stricto SENSU
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

IDENTIFICAÇÃO DE ESPÉCIES DE CARNÍVOROS (MAMMALIA, CARNIVORA) UTILIZANDO SEQÜÊNCIAS DE DNA E SUA APLICAÇÃO EM AMOSTRAS NÃO-INVASIVAS

Paulo Bomfim Chaves

PUCRS
PRPPG
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

IDENTIFICAÇÃO DE ESPÉCIES DE CARNÍVOROS (MAMMALIA, CARNIVORA) UTILIZANDO SEQÜÊNCIAS DE DNA E SUA APLICAÇÃO EM AMOSTRAS NÃO-INVASIVAS

Paulo Bomfim Chaves

Orientador: Dr. Eduardo Eizirik

DISSERTAÇÃO DE MESTRADO

PORTO ALEGRE – RS – BRASIL

2008

AGRADECIMENTOS

Ao Prof. Eduardo Eizirik, cujo prazer em compartilhar seu vasto conhecimento contribuiu para a minha formação intelectual e profissional nesses dois anos de convívio.

Aos professores do Programa de Pós-graduação em Zoologia da PUCRS e à Maria Luiza Moreira, pelo esforço coletivo, muitas vezes sutil e despercebido, que tornaram o mestrado uma experiência acadêmica proveitosa para mim.

A todos que contribuíram com amostras biológicas tornando este projeto viável, especialmente os pesquisadores do CENAP/IBAMA, Fundação Pró-Carnívoros e à Dra. Márcia Jardim (Fundação Zoobotânica do Rio Grande do Sul).

À médica veterinária Raquel Von Hohendorff por getilmente auxiliar na coleta de fezes de carnívoros do Parque Zoológico de Sapucaia do Sul/Fundação Zoobotânica do Rio Grande do Sul.

À bióloga Vanessa Graeff por compartilhar os estimulantes dias de pesquisa em campo no Centro de Pesquisas e Conservação da Natureza PRÓ-MATA, bem como pela ajuda na identificação de pêlos de carnívoros.

Aos meus companheiros estudantes dos carnívoros pela incomensurável ajuda no laboratório, particularmente Alexsandra Schneider**,** Ana Carolina Escobar, Anelisie Santos, Cristine Trinca, Gabriel Macedo, Henrique Figueiró, Manoel Rodrigues, Mirian Tsuchiya, Paulo Prates Jr., Taiana Haag e Tatiane Trigo.

Ao competente time de professores e colegas do “Genoma”. O convívio diário com vocês fez destes dois anos em uma experiência singular; enriquecedora pessoal e profissionalmente. Muito obrigado!

À Simone Lóss, pelo companherismo e amor incondicional. Si, obrigado por ser sempre um porto seguro. Sem você os dias difíceis teriam sido intoleráveis e os alegres, apenas mais um.

À minha família, pela confiança, apoio e estímulo incessantes.

Àqueles cujos nomes não estão listados aqui, mas que contribuíram de alguma forma para a conclusão deste trablaho, esta linha dos meus “Agradecimentos” reconhece a sua contribuição.

À CAPES pela bolsa de mestrado concedida.

SUMÁRIO

RESUMO

Seqüências de DNA usadas na identificação de material biológico têm alcançado considerável popularidade nos últimos anos, especialmente no contexto dos códigos de barras de DNA. Aferir a espécie de origem em amostras de pêlos, penas, peles e particularmente fezes é um passo fundamental para os interessados em estudar ecologia e evolução de diversos animais com este tipo de amostra. Este é o caso em carnívoros, cujos hábitos furtivos e baixas densidades de algumas espécies evidenciam a importância de estudos baseados em amostras não-invasivas. Entretanto a atual escassez de ensaios padronizados de identificação de carnívoros freqüentemente dificulta a aplicação dessas amostras em larga escala e comparações de resultados entre diferentes localidades. No presente estudo nós avaliamos dois segmentos curtos (<250 pb) de DNA mitochondrial (mtDNA) localizados nos genes *ATP sintase 6* e *citocromo oxidase I* com potencial de servirem como marcodres-padrão para identificação de carnívoros. Entre um e 11 indivíduos de 66 espécies de carnívoros foram seqüenciados para um ou ambos os segmentos do mtDNA e analisados usando três diferentes métodos (árvore de distância, distância genética e análise de caracteres). Em geral, indivíduos conspecíficos apresentaram menor distância genética entre si do que em relação a outras espécies, formando agrupamentos monofiléticos. Exceções notáveis foram algumas espécies que divergiram recentemente, algumas das quais ainda puderam ser identificadas pelo método de caracteres, haplótipos espécie-específicos, ou reduzindo a abrangência geográfica das comparações (restringindo a análise a uma região zoogeográfica). Análises *in silico* adicionais, usando um segmento curto do *citocromo b* freqüentemente empregado em carnívoros, também foram realizadas com o intuito de comparar o desempenho deste segmento em relação aos outros dois propostos. Nós então testamos o desempenho destes segmentos na identificação de fezes de carnívoros por meio de três estudos de caso: (i) fezes de felinos de zoológico, objetivando-se verificar o potencial de contaminação das seqüencias com DNA da presa (coelho); (ii) fezes coletadas no Cerrado brasileiro contendo restos de presas (pêlos, ossos, penas), supostamente proveniente de lobo-guará, objetivando-se investigar a eficiência de identificação do predador e ocorrência de interferência do DNA da presa na identificação; e (iii) fezes coletadas em uma reserva na Mata Atlântica, também com o objetivo de avaliar a eficiência de identificação. Apesar de diferenças em alguns aspectos de sua performance, nossos resultados indicam que os dois segmentos propostos têm um bom potencial de servir como marcadores moleculares eficientes para identificação acurada de amostras de carnívoros ao nível de espécie.

APRESENTAÇÃO

A presente dissertação de mestrado, intitulada “Identificação de espécies de carnívoros (Mammalia, Carnivora) utilizando seqüências de DNA e sua aplicação em amostras não-invasivas” é apresentada como parte dos requisitos necessários para a obtenção do grau 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 gerar uma base padronizada de seqüências de DNA de carnívoros, avaliar sua a eficiência na identificação das espécies incluídas e testar o desempenho destas seqüências em amostras de fezes em diferentes contextos. Com base nos resultados obtidos e em informações da literatura, procurou-se contribuir para a consolidação de metodologia confiável para a identificação de espécies de carnívoros e de amostras biológicas de origem desconhecida, contribuindo assim para o estabelecimento de medidas adequadas para conservação desta ordem de mamíferos.

Esta dissertação é apresentada no formato de um manuscrito científico a ser submetido ao periódico *BMC Evolutionary Biology*.

DNA barcoding meets molecular scatology: an evaluation of short mtDNA sequences for standardized species assignment of carnivore noninvasive samples

PAULO B. CHAVES, VANESSA G. GRAEFF, MARÍLIA B. LION, LARISSA R. DE OLIVEIRA and EDUARDO EIZIRIK

A ser submetido ao periódico 'BMC Evolutionary Biology'

DNA barcoding meets molecular scatology: an evaluation of short mtDNA sequences for standardized species assignment of carnivore noninvasive samples

PAULO B. CHAVES[1], VANESSA G. GRAEFF[1], MARÍLIA B. LION[2], LARISSA R. DE OLIVEIRA[3] AND EDUARDO EIZIRIK[3]

[1] Programa de Pós-graduação em Zoologia, Pontifícia Universidade Católica do Rio Grande do Sul, Faculdade de Biociências, Av. Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil.

[2] Programa de Pós-graduação em Ecologia, Universidade de Brasília, Instituto de Ciências Biológicas, Departamento de Ecologia, Asa Norte, 70910-900 - Brasília, DF, Brazil.

[3] Laboratório de Biologia Genômica e Molecular, Pontifícia Universidade Católica do Rio Grande do Sul, Faculdade de Biociências, Av. Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil.

Key words: DNA barcoding, character-based, *COI*, *ATP6*, faeces, species identification

Running title: DNA identification of Carnivores

Corresponding author: Pontifícia Universidade Católica do Rio Grande do Sul, Laboratório de Biologia Genomica e Molecular, Faculdade de Biociências. Av. Ipiranga 6681, prédio 12C, sala 172, Partenon, 90619-900 - Porto Alegre, RS - Brasil

Telefone: (51) 33203500 Extension: 4685 Fax: (51) 33203612

e-mail: eduardo.eizirik@pucrs.br

Abstract

DNA sequences for species-level identification of biological materials have achieved considerable popularity in the last few years, especially in the context of the DNA barcoding initiative. Species assignment of biological samples such as hairs, feathers, pelts and particularly faeces is a crucial step for those interested in studying ecology and evolution of many species with these samples. This is especially the case for carnivores, whose elusive habits and low densities highlight the importance of studies based on noninvasive samples. However, the current lack of standardized assays for carnivore identification often poses challenges to the large-scale application of this approach, as well as the cross-comparison of results among sites. Here we evaluate the potential of two short (<250 pb) mitochondrial DNA (mtDNA) segments located within the genes *ATP synthase 6* and *cytochrome oxidase I* as standardized markers for carnivore identification. Between one and eleven individuals of 66 carnivore species were sequenced for one or both of these mtDNA segments and analyzed using three different approaches (tree-based, distance-based and character-based), in conjunction with sequences retrieved from public databases. In most cases, conspecific individuals had lower genetic distances from each other relative to other species, resulting in diagnosable monophyletic clusters. Notable exceptions were the more recently diverged species, some of which could still be identified using diagnostic character attributes, species-specific haplotypes, or by reducing the geographic scope of the comparison (restricting the analysis to a single zoogeographic region). Additional *in silico* analyses using a short *cytochrome b* segment frequently employed in carnivore identification were also performed aiming to compare performance to that of our two focal markers. We then tested the performance of these segments in the identification of carnivore faeces via three case studies: (i) felid faeces collected in a controlled zoo experiment, aimed at assessing whether DNA from rabbit prey would contaminate the resulting sequences; (ii) field-collected faeces from the Brazilian Cerrado presumed to be from maned wolves and containing prey remains (hairs, bones, feathers), aimed at investigating the efficiency of predator identification and occurrence of prey DNA interference; and (iii) field-collected scats from an Atlantic Forest study site, also addressing the issue of PCR success rate and identification efficiency. In spite of some relevant differences in some aspects of their performance, our results indicate that both of our focal segments have a good potential to serve as efficient molecular markers for accurate species-level identification of carnivore samples.

