3).

The analysis of each locus revealed a significant difference for FH2088 locus ($\chi^2 = 13.041$; P = 0.04; Table 4).

Another study employing 14 isozyme loci for the same maned wolf samples, detected 42.9% polymorphism and an average heterozygosity of 5.6% (Moreira et al., 1998), which was similar to the results obtained for other canids (Francisco et al., 1996; Koskinen and Bredbacka, 2000; Altet et al., 2001). Nevertheless, Rodrigues (2002) detected extremely low levels of protein polymorphism (10%) for the population of maned wolves from ESECAE, which is a small conservation unit situated in the northern part of Distrito Federal. The observed heterozygosity values in the latter study were low (1.4%), when compared to other canids.

Table 2. Measures of genetic variability and allele frequencies for each short-tandem repeat locus analyzed in the different maned wolf region samples and in the total population sample of Brazil.

Locus	Alleles/ H_O	DF/GO	BH	Triângulo Mineiro	São Paulo	Total population
FH2088		(29)	(11)	(11)	(15)	(66)
	1	0.552*	0.273	0.364	0.333	0.424
	2	0.414	0.636*	0.409*	0.500*	0.470*
	3	0.034	0.091	0.227	0.167	0.106
	$H_{(DC)}$	0.517	0.455	0.455	0.333	0.455
	$H_{(unb)}$	0.532	0.537	0.680	0.632	0.593
	PIC	0.418	0.444	0.574	0.535	0.500
FH2010		(29)	(11)	(11)	(15)	(66)
	1	0.190	0.045	0.182	0.167	0.159
	2	0.362*	0.455*	0.455*	0.200	0.356
	3	0.328	0.364	0.318	0.533*	0.379*
	4	0.103	0.091	0.045	0.067	0.083
	5	0.017	0.045	0.000^{+}	0.033	0.023
	$H_{(DC)}$	0.793	0.364	1.000	0.533	0.697
	$H_{(unb)}$	0.727	0.680	0.688	0.664	0.702
	PIC	0.663	0.586	0.593	0.598	0.642
FH2006		(27)	(11)	(9)	(14)	(63)
	1	0.185	0.273	0.500*	0.464*	0.317
	2	0.481*	0.364*	0.278	0.286	0.389*
	3	0.259	0.364*	0.222	0.250	0.262
	4	0.074**	0.000	0.000	0.000	0.032
	$H_{(DC)}$	0.741	0.727	0.778	0.714	0.746
	$H_{(unb)}$	0.674	0.693	0.660	0.664	0.684
	PIC	0.606	0.587	0.553	0.568	0.615
FH2144		(29)	(10)	(10)	(15)	(64)
	1	1.000	1.000	1.000	1.000	1.000
	$H_{(DC)}$	0.000	0.000	0.000	0.000	0.000
	$H_{(unb)}$	0.000	0.000	0.000	0.000	0.000
	PIC	0.000	0.000	0.000	0.000	0.000
FH2054		(32)	(11)	(9)	(15)	(67)
	1	0.250	0.318	0.278	0.300	0.276
	2	0.453*	0.364*	0.500*	0.400*	0.433*
	3	0.203	0.273	0.056	0.133	0.179
	4	0.078	0.045	0.167	0.167	0.104
	5	0.016**	0.000	0.000	0.000	0.007
	$H_{(DC)}$	0.813	0.727	1.000	0.733	0.806
	$H_{(unb)}$	0.695	0.723	0.680	0.729	0.699
	PIC	0.633	0.627	0.583	0.652	0.641

**Private allele (found in just one population); *most frequent allele in each locus; $^{+}$absent allele in just one population.

Table 3. Results of the chi-square test (χ^2) for samples of each pair of maned wolf populations according to Brazilian region.