Introduction

Many of the currently recognized 287 carnivore species are sympatric, nocturnal and elusive (Wozencraft 2005), making it sometimes difficult to investigate their habits or to obtain unmistakably identified biological material for ecological and genetic studies (Davison *et al.* 2002, Palomares *et al.* 2002). Although some species are fairly abundant (*e.g.* coyotes, raccoons, and some foxes), many are naturally rare (*e.g.* the pampas cat), or currently face threats that eventually make them scarce in some localities (*e.g.* the jaguar). While many carnivores are remarkably hard to observe, their scats (faeces) may be fairly common to encounter in the field. Faeces can provide information on diet, physiology, geographic distribution, habitat use and parasite load, which are often problematic to collect employing other means. In addition, since faeces contain cells that are sloughed off from the gut wall (Albaugh *et al*. 1992), they are now a widely used source of DNA for studies employing molecular markers. Advances in the efficacy and reliability of DNA extraction protocols in the last 15 years have allowed researchers to apply molecular techniques based on faecal DNA to carry out comprehensive genetic and ecological analyses of free-ranging populations, addressing issues such as phylogeography, demographic history, hybridization events, inbreeding effects, mating systems, dispersal patterns and social structure (DeSalle & Amato 2004). Genetic surveys that rely on scat samples as the source of DNA are commonly named molecular scatology studies (Reed *et al.* 1997, Kohn & Wayne 1997). In a broader context, scats have proven to be a powerful, noninvasive source of information on carnivore communities, not only because they are easier to collect and less disturbing to the animals than other materials, but also because their international transport is exempt from CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) control for Appendix I species (Gerloff *et al.* 1995).

Identifying faeces at the species level has always been a key requirement for all further analyses in which scats are the source of information, such as carnivore behavioral ecology and food habits (Major *et al.* 1980, Johnson *et al.* 1984). Although faecal identification has been historically performed using features such as size, shape, scent, or dietary content (Zuercher *et al*. 2003, Prugh & Ritland 2005, Napolitano *et al.* 2008), these approaches have been found on several occasions to be limited and/or error-prone for at least some groups of carnivores (Hansen & Jacobsen 1999, Bulinski & McArthur 2000, Farrell *et al.* 2000, Davison *et al.* 2002, Reed *et al*. 2004, Onorato *et al*. 2006, Fernandes *et al. in press*). Therefore, morphology-based identification approaches will probably not be

fully effective in areas where multiple carnivores co-occur (many of which have indistinguishable scats), nor will they be amenable to comprehensive standardization across studies or field sites. An alternative method is to use macro- and microscopic features of self-ingested predator guard hairs to identify carnivore scats. Although this technique has been shown to be informative in several cases (*e.g.* Onorato *et al*. 2006), at least two problems prevent it from being employed as a global standard for a large number of species: (i) carnivore species vary extensively in the degree and pattern of self-cleaning behavior, so that their faeces often do not contain predator hairs; and (ii) the number of morphological characters currently surveyed in these hair-based approaches is limited, leading to the observation that not all species can be distinguished using these features (Harrison 2002, V. Graeff *et al*. unpublished data).

In this context, short diagnostic mitochondrial DNA (mtDNA) sequences (<250 bp), recently referred to as minimalist DNA barcodes or mini-barcodes (Hajibabaei *et al*. 2006), can be a powerful tool for identifying carnivore scats at the species level (Farrell *et al.* 2000, Mills *et al.* 2000, Davison *et al.* 2002). In addition to presenting higher amplification success than nuclear segments (since each cell can contain hundreds or thousands of mtDNA copies) or long mtDNA fragments (since PCR efficiency is higher with shorter products, especially using a template of degraded DNA), mini-barcodes tend to have high inter-specific divergence and less homoplasy than microsatellite markers (occasionally used for species identification – *e.g.* Pilot *et al.* 2007). They are therefore suitable markers for species identification, even when DNA quality is low, such as in the case of noninvasive samples (Hajibabaei *et al*. 2006, Broquet *et al.* 2007).

In addition to their application in noninvasive samples, mini-barcodes can be employed to identify artifacts made of (or materials derived from) endangered species, and thus to aid in the monitoring of market products (*e.g.* fur and souvenirs) and international trading that threaten these organisms, by providing a fast and specialist-independent identification tool. The application of such an approach can enhance our ability to curb illegal traffic operations, as well as contribute to other relevant forensic and management issues, such as the identification of livestock predators (Bartlett & Davidson 1992, Palumbi & Cipriano 1998, Farrell 2000, 2001, Breuer 2005). Short diagnostic segments may also be useful in other research endeavors that rely on biological materials containing DNA of poor quality and quantity, such as hairs, museum skins, bones, and palaeontological remains,

120 which are often employed in conservation genetics and molecular evolution investigations (Wayne *et*
121 *al.* 1999, Pääbo *et al.* 2004).

122 Although many studies have attempted to assign faeces to species using molecular
123 approaches, and to thus characterize local carnivore communities or to track some species in a given
124 habitat, none to date has focused on establishing a standardized method that can be used for this
125 mammalian group worldwide. Instead, several papers have addressed faecal species assignment in
126 carnivores employing different methods such as thin-layer chromatography (TLC) of faecal bile acids
127 (Fernandez *et al*. 1997, Khorozyan *et al*. 2007) and a diverse array of PCR primers, genomic
128 segments and molecular approaches, including PCR-RFLP (Paxinos *et al.* 1997, Hansen & Jacobsen
129 1999, Riddle *et al.* 2003, Gómez-Moliner *et al.* 2004, Vercillo *et al.* 2004, Colli *et al.* 2005, López-
130 Giráldez *et al.* 2005, Nagata *et al.* 2005, Bidlack *et al.* 2007, Lucentini *et al.* 2007), various mtDNA
131 sequences (Wasser *et al.* 1996, Farrell *et al.* 2000, Mills *et al.* 2000, Davison *et al.* 2002, Sugimoto *et*
132 *al.* 2006, Mukherjee *et al.* 2007), nuclear VNTRs (Reed *et al.* 1997, Domingo-Roura 2002, Wan *et al*.
133 2003, Pilot *et al.* 2007) and species-specific primers (Palomares *et al.* 2002, Dalén *et al.* 2004,
134 Fernandes *et al. in press*) (Figure 1). The current lack of standardized molecular assays can be seen
135 as an obstacle to the development of more rigorous and comparable strategies for species-level
136 identification. In particular, sequence-based identification approaches should become more efficient
137 and reliable if different studies employ the same genomic segments, so that multiple groups can
138 contribute to the growth of common data bases, which in turn should enhance the probability that all
139 extant variation in that region will be sampled, aiding in reliable identification and error-checking.
140 However, until now the trend has been for independent development of markers, with lack of overlap
141 among the various segments employed even in a single gene, such as the *cytochrome b* (Figure 2).

142 Standardization of universal segments and primers suitable for carnivore species identification
143 worldwide can clearly help save time spent in initial steps of molecular scatology studies. In addition,
144 standardized DNA sequences can be easily deposited and recovered from DNA data bases such as
145 GenBank and BOLD (Barcode of Life Data System) (Ratnasingham & Hebert 2007), enhancing the
146 link between ascertained voucher specimens, museum collections and molecular identification tools,
147 and thus improving the reliability and potential for cross-comparison of species assignments for field-
148 collected samples in different regions or studies. In spite of the clear potential for mutual benefit in the
149 integration between the DNA barcoding approach (species level identification through the use of

standardized DNA sequences) and the application of molecular scatology for improving our understanding of wildlife biology, this interface has not yet been explored in a systematic manner, to verify the feasibility of developing a comparable, reliable and rigorous strategy for the identification of noninvasive samples.

Here we report an in-depth investigation of the performance of two short mtDNA sequences for carnivore species assignment, employing a large data base that covered much of the family-level diversity of the Carnivora, and included recently diverged sister-taxa and species with complex evolutionary histories (*e.g.* deep phylogeographic partitions). Our study focused on two central questions: (i) Do short mtDNA sequences have sufficient inter-specific divergence to discriminate among carnivore species with high sensitivity and specificity?; and (ii) Are these segments effective for carnivore species identification using faecal DNA (which is not only often degraded and present at low concentrations, but also comprises a mixture derived from the predator, prey, parasites, and endosymbiont microorganisms)? To address these questions, we initially assembled and analyzed comprehensive DNA reference alignments for two segments, and then investigated their performance for the species level identification of faeces collected from known captive animals, as well as unidentified scat samples collected in the field.

Materials and Methods

Background on the selected genomic segments

Several studies involving the identification of carnivore species have focused on the use of coding and non-coding mtDNA segments such as *cytochrome b* (*cytb*), control region and 16S rRNA (Figure 1 and Table S1), mainly because mtDNA is a more abundant template than nuclear segments, and it exhibits relatively high mutation rates. The latter property, along with its smaller effective population size relative to the nuclear genome, leads to a higher probability of identifying species-specific diagnostic sites even in short DNA segments (Hajibabaei *et al.* 2007a). Although no study to date has used the *cytochrome c oxidase I* gene (*COI*) for this purpose, a specific 658 bp portion of *COI*, currently referred to as the standardized metazoan DNA barcode, has been shown to be one of the most variable coding sequences within the mtDNA, and thus diagnostic for many animal species (Hebert *et al*. 2003, Mueller 2006, but see Rubinoff *et al*. 2006 for a critical viewpoint). Recently, Hajibabaei *et al*. (2006, 2007b) have demonstrated that even short (< 200 bp) *COI* and *cytb* segments,

or mini-barcodes, can deliver species-level identification with high efficiency and can be useful in samples whose DNA is degraded.

In a parallel effort, our laboratory has developed an assay which employs a short (*ca.* 130 bp) segment of the *ATP synthase 6* (*ATP6*) gene (including a portion that overlaps with *ATP8*) to identify carnivore scats, which was initially applied in a study focusing on puma (*Puma concolor*) *vs.* jaguar (*Panthera onca*) samples (Haag *et al*. in prep.). This segment was originally selected for being variable among available carnivore species and also positioned outside the *Panthera* nuclear mtDNA insertion (*numt*) of ~12.5 Kb, which encompasses both *COI* and *cytb* genes (Kim *et al.* 2006), and also the *Felis catus numt*, which contains *COI* (Lopez *et al.* 1996). In the former case, amplification of the nuclear copy in lieu of (paralogous sequence) or along with the mtDNA copy (chimeric sequence), might hinder identification of jaguars, leopards, lions, tigers and snow leopards (see Figure 2).

We thus started from the premise that three mtDNA segments (*cytb*, *COI* and *ATP6*), or sub-regions of them, could be used to develop standardized markers for carnivore identification. Each of them presents a set of assets and limitations: (i) *cytb* has already been used in several studies for carnivore identification, although no standardized segment has been fully established, nor has its resolving power been assessed in a systematic manner; (ii) *COI* has not yet been employed for this purpose, but its promise resides in the vast worldwide effort to accumulate reliable sequences of the DNA barcode portion for all animal species (in many cases linked to voucher specimens), and its resolving power shown for other taxa; and (iii) *ATP6* has been successfully used in our laboratory for carnivore scat identification, but its resolving power has not been tested in a broader context. While the *ATP6* segment employed in our assays is already quite short, an equivalent fragment should be established for *COI* and *cytb*. We thus focused our empirical investigation on a comprehensive comparison of the *ATP6* marker and a sub-segment of the *COI* barcode, and also performed an *in silico* assessment of their informative power relative to a short segment of the *cytb* often used in molecular scatology (Farrell *et al.* 2000).

Database assembly and primer design

For the *COI* segment, all 184 DNA sequences of the barcode-portion of this gene belonging to a carnivore available in the BOLD data base were downloaded. The 658 bp alignment flanked by Folmer *et al.*'s (1994) primers was screened for the most polymorphic 200 bp-long segment using

DnaSP 4.0 (Rozas *et al.* 2003) by moving a "sliding window" one 1 bp forward from the first 200 bp segment until the last. This resulted in 459 segments of 200 bp analyzed for the number of variable sites. The absolute number of variable sites was recorded and plotted on a graph (Figure 3a). In order to eliminate a possible bias due to the excess of some over-represented species, the same analysis was also performed using an alignment containing only one representative sequence of each one of 65 species included in the initial data set (Figure 3a). After detecting the most variable 200-bp segment within the *COI* barcode segment, we searched for a conserved portion nearby to anchor the primers. Since the best segments were found to be in the 3' portion of the alignment, and the frequently used nearby reverse primer HCO2198 (Folmer *et al*. [1994]) displayed a good match with available carnivore sequences, we decided to keep this primer, and to only design a new internal forward primer. We then selected 13 sequences that were representative of carnivore evolutionary diversity to use as an operational alignment for the design of a primer with a conserved 3' annealing position (Figure S1).

To evaluate the informative power of the *ATP6* segment relative to that of *cytb* and *COI*, we compared the variability of a longer fragment containing *ATP6+ATP8* (so as to go beyond the boundaries of the marker applied by Haag *et al*. [in prep.] in both directions) to that of the latter two regions. To accomplish this, we downloaded 50 complete carnivore mitochondrial genomes and created an alignment of each one of these three segments, which was analyzed using a sliding window approach as described above. The initial portion of the *ATP8-ATP6* segment was found to be highly variable among species, and actually more so than any equivalent segment of *cytb* or *COI* (Figure 3b). Since the *ATP6* marker described by Haag *et al.* (in prep.) to distinguish puma *vs.* jaguar scats already lay in this variable region (see Figure 3b), we chose to keep the same segment, but slightly changed the annealing position of the forward primer. This was done to increase the probability of specific amplification in all carnivore families, since we observed that the primer ATP6-DF2 (Haag *et al*. in prep.) did not have a perfect match near its 3' end when compared to the broad sample of carnivore sequences evaluated here. Subsequently, empirical tests led us to also design a new reverse primer for this segment (see Results), in a continuous attempt to achieve efficient amplification across all carnivores.

New primers for the *COI* and *ATP6* markers were designed using Primer3 (Rozen & Skaletsky 2000) aiming to maximize amplification of carnivore DNA. This was attained by selecting annealing

targets with the maximum possible number of matches with all carnivores in the reference DNA alignment. As it was impossible to find primer anchor sites with zero variability, a set of criteria was applied to conduct primer design. For example, when a position in the alignment was variable among carnivores, we preferred the bases to form G-T bonds, which are not as stable as A-T but are considerably better than a C-A bond (Palumbi 1996). The frequency of the identified variants was also taken into account, so that more attention was devoted to sites exhibiting extensive variation across many carnivores relative to those in which variation was seen in few species. In addition, sites that showed variation only in pinnipeds (marine carnivores or Pinnipedia) were not considered to be a serious issue, as the focus of this effort were the more speciose terrestrial families of this order. Finally, we also attempted to keep annealing temperatures as high as possible to increase PCR specificity and to thus minimize the probability of amplification of prey DNA (King *et al.* in press).

In addition to *ATP6* and *COI*, we comparatively assessed the informative power of the 110 bp *cytochrome b* segment described by Farrell *et al.* (2000) and used by other authors to identify carnivore scats. The amplicon size of this segment is 170 bp, but the primers span a total of 60 bases, so that the information content of the fragment is restricted to the central 110 bp. For this set of analyses, we downloaded all 2,836 sequences found in a GenBank search with the terms “Carnivora” and “*cytochrome b*”. In a first visual inspection, non-carnivore sequences, Pinniped sequences, and the excess of sequences of some species (*e.g. Canis familiaris* and *Vulpes vulpes*) were removed, resulting in a file containing 1,827 sequences. This file was partitioned into three subsets of 609 sequences each, prior to independent alignment which was performed in three separate PCs. Each one of these three files contained the exact Farrell *et al.*’s segment in the first line to facilititate finding the block in the alignment to be analyzed. Aligned files were then combined again and only the 110 bp block was kept. In a final attempt to reduce redundancy, we searched for identical haplotypes within each species and kept only one representative per haplotype in the alignment, resulting in a final file containing 703 sequences of terrestrial carnivores.

Laboratory procedures

An extensive DNA sequencing effort was carried out to generate the *COI* and *ATP6* carnivore data bases. A panel of 33 carnivore species belonging to seven families was selected to compose a reference sequence alignment, which was complemented by data retrieved from public data bases.

For each of the focal 33 species, we sequenced one to eleven representatives of known geographic origin (using tissue samples such as blood, liver, skin or muscle), making up a total of 206 analyzed individuals. Whenever possible, we selected individuals that maximized the geographic representation of each species, in order to account for the extant genetic diversity and possible phylogeographic partitions occurring in these taxa, which could potentially hamper their accurate identification with respect to close relatives. The primers were subsequently tested for PCR amplification and sequencing in 35 other carnivore species (representing seven additional families) to assess their performance in a broad phylogenetic spectrum within this mammalian order.

Total genomic DNA was extracted from tissues with standard proteinase K/phenol-chloroform protocol (Sambrook *et al.* 1989). All PCR reagent cocktails, excluding DNA, were assembled in a dedicated room physically isolated from DNA extracts and PCR products. This room was periodically sterilized through 15 min overhead UV radiation. PCR reactions were performed in a PTC-100 thermocycler (MJ Reasearch) with the following conditions: *ATP6* – PCR reactions contained 1 to 5 μl of template DNA (empirically diluted), 1X PCR buffer, 100 μM of each dNTP, 8.0 pmol of each primer, 1.5 mM $MgCl_2$, 1.0 U of Taq DNA Polymerase (Invitrogen) and dH_2O to complete a 20 μl final volume. Cycling temperatures were: initial denaturation at 94ºC/3 minutes, followed by 94ºC/45 seconds, 60ºC/45 sec (touchdown - 1ºC/10 cycles), 72ºC/1 min 30 sec, and 30 cycles at 94ºC/45 sec, 50ºC/45 sec, 72ºC/1 min 30 sec, and a final extension at 72ºC/3 min. *COI* – reactions were set up with the same amount of reagents with *ATP6*, except $MgCl_2$ (2.0 mM). Cycling conditions were: initial denaturation at 96ºC/1 min, followed by 40 cycles of 94ºC/30 sec, a within-cycle decreasing annealing step of 50ºC/20 sec, 48ºC/05 sec, 46ºC/05 sec, 44ºC/05 sec, 42ºC/05 sec, 40ºC/20 sec, extension at 72ºC/1 min 30 sec, and a final extension step at 72ºC/3 min. Blank PCR controls were used in all reactions to monitor the occurrence of contamination.