Loci	Allele No.	χ^2	d.f.	P
DF/GO & Belo Horizonte				
FH2088	3	5.293	2	0.07090
FH2010	5	3.157	4	0.53184
FH2006	4	3.272	3	0.35153
FH2054	5	1.543	4	0.81908
Total		13.265	13	0.42755
DF/GO & Triângulo Mineiro				
FH2088	3	7.905	2	0.01921*
FH2010	5	1.354	4	0.85216
FH2006	4	7.779	3	0.05081
FH2054	5	3.311	4	0.50719
Total		20.348	13	0.08686
DF/GO & São Paulo				
FH2088	3	6.651	2	0.03596*
FH2010	5	4.374	4	0.35772
FH2006	4	8.905	3	0.03058*
FH2054	5	2.848	4	0.58353
Total		22.779	13	0.04441*
Belo Horizonte & São Paulo				
FH2088	3	1.116	2	0.57240
FH2010	5	5.226	4	0.26486
FH2006	3	1.954	2	0.37649
FH2054	4	2.956	3	0.39848
Total		11.252	11	0.42242
Belo Horizonte & Triângulo Mineiro				
FH2088	3	2.658	2	0.26469
FH2010	5	3.200	4	0.52493
FH2006	3	2.248	2	0.32496
FH2054	4	4.610	3	0.20271
Total		12.716	11	0.31228
São Paulo & Triângulo Mineiro				
FH2088	3	0.503	2	0.77749
FH2010	5	4.850	4	0.30301
FH2006	3	0.067	2	0.96705
FH2054	4	0.930	3	0.81830
Total		6.350	11	0.84901

*Significance level: $P \leq 0.05$. DF = Distrito Federal; GO = Goiás.

Table 4. Results of the chi-square test (χ^2) for all loci among the four Brazilian maned wolf populations.

Loci	Allele No.	χ^2	d.f.	P
FH2088	3	13.041	6	0.04239*
FH2010	5	9.986	12	0.61722
FH2006	4	15.575	9	0.07630
FH2054	5	8.727	12	0.77527
Total		46.727	39	0.18475

*Significance level: $P \leq 0.05$.

Another genetic assay involved 20 protein loci in 28 maned wolves from an area in northeastern São Paulo State. Although the area was divided into three sub-areas, considering the Mogi-Guaçu and Pardo Rivers as possible barriers to gene flow, the polymorphism and heterozygosity levels found were similar to those observed by Moreira et al. (1998) for the maned wolf and by other authors for other species of free-living canids, and the samples of each sub-area and the total sample exhibited genotype frequencies consistent with the genetic equilibrium model showing no evidence of inbreeding, nor population subdivision (De Mattos et al., 2004).

Although the results obtained for isozymes and microsatellite markers showed a considerable genetic diversity in maned wolf populations (except the one studied by Rodrigues, 2002), mtDNA markers suggest that this species shows the lowest mtDNA diversity (Π = 0.0026), of all the carnivores already investigated (Prates Jr et al., 2004).

Considering the analysis for the four polymorphic loci in the total maned wolf population, the mean allele number (A) was 4.3 (± 0.5) and the mean expected heterozygosity (H_E), 0.669 (± 0.026). These results are in accordance with those observed for *Lycaon pictus (*A = 3.9; H_E = 0.61) (Girman et al., 2001), *Canis lupus (*A = 4.5; H_E = 0.62) (Roy et al., 1994), *Canis rufus (*A = 5.3; H_E = 0.55), and *Canis mesomelas (*A = 5.0; H_E = 0.67) (Wayne, 1996). *Canis simensis*, probably the most endangered canid, with fewer than 500 individuals remaining in small and highly isolated populations restricted to Ethiopian highlands, showed lower values for the number of alleles and heterozygosity *(*A = 2.4; H_E = 0.24; Gottelli et al., 1994) than those found for the maned wolf. It is important to note that three of the five species shown above are considered threatened by IUCN (*C. rufus*, *C. simensis* and *L. pictus*) (Sillero-Zubiri et al., 2004), while the other two are protected by the U.S. government (Wayne, 1996).