Amplification products were run in 1% agarose/TBE gels stained with ethidium bromide or GelRed (Biotium). PCR products showing a single band of the predicted size were purified using Polyethylene glycol precipitation (20% PEG 8000, 2.5 M NaCl) followed by 70% ethanol washing and water elution in a 10 μl final volume (only for *COI*). We observed that most attempts of purifying the *ATP6* segment with PEG 8000 resulted in loss of PCR products in the process, probably because PEG 8000 is inefficient at precipitating short DNA molecules (Paithankar & Prasad 1991). The *ATP6* segment was thus sequenced without the need of purification by using 0.5 to 1.0 μl of PCR for cycle-

sequencing reactions. Sequencing of both strands was performed in a MegaBACE1000 automatic system using 5 pmol of primer, 2-5 μl for purified PCR, and the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) to a final volume of 10 μl as recommended by the kit manufacturer.

Faecal DNA case studies

We tested the suitability of the *COI* and *ATP6* segments in faecal DNA identification of carnivores in order to evaluate their efficiency in low quality/quantity samples, as well as to verify the occurrence of interference of prey DNA on predator identification. Scats were collected in various settings (see below) and stored at – 20º C in 50 ml or 15 ml polypropylene vials containing either silica beads or 94-100% ethanol. DNA was purified with the QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's protocol in a separate laboratory area, within a UV-sterilized laminar flow hood dedicated to noninvasive DNA extraction. We prioritized the surface of scat samples for DNA isolation as it seems to contain more predator cells than the inner portion (Ball *et al.* 2007). From each faecal sample, one or both mtDNA segments were PCR-amplified as previously described.

Case study 1 – Identifying felid faeces in a controlled zoo experiment: Nineteen felids belonging to six different species (three servals [*Leptailurus serval*], two ocelots [*Leopardus pardalis*], four jaguars [*Panthera onca*], two leopards [*Panthera pardus*], three tigers [*Panthera tigris*] and five pumas [*Puma concolor*]), kept at a zoo in southern Brazil (Parque Zoológico de Sapucaia do Sul/FZB-RS, Rio Grande do Sul state) were fed with rabbit one day prior to sample collection. Fresh scats were collected the following morning during the routine clean-up procedure. These samples were used to assess the interference of prey DNA, which is likely co-extracted with predator genomic material, in downstream PCR amplification and DNA sequencing.

Case study 2 – Field-collected maned wolf faeces: In the course of a field study of maned wolves (*Chrysocyon brachyurus*) in the Cerrado biome of central Brazil, 167 faeces suspected to be from this canid were collected from January to August 2006 in three nature reserves near the Brazilian capital, Brasília (Parque Nacional de Brasília 15º 40' 43" S/48º 11' 53" W, Estação Ecológica de Águas Emendadas 15º 37' 28" S/47º 40' 15" W and Fazenda Águas Limpas 16° 1' 34" S/48° 3' 45"W). Of

these samples, we selected 82 scats in which prey remains (*e.g.* bones, feathers, claws, scales, and hairs) could be visually identified. This indicated that these samples should contain a substantial amount of DNA originating from vertebrate prey, thus providing an opportunity for co-amplification along with the predator template. After DNA extraction from each of these faecal samples, their prey content was visually analyzed to assess whether non-carnivore DNA-based identification matched the observed dietary items. This was performed by washing the scats in water over a 0.1 mm sieve, sifting away the bile powder, and separating the prey contents manually. Contents were then dried in an oven at 60 ºC, stored in paper envelopes and then broadly classified as bones, hairs, feathers, scales, teeth or some specific body part (*e.g.* foot or beak). No detailed identification of the prey items was attempted, as the primary goal of this exercise was to assess whether prey DNA was being picked up by the PCR-based assay.

Case study 3 – Field-collected faeces from an Atlantic Forest study site: The Pró-Mata Research Center (Pró-Mata RC, 29° 29' 27" S/50° 11' 15" W) is a natural reserve located in the northeastern portion of Rio Grande do Sul state, southern Brazil. A diverse carnivore community, including felids (*Puma concolor* and three smaller cat species), canids (*Cerdocyon thous* and *Lycalopex gymnocercus*), two procyonids (*Nasua nasua* and *Procyon cancrivorus*) and at least one mustelid (*Eira barbara*), is known to occur in this area. Given the results observed in Case study 2 (see above and the "Results" section), we chose to further test the field perfomance of the *ATP6* marker using faecal samples collected at this study site, with its different carnivoran fauna and weather conditions (*i.e.* considerably more humid than the Cerrado). For that purpose, nineteen scats were collected opportunistically along roads and trails in this area in the year of 2006, and analyzed using this molecular assay to verify the precision of species assignment and the occurrence of potential prey contamination.

Data analyses

Sequences were visually edited and aligned using the programs BIOEDIT (Hall 1999) and CLUSTALW (Thompson *et al*. 1994). The degree of sequence similarity between species was assessed using the neighbor-joining (NJ) algorithm (Saitou & Nei 1987) as implemented in MEGA 3.1 (Kumar *et al*. 2004) with the Kimura-2-parameter (K2P) model (Kimura 1980) for DNA sequence

evolution. The degree of information support for clusters (or clades) was assessed for each segment separately and concatenated by bootstrap resampling of 1000 pseudodoreplicates (Felsenstein 1985). Sequences were considered to belong to the same species if all individuals *a priori* attributed to each species formed monophyletic groups or when the maximum intraspecific K2P distances was lower than the minimum interspecific distance calculated.

For sequences of known species which formed unresolved or weakly supported groups, we alternatively used a character-based approach (DeSalle *et al.* 2005) slightly modified from Rach *et al*. (2008), through which we searched for characteristic attributes (CAs) that distinguish closely-related species or those with sympatric distributions that could be misidentified with other methods. More specifically, we visually searched for diagnostic base pair mutations (or CAs) that were present in all individuals of one species but not in its closest outgroups in the distance tree. For instance, *Conepatus chinga* and *C. semistriatus* were not reciprocally monophyletic in any of the distance-based trees (*ATP6*, *COI* analyzed separately or concatenated), but we were able to find four diagnostic transitions in the *COI* segment that differentiate them unambiguously (see Results). This procedure relies on the same assumption made for PCR-RFLP assays, in which a restriction site is not shared between species but is present in all individuals of the same species. The advantage here is that a combination of CAs can be observed even if they are spread along a short stretch of DNA, whilst a restriction site must be a specific combination of characters in sequential order. Finally, sequences that did not group into any carnivore clade in the neighbor-joining tree were submitted to a BLAST search (Altschul *et al*. 1997) in order to identify the possible species or any closer relative. This analysis was particularly necessary for the highly divergent *COI* sequences observed in some maned wolf scats, likely derived from prey material (see Results).

Results

Evaluation of candidate segments and primer design

When the *ATP8-ATP6, cytb* and *COI* complete sequences were compared (Figure 3b), *ATP8-ATP6* displayed a peak of variable sites within its initial portion (first 250 segments of 200 bp each) while *cytb* was found to be more diverse in its intermediate region (between segments 515 and 570) and the *COI* barcode showed more variation in its final section (the last 60 segments). One initial observation emerging from this analysis was that the selected 110-bp segment of the *cytb* gene is not

located within the most variable portion of this locus (Figure 3b), suggesting that the identification potential of *cytb* may not have been fully explored with this marker. The analysis of the *COI* barcode fragment revealed a consistent pattern with all three alignments evaluated here (each of them including a different species composition – see Figure 3), supporting the conclusion that the 3' end presents more variable sites per 200 bp segment than any other portion of this segment (Hajibabaei *et al*. [2006 and 2007b] have also identified such a trend in other animal groups). To compare the variability in this selected *COI* region (absolute number of variable sites [V]=90) to that observed in the 110-bp *cytb* fragment evaluated here, we analyzed all 178 *cytb* segments of 200 bp that include this shorter region. The resulting mean variability (V=93.3, SD=2.1) was comparable to that recorded in the *COI* marker, indicating that the information content of both segments should be similar. In contrast, the variability of the *ATP6* region was considerably higher (see Figure 3b), highlighting the potential of this segment for discriminating recently diverged carnivore species.

The best primer pair found to amplify a short segment near the selected portion of *COI* was BC-F2 and HCO2198 (Folmer *et al*. 1994) (Table 1), producing a 239-bp amplicon (187 bp with primers excluded). Our focal *ATP6* segment was initially amplified and sequenced with primers ATP6-DF3 and ATP6-DR1 (Table 1), producing a 172-bp fragment (126 bp with primers excluded). As the analyses progressed, we empirically observed that *Procyon cancrivorus* samples were never amplified with this *ATP6* pair. Aligning the primers with the available *Procyon lotor* mtDNA genome (GenBank accession AB297804) we found mismatches in the first three bases at the 3' end of the primer ATP6-DR1. Therefore we designed a new reverse primer (ATP6-DR2) eight bases downstream from the previous oligonucleotide (see Table 1), with a 3' position that was invariable in a group of 49 different carnivore sequences retrieved from GenBank, including representatives of 11 families. Although this latter combination of primers produced an analyzable fragment of 134 bp (179 bp with primers), we only considered the 126 bp core segment that had been previously produced with the ATP6-DF3/DR1 combination for the majority of the included species. This was done so as to have a consistent data set for all species, minimizing the impact of missing information on the analyses.

The *Procyon cancrivorus* samples could be successfully amplified and sequenced with the ATP6-DF3/DR2 pair, which was then also evaluated in a broader survey of carnivoran lineages. This exercise consisted of sequencing one individual each of 35 additional carnivore species, representing 14 of the 15 currently recognized families in the Carnivora (Table S3). Eighteen of these samples

420 could be successfully amplified and sequenced with the pair ATP6-DF3/DR2, while 13 others worked
421 better using ATP6-DR1 as the reverse primer. This may indicate that the alternative use of both
422 reverse primers may be required to maximize the success rate when amplifying a diverse array of
423 carnivore species. Several faecal samples from case studies 2 and 3 were also evaluated using the
424 ATP6-DR2 reverse primer, leading to similar results as had been obtained using ATP6-DR1 (see
425 below).

426 In an equivalent effort to expand the reference database by adding this second panel of
427 carnivore species, the *COI* marker could be successfully sequenced for 24 out of 35 taxa (Table S3).
428 Only the representative of the family Nandiniidae (*Nandinia binotata*) and one felid species, *Profelis*
429 *aurata*, were not amplified for either the A*TP6* or *COI* segments. This probably does not rule out the
430 possibility of having success with these species in future attempts with these markers, since only one
431 DNA sample was available for each of them, in both cases having been stored for several years.
432
433 *Carnivore data sets*

434 Of a total of 206 tissue samples used to set up the initial reference data base containing 33
435 species of seven families, 180 were sequenced for the *ATP6* segment and 155 for the *COI* marker
436 (Table S2). All sequences generated here are retrievable from GenBank under accession numbers
437 XXXXXXXX-XXXXXXXX. These data sets were then complemented by all sequences generated in
438 the second round of amplification characterizing the additional 35 species (Table S3), as well as data
439 derived from the scat samples analyzed in the three case studies, and also all pertinent GenBank
440 entries. This effort led to final data sets of 448 sequences (110 species) for the *ATP6* marker and 419
441 (105 species) for the *COI* segment (Figure 4).

442 The ability of these two segments to identify carnivore species was initially investigated using
443 a tree-based approach (neighbor-joining clustering based on a simple distance matrix), which showed
444 that both markers could correctly discriminate most of the included taxa (Figure 4). Seventy-six
445 species could be tested for monophyly (*i.e.* at least two individuals of it were represented in the data
446 set) with *ATP6*, of which 65 (86%) formed monophyletic groups (bootstrap support [BS]: 15-99%). If a
447 BS threshold of 50% was established as a measure of robustness, 63 species (83%) could be
448 considered to be unambiguously identified with *ATP6*. An equivalent assessment of the *COI* marker
449 included 73 species, of which 62 (85%) formed monophyletic clusters (BS: 53-100%), indicating a

similar potential for correctly identifying unknown carnivore samples using both mtDNA segments. Sixty-one species could be assessed with both markers, revealing that they were congruent in 56 cases. In the five discordant species (monophyly observed with only one of the segments), one favored *COI* (margay was monophyletic with *COI* but not with *ATP6*) while the other four favored *ATP6* (South American gray fox, brown bear, domestic cat and lion) (see Figure 4 and Table 2).

We then performed a more detailed assessment of the performance of both segments focusing on the cases of discordance between them, as well as groups of closely related carnivores that were sampled in this study (Table 2). In most cases this involved Neotropical taxa, sometimes also including close relatives from other regions (*e.g.* in the *Panthera* genus). For this in-depth analysis, we compared the three methods commonly used to distinguish species in DNA barcoding initiatives (tree-based, distance-based and character-based approaches). It was apparent that the tree- and distance-based approaches tend to be congruent in all cases of success or failure, while the character-based method was successful in all cases resolved by the former methods, but also offered additional resolution for some species. This was particularly the cases of margay/ocelot, domestic cat/sand cat, pantherine felids, South American and Australian fur seals, Neotropical skunks, and some South American foxes (see Table 2). When the *ATP6* and *COI* segments were concatenated, the general pattern obtained with each fragment segment separately was maintained, with an improvement in bootstrap support (data not shown). Also, the ocelot (*Leopardus pardalis*) and margay (*L. wiedii*) became reciprocally monophyletic groups in the concatenated analysis, while this pattern was not obtained with either the *ATP6* or *COI* segments alone.

Comparing the tree-based to the character-based species identification method in four genera that were particularly challenging (Figure 4), we observed the following patterns: *Lycalopex* – of the five species analyzed, *L. vetulus* (hoary fox) was clearly distinguishable with both *ATP6* and *COI* using both methods; *L. griseus* (chilla fox) was distinguished from all its congeners by one diagnostic character in *ATP6*; one additional site in *ATP6* and two in *COI* distinguished *L. griseus* from *L. fulvipes* (Darwin's fox) and *L. culpaeus* (culpeo); the challenging species whose identification remain ambiguous is the trio culpeo, Darwin's fox and *L. gymnocercus* (pampas fox) as they could not be distinguished by any analysis. *Conepatus* – the two sampled hog-nosed skunk species (*C. chinga* and *C. semistriatus*) were not discernible in the tree as reciprocally monophyletic groups, but four transitions in *COI* were diagnostic between them. *Leopardus* - in spite of the ocelot and the margay

not forming reciprocally monophyletic groups with either segment, we could observe seven diagnostic characters between them (one in *ATP6* and six in *COI*). *Panthera* – jaguar (*P. onca*) samples did not form a monophyletic group in the tree but they displayed one characteristic attribute (CA) in *ATP6*; a group of six CAs in the *COI* segment distinguished leopard (*P. pardus*), lion (*P. leo*) and tiger (*P. tigris*) samples.

For the *cytb* analysis, the alignment of 703 sequences contained representatives of 164 terrestrial carnivore species recognized by Wozencraft (2005). Most of these species (n=109) were represented by more than one sequence each, and of those 76 (70%) formed monophyletic groups, 71 (65%) of which exhibited > 50% bootstrap support. However, we identified 37 species out of the total data set (23%) in which at least one individual seemed to have been misidentified. The majority of misidentified cases were found in very speciose genera such as *Genetta*, *Lycalopex, Martes and Mustela* (data not shown).

Case studies with faecal samples

Case study 1 - Zoo Carnivore faeces

Of the 38 attempts to obtain sequences from felid scats that contained prey (rabbit) remains, 29 were positive (76%). PCR success was slightly higher (82%) because three samples resulted in an amplicon but sequencing failed (Table S4). All successfully sequenced faecal samples (*ATP* = 16, *COI* = 13) resulted in the expected felid species, with no interference of prey DNA, in spite of compelling evidence of prey remains such as bones and hairs in those scats (data not shown). The *Panthera tigris COI* sequence was shorter than expected (116 bp), most likely due to ordinary sequencing error rather than interference of alien DNA.

Case study 2 - Maned wolf faeces

The 82 samples selected for this study were initially analyzed with the *ATP6* marker, resulting in good sequences for 65 of these samples (79%). Three of these samples were identified as originating from domestic dogs (see Figure 4) and thus excluded from the *COI* assessment and prey content analysis. Out of the remaining 79 samples, only 44 (56%) could be successfully sequenced with *COI*. If positive PCRs resulting in dissatisfactory sequences were also taken into account, the success rate of the *COI* marker reached 70%, still lower than that observed for the *ATP6* segment.

Fifteen samples (19%) were negative for both *ATP6* and *COI* PCR amplifications, suggesting that either there was no suitable DNA template or that extracts contained PCR inhibitors. In addition to the three samples identified as produced by domestic dogs (PN145, PN150 and ES177), two others were also found to have been deposited by a carnivore other than the presumed maned wolf (*Puma concolor* for sample FAL25, and *Felis catus* for ES10) (see Figure 4), corroborating previous findings that even experienced field researchers can misidentify scats on the basis of their appearance.

Seventy-four of the 79 faeces analyzed for dietary material contained prey remains of animal origin. The most common items were feathers (41/79, 52%), bones (39/79, 49%) and hairs (33/79, 42%). We saw no evidence of amplification of prey DNA with the *ATP6* segment, but in 11 samples (ten of which contained visually identifiable prey material), the *COI* sequences likely originated from prey material, as inferred using a BLAST search (Table S5). This analysis indicated that the amplified prey DNA likely belonged to marsupials, bats, rodents and fish, but it was impossible to identify them to species level due to the low observed identity relative to GenBank sequences. Although feathers were the most frequent evidence of animal prey consumption, no sequence closely related to birds was obtained, indicating no interference of this prey group in the predator identification.