The genetic variability results for this maned wolf sample, which is considered threatened with extinction, did not show any loss of variability when compared to studies of other canids (Francisco et al., 1996; Koskinen and Bredbacka, 2000; Altet et al., 2001).

IMPLICATION FOR CONSERVATION

The results obtained should be considered with caution, since they reflect the genetic variability of the maned wolf in a sample that was collected ten years ago. Despite the fact that no significant reduction of the genetic variability for the species was found, it does not mean that this process is not underway. The present study represents a base of comparison for future evaluations of the conservation status of the species. The insularization process of Cerrado

areas has accelerated in the last years, and if effective conservation measures are not soon adopted, future evaluations could reveal a severe threat to the species' survival. The results obtained in the present study open a new perspective in conservation genetic studies of the maned wolf. The use of a larger sample in the analysis of new short-tandem repeat markers may not only provide greater robustness to the results obtained, but also assist in the elaboration and execution of strategies in conservation programs and management of the maned wolf, such as those developed by Pró-Carnivoros and the AZB (Brazilian Zoo Association).

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REFERENCES

Altet L, Francino O and Sanchez A (2001). Microsatellite polymorphism in closely related dogs. *J. Hered.* 92: 276-279.

Blanquer-Maumont A and Crouau-Roy B (1995). Polymorphism, monomorphism, and sequences in conserved microsatellites in primate species. *J. Mol. Evol.* 41: 492-497.

Breen M, Jouquand S, Renier C, Mellersh CS, et al. (2001). Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Res.* 11: 1784-1795.

Bruford MW and Wayne RK (1993). Microsatellites and their application to population genetic studies. *Curr. Opin. Genet. Dev.* 3: 939-943.

Coltman DW, Bowen WD and Wright JM (1996). PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. *Mol. Ecol.* 5: 161-163.

De Mattos PS, Del Lama MA, Toppa RH and Schwantes AR (2004). Populational genetic structure of free-living maned wolves (*Chrysocyon brachyurus*) determined by proteic markers. *Braz. J. Biol.* 64: 639-644.

Dietz JM (1984). Ecology and social organization of the maned wolf (*Chrysocyon brachyurus*). *Smithson. Contrib. Zool.* 392: 1-51.

FitzSimmons NN, Moritz C and Moore SS (1995). Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Mol. Biol. Evol.* 12: 432-440.

Francisco LV, Langston AA, Mellersh CS, Neal CL, et al. (1996). A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mamm. Genome* 7: 359-362.

Galbusera P, Dongenand ST and Matthysen E (2000). Cross-species amplification of microsatellite primers in passerine birds. *Conserv. Genet.* 1: 163-168.

Gemmell NJ, Allen PJ, Goodman SJ and Reed JZ (1997). Interspecific microsatellite markers for the study of pinniped populations. *Mol. Ecol.* 6: 661-666.

Girman DJ, Vila C, Geffen E, Creel S, et al. (2001). Patterns of population subdivision, gene flow and genetic variability in the African wild dog (*Lycaon pictus*). *Mol. Ecol.* 10: 1703-1723.

Gonela A (2003). Aplicação de marcadores microssatélites de *Sus scrofa* doméstica na caracterização genética de populações de *Sus scrofa* sp (porco-monteiro) e *Tayassu pecari* (queixada). Doctoral thesis, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto.

Gottelli D, Sillero-Zubiri C, Applebaum GD, Roy MS, et al. (1994). Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Mol. Ecol.* 3: 301-312.

Guyon R, Lorentzen TD, Hitte C, Kim L, et al. (2003). A 1-Mb resolution radiation hybrid map of the canine genome. *Proc. Natl. Acad. Sci. USA* 100: 5296-5301.