Case study 3 - The Pró-Mata RC faeces

All 19 samples could be positively identified using the *ATP6* segment, and no interference of prey DNA was detected. Eight scats clustered in the crab-eating fox (*Cerdocyon thous*) group, ten were placed in the oncilla (*Leopardus tigrinus*) cluster, and one in the domestic dog/gray wolf (*Canis familiaris*) group. The identification of one sample as originating from a domestic dog is noteworthy, and indicates that it was likely produced by one of the four dogs that were observed near the study area during field work. Further analyses of these and other samples collected at this study site will be published elsewhere (Graeff *et al*. in prep.).

Discussion

Short stretches of mtDNA sequences have been shown to be very useful to identify biological samples derived from carnivore species (*e.g*. Palomares *et al*. 2002, Wetton *et al*. 2004, Zuercher *et al*. 2003). Here we have performed an in-depth investigation of this potential applying a “DNA barcoding” framework, and suggest that standardization of one or a few segments would be helpful to

accumulate comparable and reliable data, and to further evaluate the feasibility of accurate species-specific diagnosis on a worldwide scale. This would be particularly relevant as a means to promote rapid and accurate surveys of current geographic ranges of all carnivore species, as well as monitoring their shifting habitat occupancy in the face of human disturbance.

Several molecular methods for carnivore species identification have been historically suggested since the 1980s, especially those to distinguish faeces for further studies. Thin-layer chromatography of faecal bile acids, although recently used for some distantly related species (Taber *et al*. 1997, Ray & Sunquist 2001) has been shown to be unreliable due to intraspecific variation of bile acids depending on individual diet (Quinn & Jackman 1994, Jiménez *et al*. 1996). One practical shortcoming of implementing a large-scale TLC protocol for laboratories wherein DNA studies are being employed is that setting up the structure with reagents and equipment necessary for TLC assays may be less attractive than DNA-based methods. With respect to DNA-based protocols, it is noteworthy that a diverse array of DNA segments and methods are currently in use for carnivore species identification. One can find in the literature groups of species (*e.g. Martes*, *Mustela*, *Canis, Panthera*) that present many different approaches to distinguish their faeces, suggesting that research groups are often not employing protocols that have already been developed by others. This observation illustrates the issue that cross-laboratory validation of methods and results is not taking place on a regular basis, which limits the prospect of integration, comparisons among study sites and improvements in reliability. Microsatellites, in particular, are less amenable to such integration since they are prone to homoplasy and often show overlapping allelic ranges among species (Nauta & Weissing 1996). Moreover, for noninvasive DNA sampling, these markers tend to yield low amplification success and would often result in the loss of roughly 50% of identifiable samples, against 30-10% with mtDNA sequences (Broquet *et al*. 2007). However microsatellites would remain an option in the few cases where they can distinguish very closely related species that are not discernible using mtDNA markers.

To date, the most extensive attempt to characterize a carnivore community through faecal samples involved 16 species of six families (Fernandes *et al. in press*). Although this is a remarkable advance, a primer-specificity based method designed for a local carnivore community (Kurose *et al*. 2005, Fernandes *et al. in press*) cannot be extended to all extant 287 carnivore species representing 15 different families (Wozencraft 2005). This is because an underlying requirement to design species-

specific primers is to gather sequences for all the species with exclusive mutations in each of them. This also remains true for methods based on TLC, PCR-RFLP and microsatellites. In fact, even the primers presented here may have to be modified to cover all species (see below). However, the likelihood of them being simultaneously inserted in nuclear copies (*numts*) is lower than if they were tandemly arranged, and that at least one of them will likely suit the vast majority of molecular scatology studies to come.

The amplification success of the *ATP6* and *COI* segments was similar to that reported in several papers reporting on noninvasive samples (Broquet *et al*. 2007 and references therein), and the primer sets employed here were shown to perform well in a broad sample of carnivores. Better amplification of shorter segments has been commonly reported (*e.g.* Broquet *et al*. 2007) and that is one of the probable reasons why *ATP6* (172 bp) PCRs were more successful than those of *COI* (239 bp). The reported amplification success of the *cytb* fragment assessed here varied extensively among published studies, from relatively low rates of 59-60% (20/34 samples in Farrell *et al*. [2000], 12/20 in Miotto *et al*. [2007]) to higher standards of 83-89% (40/48 in Bhagavatula & Singh [2006], 55/62 in Adams *et al*. [2007]). Such variation is likely due to a C-A mismatch in the second base near the 3'end of forward primer in several carnivore species.

In addition to the success rate measured by the frequency of positive amplifications and reliable sequences, another relevant aspect to be considered is the likelihood of identifying prey rather than predator DNA. Onorato *et al*. (2006) reported DNA amplification of elk (*Cervus elaphus*) and whitetail deer (*Odocoileus virginianus*) in 11 out of 88 (13%) putative carnivore faeces using the *cytb* segment described by Farrell *et al*. (2000). Rabbits (Leporidae) and rodents (Muridae) were also reported to amplify in 8% (5/62) putative carnivore scats with Farrell *et al*.'s primers (Adams *et al*. 2007). While no prey amplification was observed with *ATP6*, this issue did indeed arise with our *COI* marker (see Case Study 2). The high rate of amplification of prey DNA with the *COI* segment may be attributed to three factors: first, the reverse primer (HCO2198) has been used as a universal primer to amplify DNA from marine invertebrates, insects, and vertebrates (Folmer *et al*. 1994, Hebert *et al*. 2003); second, the annealing step that produced the best PCR yields required low temperatures (50-40 ºC), increasing the probability of non-specific matches to take place; and third, although the 3' end in the forward primer is conserved in the *Chrysocyon brachyurus* reference sequence used to design the primer (see figure S1), six mismatches at other sites may be interfering negatively (Housley *et al.*

600 2006), particularly because there is likely “annealing competition” in a population of prey and predator
601 DNA molecules. The observation that both markers that present more liability to prey DNA
602 contamination (*cytb* and *COI*) employ conserved/universal reverse primers illustrates the expected
603 trade-off between the use of universal markers (usually implying relaxed PCR conditions) and
604 amplification of non-target DNA. Further primer design and empirical optimizations will thus likely be
605 required for standardized and reliable faecal DNA studies of carnivores employing these two
606 segments.

607 Another aspect to be considered is the informative content of the marker, as expressed by its
608 variability in the target group and discriminatory power as related to within- *versus* between-species
609 divergence. In fungi and salamanders, the *ATP8* and *ATP6* genes have been shown to evolve at a
610 slower pace when compared to *cytb* and *COI* (Min & Hickey 2007, Mueller 2007). Interestingly, our
611 results indicate that carnivores exhibit a seemingly opposite pattern, with the focal *ATP6* segment and
612 adjacent portions of this gene being considerably more variable than *COI* or *cytb* (Figure 3b). The
613 discriminatory power of this segment (as measured by species monophyly, bootstrap support and
614 diagnostic characters) was also quite promising, but as whole quite comparable to that of *COI* (see
615 Table 2).

616 In some cases in which unambiguous global species assignment employing these approaches
617 was not possible with one or both markers (*e.g. Panthera* felids and South American foxes), we note
618 that geographic information can be applied to restrict the scope of the comparison. For example, if a
619 scat collected in the Neotropical region clusters in the *Panthera* group, one can infer that it belongs to
620 *Panthera onca* since the jaguar is the only species of the genus that currently occurs in the area.
621 Likewise, *Lycalopex vetulus*, *Lycalopex gymnocercus* and *Cerdocyon thous* can be promptly identified
622 in large areas of South America since they form reciprocally monophyletic clades with respect to each
623 other, even though some other sympatric foxes of the genus *Lycalopex* seem not to be discernible
624 with these markers. This recently diverged clade of South American foxes indeed presented the
625 biggest challenge for species diagnosis among all the taxa analyzed in this study, and included the
626 only instances of inter-specific haplotype sharing observed in both of our datasets: one *ATP6*
627 haplotype was shared between individuals of *L. gymnocercus* and *L. culpaeus*, while the latter species
628 also shared one *COI* haplotype with *L. fulvipes*. In the case of such very recent radiations, the
629 character-based approach appears to be the most promising, as it seems to be able to differentiate at

630 least *L. griseus* from its other congeners. Further sampling of these species will be required to better
631 characterize their intra-specific diversity and to assess the reliability of diagnostic sites or haplotypes.
632 In a broader context, distance-based or phenetic methods used in DNA barcoding have been
633 documented to present limitations for species assignment (Witt *et al*. 2006), especially due to high
634 substitution rates in the mtDNA molecule, which often lead to overlaps between intra- and interspecific
635 distances (as observed in Table 2). However, in cases where there are several sequences to be
636 analyzed, a distance-based clustering method can be undertaken to first identify species that form
637 monophyletic groups and will probably need no further inspection for reliable identification of unknown
638 samples. Alternatively, for closely-related species in which monophyletic grouping cannot be achieved
639 or is weakly supported with short sequences, a character-based analysis may deliver unambiguous
640 identifications via species-specific character states. Character-based identification can be seen as a
641 refinement of PCR-RFLP methods. In PCR-RFLP, diagnosis depends on the presence or absence of
642 a combination of species-specific restriction sites that usually vary from four to six base pairs. The
643 probability of finding a set of restriction sites that is diagnostic for a group of species in one DNA
644 segment is negatively related to the number of taxa, and correlates positively with the size of the
645 fragment and the number of enzymes used. Hence, setting up a PCR-RFLP assay to identify a large
646 group of species, such as carnivores, would require extensive tests with several restriction
647 endonucleases and DNA segments, and is likely to be impossible due to the presence of
648 polymorphisms in restriction sites within species, and the need to use large DNA fragments,
649 hampering its application in noninvasive samples.
650 In recently diverged species potentially showing incomplete lineage sorting, it is possible that
651 any short sequence will fail to provide unambiguous identification. Even though we did not observe
652 monophyletic groups for all the investigated species, a standardized approach has the advantage that
653 accumulating sequences in databases from several research groups may eventually lead to an almost
654 complete representation of the molecular diversity of these segments in each species (*i.e.* all or most
655 of its haplotypes), especially those presenting low intraspecific diversity, small population size,
656 restricted distribution and also taxa that are more easily sampled for broad genetic studies. Therefore,
657 if haplotypes are not shared among species, any new sample collected will necessarily match a known
658 haplotype for a single species in the data base. In addition, the improvement of analytical tools and the
659 joint analysis of other characters (*e.g.* geography, morphology, ecology and reproduction) may in the

660 future increase our ability to use full-length and mini-barcodes to distinguish species and possibly to
661 describe new ones in an integrative framework (DeSalle *et al.* 2005, Will *et al*. 2005).
662
663 **Methodological guidelines**
664 For those interested in species identification using one of these three mtDNA segments, we
665 summarize below the advantages and limitations of each of them, and provide some guidelines for
666 their application:
667
668 ***ATP6***: This segment delivered reliable identification in most cases where *COI* and *cytb* segments also
669 succeeded. At least in carnivores, the 126 bp *ATP6* segment used here seems to evolve at a higher
670 rate between species whereas low divergence rates are observed within species, which is essential to
671 avoid misidentifications due to homoplasy. Amplification of predator faecal DNA with primers ATP6-
672 DF3/DR2 or DR1 is achieved in high success rates with no evidence of prey DNA contamination. This
673 segment lies outside *Panthera* and *Felis* nuclear insertions, reducing the opportunities for amplification
674 of paralogous sequences. One disadvantage is that few carnivore sequences of this segment are
675 presently deposited in public databases, requiring generation of reference data prior to use in faecal
676 studies of most carnivore communities. Therefore, an important step in future studies with *ATP6* is to
677 set up reference sequences from tissue samples prior to identification of scats. To keep costs to a
678 minimum, identifications seem to remain robust even when either the first or the last 20 base pairs are
679 removed from the alignment. Hence, if only one DNA strand is to be sequenced, we recommend that
680 the light strand be selected, since the last 20 bp are recovered more reliably and this portion displays
681 more diagnostic characters than the first ones (data not shown). As we observed that the PCR
682 purification protocol using PEG 8000 presented low sucess for this segment, we recommend the use
683 of other protocols (*e.g.* ExoSAP, filter columns) or to use unpurified products for sequencing. High
684 quality sequences could be obtained for *ATP6* without PCR purification, in which case 0.5 to 1.0 μl of
685 a strong product should be used.
686 ***COI***: The main advantage of using the *COI* segment is the existence of a systematized repository of
687 sequences – the Barcode of Life Data System – which is an increasing database largely constructed
688 with sequences tied to voucher specimens, allowing one to track and check dubious records. It will
689 also allow researchers to verify whether character-based identifications remain robust or not after

addition of new specimens. Hence it is advisable to prospective researchers on *COI* to include reference sequences following the guidelines of BOLD/CBOL whenever possible. The unexpected number of prey DNA amplification with the primer set developed in the present study points out the need of further primer optimization for faecal DNA assessments. Similar to *ATP6*, sequencing the light strand is recommended for single-strand sequencing attempts.

Cytb: For the 110 bp segment, at least one individual of 164 terrestrial carnivore species is readily available on GenBank for comparison of faecal DNA. Although sparsely tested in few species, the set of primers seems to reliably amplify the predator DNA but with some degree of prey DNA interference. One shortcoming of this marker as presently applied is that 60 nucleotides out of the 170 bp amplified segment correspond to primers, and are thus not informative. Also, we observed apparent misidentifications in 37 out of 164 species (23%), highlighting the possibility that some sequences deposited in public databases may not be reliably identified. This implies that, although more species have already been sequenced for this segment relative to the other two markers, a similar amount of work will likely be required to build a large database containing reliable sequences from all extant carnivores.

Final remarks

Overall, our results indicate that short mtDNA segments are viable identification tools for most carnivore species, and that standardization of primer sets and PCR conditions should be feasible across the Carnivora. It is likely that a combination of two or more segments will be required for reliable identification of all carnivore species, and that in some exceptional cases where divergence has been extremely recent more complex genomic approaches will be required. However, we foresee that even one of the segments characterized here alone could be standardized to perform reliable identification of the vast majority of carnivore species, especially if further sampling affirms our observation of virtually no haplotype sharing among species. Even if a combination of primers is required for large scale amplification of this standardized segment across all species, we believe that this is an advantageous approach to enhance the speed, reliability and geographic scope of carnivore species identification, thus aiding in the enhanced acquisition of knowledge on this ecologically important mammalian group.

References

Adams JR, Lucash C, Schutte L, Waits LP (2007) Locating hybrid individuals in the red wolf (Canis rufus) experimental population area using a spatially targeted sampling strategy and faecal DNA genotyping. *Molecular Ecology*, **16**, 1823-1834.

Albaugh GP, Iyengar V, Lohan A, *et al*. (1992) Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. *International Journal of Cancer*, **52**, 347–350.

Altschul SF, Madden TL, Schaffer AA *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**, 3389-3402.

Bartlett SE, Davidson WS (1992) FINS (forensically informative nucleotide sequencing): a procedure for identifying the animal origin of biological specimens. *Biotechniques*, **12**, 408-411.

Bhagavatula J, Singh L (2006) Genotyping faecal samples of Bengal tiger Panthera tigris tigris for population estimation: A pilot study. *BMC Genetics*, **7**, 48.

Bidlack AL, Reed SE, Palsbøll PJ, Getz WM (2007) Characterization of a western North American carnivore community using PCR-RFLP of cytochrome *b* obtained from fecal samples. *Conservation Genetics*, **8**, 1511-1513.

Broquet T, Ménard N, Petit E (2007) Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conservation Genetics*, **8**, 249-260.

Bulinski J, McArthur C (2000) Observer error in counts of macropod scats. *Wildlife Research* 27, 277-282.

Colli L, Cannas R, Deiana AM *et al.* (2005) Identification of mustelids (Carnivora: Mustelidae) by mitochondrial DNA markers. *Mammalian Biology*, **70**,384-389

Dalén L, Gotherstrom A, Angerbjorn A (2004) Identifying species from pieces of faeces. *Conservation Genetics*, **5**,109-111.

Davison A, Birks JDS, Brooke RC, Braithwaite STC, Messenger JE (2002) On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. *Journal of Zoology*, **257**,141-143.

Domingo-Roura X (2002) Genetic distinction of marten species by fixation of a microsatellite region. *Journal of Mammalogy*, **83**, 907-912.

752 Farrell LE, Roman J, Sunquist ME (2000) Dietary separation of sympatric carnivores identified by
753 molecular analysis of scats. *Molecular Ecology*, **9**, 1583-1590.
754 Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*,
755 **39**, 783-91
756 Fernandes CA, Ginja C, Pereira I *et al.* (*in press*) Species-specific mitochondrial DNA markers for
757 identification of non-invasive samples from sympatric carnivores in the Iberian Peninsula.
758 *Conservation Genetics*.
759 Fernandez GJ, Corley JC, Capurro AF (1997) Identification of cougar and jaguar feces through bile
760 acid chromatography. *Journal of Wildlife Management*, **61**, 506-510.
761 Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of
762 mitochondrial cytochrome *c* oxidasesubunit I from diverse metazoan invertebrates. *Molecular*
763 *Marine Biology and Biotechnology*, **3**, 294-299.
764 Gerloff U, Schlotterer C, Rassmann K *et al.* (1995) Amplification of hypervariable simple sequence
765 repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*).
766 *Molecular Ecology*, **4**, 515-518.
767 Gómez-Moliner BJ, Cabria MT, Rubines J *et al.* (2004) PCR-RFLP identification of mustelid species:
768 European mink (*Mustela lutereola*), American mink (*Mustela vison*) and polecat (*Mustela*
769 *putorius*) by analysis of excremental DNA. *Journal of Zoology*, **262**, 311-316.
770 Hajibabaei M, Smith MA, Janzen DH *et al.* (2006) A minimalist barcode can identify a specimen whose
771 DNA is degraded. *Molecular Ecology Notes*, **6**, 959-964.
772 Hajibabaei M, Singer GA, Hebert PD, Hickey DA (2007a) DNA barcoding: how it complements
773 taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, **23**, 167-172.
774 Hajibabaei M, Singer GA, Clare EL, Hebert PD (2007b) Design and applicability of DNA arrays and
775 DNA barcodes in biodiversity monitoring. *BMC Biology,* **5**, 24.
776 Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for
777 Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95-98.
778 Hansen MM, Jacobsen L (1999). Identification of mustelid species: otter (*Lutra lutra*), American mink
779 (*Mustela vison*) and polecat (*Mustela putorius*), by analysis of DNA from faecal samples.
780 *Journal Zoology*, **247**, 177-181.