Hille A, Janssen IA, Menken SB, Schlegel M, et al. (2002). Heterologous amplification of microsatellite markers from colubroid snakes in European natricines (Serpentes: Natricinae). *J. Hered.* 93: 63-66.

Koskinen MT and Bredbacka P (2000). Assessment of the population structure of five finnish dog breeds with microsatellites. *Anim. Genet.* 31: 310-317.

Langguth A (1975). Ecology and evolution in south America canids. In: The Wild Canids (Fox MW, ed.). Van Nostrand Reinhold Co., New York, 192-206.
Marshall TC, Slate J, Kruuk LE and Pemberton JM (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7: 639-655.
Moore SS, Sargeant LL, King TJ, Mattick JS, et al. (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* 10: 654-660.
Moreira JR, Klautau-Guimarães N, Contel EPB and De Bem AR (1998). Estudo preliminar da variabilidade genética do lobo-guara (*Chrysocyon brachyurus*). Comunicado Técnico, *EMBRAPA/CENARGEN* 30: 1-10.
Nguyen TT, Menetrey F, Genini S, Nguyen VL, et al. (2005). Application of bovine microsatellite markers on Saola (*Pseudoryx nghetinhensis*). *J. Anim. Breed. Genet.* 122: 195-198.
Pepin L, Amigues Y, Lepingle A, Berthier JL, et al. (1995). Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity* 74: 53-61.
Prates PH Jr, Grisolia MNKG, Moreira JR and Bonatto SL (2004). Diversidade no DNA mitocondrial, história das populações e conservação do *Chrysocyon brachyurus* (Illiger, 1811). XXV Congresso Brasileiro de Zoologia, Brasília.
Primmer CR, Moller AP and Ellegren H (1996). A wide-range survey of cross-species microsatellite amplification in birds. *Mol. Ecol.* 5: 365-378.
Queller DC, Strassmann JE and Hughes CR (1993). Microsatellites and kinship. *Trends Ecol. Evol.* 8: 285-288.
Rodrigues FHG (2002). Biologia e conservação do lobo-guará na estação ecológica de águas emendadas, DF. Doctoral thesis, Universidade Estadual de Campinas, Campinas.
Rodrigues FM, Telles MP, Resende LV, Soares TN, et al. (2006). Transferability of short tandem repeat markers for two wild *Canid* species inhabiting the Brazilian cerrado. *Genet. Mol. Res.* 5: 846-850.
Roy MS, Geffen E, Smith D, Ostrander EA, et al. (1994). Patterns of differentiation and hybridization in north American wolflike canids, revealed by analysis of microsatellite loci. *Mol. Biol. Evol.* 11: 553-570.
Sanguinetti CJ, Dias NE and Simpson AJ (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 914-921.
Scribner KT, Gust JR and Fields RL (1996). Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Can. J. Fish. Aquat. Sci.* 53: 833-841.
Sillero-Zubiri C, Hoffmann CM and MacDonald DW (2004). Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan. IUCN/SSC Canid Specialist Group, Gland, Switzerland and Cambridge.
Swofford DL and Selander RB (1981). A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Centre for biodiversity, Illinois Natural History Survey, Champaign.
Vieira EM (1996). Highway mortality of mammals in central Brazil. Ciência cultura. *J. Braz. Assoc. Adv. Sci.* 48: 270-272.
Wayne RK (1996). Conservation genetics in the *Canidae*. In: Conservation genetics: case histories from nature (Avise J and Hamrick J, eds.). Chapman & Hall, New York, 75-118.
Williamson JE, Huebinger RM, Sommer JA, Louis EE Jr, et al. (2002). Development and cross-species amplification of 18 microsatellite markers in the Sumatran tiger (*Panthera tigris* sumatrae). *Mol. Ecol. Notes* 2: 110-112.
Wintero AK and Fredholm M (1995). Three porcine polymorphic microsatellite loci (S0075, S0151, S0158). *Anim. Genet.* 26: 125-126.