781 Harrison RL (2002) Evaluation of microscopic and macroscopic methods to identify felid hair. Wildlife
782 *Society Bulletin*, **30**, 412-419.
783 Hebert PDN, Cywinski A, Ball SL, DeWaard JR (2003) Biological identifications through DNA
784 barcodes. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **270**, 313–
785 321.
786 Housley DJE, Zalewski ZA, Beckett SE, Venta PJ. Design factors that influence PCR amplification
787 success of cross-species primers among 1147 mammalian primer pairs. *BMC Genomics*, **7**,
788 253.
789 Jiménez JE, Yanez JL, Jaksic FM (1996) Inability of thin-layer chromatography to distinguish feces
790 from congeneric foxes by their bile acid contents. *Acta Theriologica* **41**, 211-215.
791 Johnson MK, Belden RC, Aldred DR (1984) Differentiating mountain lion and bobcat scats. *Journal of*
792 *Wildlife Management*, **48**, 239-244.
793 Kocher TD, Thomas WK, Meyer A*, et al.* (1989) Dynamics of mitochondrial-dna evolution in animals -
794 amplification and sequencing with conserved primers. *Proceedings of the National Academy of*
795 *Sciences of the United States of America*, **86**, 6196-6200.
796 Khorozyan IG, Cazon A, Malkhasyan AG, Abramov AV (2007) Using thin-layer chromatography of
797 fecal bile acids to study the leopard (Panthera pardus ciscaucasica) population. *Biology Bulletin*,
798 **34**, 361-366.
799 Kim J, Antunes A, Luo S *et al.* (2006) Evolutionary analysis of a large mtDNA translocation (*numt*) into
800 the nuclear genome of the *Panthera* genus species. *Gene*, **366**, 292-302.
801 Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through
802 comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111-120.
803 Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics
804 Analysis and sequence alignment. *Briefings in Bioinformatics*, **5**,150-163.
805 Kurose N, Masuda R, Tatara M (2005) Fecal DNA analysis for identifying species and sex of sympatric
806 carnivores: a noninvasive method for conservation on the Tsushima Islands, Japan. *Journal of*
807 *Heredity*, **96**, 688-697.
808 Lopez JV, Cevario S, O'Brien SJ (1996) Complete nucleotide sequences of the domestic cat (*Felis*
809 *catus*) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear
810 genome. *Genomics*, **33**, 229-246.

López-Giráldez F, Gómez-Moliner BJ, Marmi J, Domingo-Roura X (2005). Genetic distinction of American and European mink (*Mustela vison* and *M. lutreola*) and European polecat (*M. putorius*) hair samples by detection of a species-specific SINE and a RFLP assay. *Journal Zoology*, **265**, 405-410.

Lucentini L, Vercillo F, Palomba A, Panara F, Ragni B (2007) A PCR-RFLP method on faecal samples to distinguish *Martes martes*, *Martes foina*, *Mustela putorius* and *Vulpes vulpes*. *Conservation Genetics*, **8**,757-759.

Major M, Johnson MK, Shaw Davis W, Kellogg TF (1980) Identifying scats by recovery of bile acids. *Journal of Wildlife Management*, **44**, 290-293.

Mills LS, Pilgrim KL, Schwartz MK *et al* (2000) Identifying lynx and other North American felids based on mtDNA analysis. *Conservation Genetics*, **1**, 285-288.

Min XJ, Hickey DA (2007) Assessing the effect of varying sequence length on DNA barcoding of fungi. *Molecular Ecology Notes*, **7**, 365-373.

Miotto RA, Rodrigues FP, Ciocheti G, Galetti PM (2007) Determination of the minimum population size of pumas (Puma concolor) through fecal DNA analysis in two protected cerrado areas in the Brazilian Southeast. *Biotropica*, **39**, 647-654.

Mueller RL (2006) Evolutionary rates, divergence dates, and the performance of mitochondrial genes in Bayesian phylogenetic analysis. *Systematic Biology*, **55,** 289-300.

Mukherjee N, Mondol S, Andheria A, Ramakrishnan U (2007) Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. *Conservation Genetics*, **8**, 1465-1470.

Nagata J, Aramilev VV, Belozor A *et al.* (2005) Fecal genetic analysis using PCR-RFLP of cytochrome b to identify sympatric carnivores, the tiger Panthera tigris and the leopard Panthera pardus, in far eastern Russia. *Conservation Genetics* **6**, 863-865.

Nauta MJ, Weissing FJ (1996) Constraints of allele size at microsatellite loci: Implications for genetic differentiation. *Genetics*, **143**, 1021-1032.

Onorato D, White C, Zager P, Waits LP (2006) Detection of predator presence at elk mortality sites using mtDNA analysis of hair and scat samples. *Wildlife Society Bulletin*, **34**, 815-820.

Pääbo S, Poinar H, Serre D *et al.* (2004) Genetic analyses from ancient DNA. *Annual Review of Genetics*, **38**, 645-679.

840 Paithankar KR, Prasad KSN (1991) Precipitation of DNA by polyethylene glycol and ethanol. *Nucleic*
841 *Acids Research*, **19**, 1346.
842 Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Molecular Systematics, 2nd
843 edn. (eds. Hillis DM, Moritz C and Mable BK). pp. 205-221. Sinauer Associates, Massachusetts.
844 Palumbi SR, Cipriano F (1998) Species identification using genetic tools: the value of nuclear and
845 mitochondrial gene sequences in whale conservation. *Journal of Heredity* **89**, 459-464.
846 Palomares F, Godoy JA, Piriz A, Johnson WE (2002) Faecal genetic analysis to determine the
847 presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx.
848 *Molecular Ecology*, **11**, 2171-2182
849 Paxinos E, McIntosh C, Ralls K, Fleischer R (1997) A non invasive method for distinguishing among
850 canid species: amplification and enzyme restriction of DNA from dung. *Molecular Ecology*, **6**,
851 225-234
852 Pilot M, Gralak B, Goszczynski J, Posłuszny M (2007) A method of genetic identification of pine
853 marten (*Martes martes*) and stone marten (*Martes foina*) and its application to faecal samples.
854 *Journal of Zoology*, **271**, 140-147.
855 Prugh LR, Ritland CE (2005) Molecular testing of observer identification of carnivore feces in the field.
856 *Wildlife Society Bulletin*, **33**, 189-194.
857 Quinn T, Jackman WR (1994) Influence of diet on detection of fecal bile-acids by thin-layer
858 chromatography. *Journal of Wildlife Management* **58**, 295-299.
859 Rach J, Desalle R, Sarkar IN, Schierwater B, Hadrys H (2008) Character-based DNA barcoding allows
860 discrimination of genera, species and populations in Odonata. *Proceedings of the Royal Society*
861 *of London Series B, Biological Sciences*, **275**, 237-247.
862 Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System
863 (www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355-364.
864 Ray JC, Sunquist ME (2001) Trophic relations in a community of African rainforest carnivores.
865 *Oecologia*, **127**, 395-408.
866 Reed JE, Baker RJ, Ballard WB, Kelly BT (2004) Differentiating Mexican gray wolf and coyote scats
867 using DNA analysis. *Wildlife Society Bulletin* **32**, 685-692.

Reed JZ, Tollit DJ, Thompson PM, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, **6**, 225-234.

Riddle AE, Pilgrim KL, Mills LS, McKelvey KS, Ruggiero LF (2003). Identification of mustelids using mitochondrial DNA and non-invasive sampling. *Conservation Genetics*, **4**, 241-243.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496-2497.

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365-386. Humana Press, New Jersey.

Rubinoff D, Cameron S, Will K (2006) A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *Journal of Heredity*, **97**, 581-594.

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406-425.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Sugimoto T, Nagata J, Aramilev VV *et al.* (2006) Species and sex identification from faecal samples of sympatric carnivores, Amur leopard and Siberian tiger, in the Russian Far East. *Conservation Genetics*, **7**, 799-802.

Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627-641.

Taber AB, Novaro AJ, Neris N, Colman FH (1997) The food habits of sympatric jaguar and puma in the Paraguayan Chaco. *Biotropica*, **29**, 204-213.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.

Vercillo F, Lucentini L, Mucci N *et al*. (2004) A simple and rapid PCR-RFLP method to distinguish *Martes martes* and *Martes foina*. *Conservation Genetics*, **5**, 869-871.

Wan QH, Fang SG, Chen GF*, et al.* (2003) Use of oligonucleotide fingerprinting and faecal DNA in identifying the distribution of the Chinese tiger (Panthera tigris amoyensis Hilzheimer). *Biodiversity and Conservation*, **12**, 1641-1648.

Wasser SK, Houston CS, Koehler GM, Cadd GG, Fain SR (1997) Techniques for application of faecal DNA methods to field studies of Ursids. *Molecular Ecology*, **6**, 1091-1097.

Wayne RK, Leonard JF, Cooper A (1999) Full of sound and fury: the recent history of ancient DNA. *Annual Review of Ecology and Systematics*, **30**, 457-477.

Wetton JH, Tsang CSF, Roney CA, Spriggs AC (2004) An extremely sensitive species-specific ARMs PCR test for the presence of tiger bone DNA (vol 126, pg 137, 2002). *Forensic Science International* **140**, 137-+.

Witt JD, Threloff DL, Hebert PD (2006) DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology*, **15**, 3073-3082.

Wozencraft WC (2005) Order Carnivora. In: Mammal Species of the World, 3rd edn (eds. Wilson DE, Reeder DM), pp 532-628. The Johns Hopkins University Press, Maryland.

Zuercher GL, Gipson PS, Stewart GC (2003) Identification of carnivore feces by local peoples and molecular analyses. *Wildlife Society Bulletin* **31**, 961-970.

Acknowledgements

We are especially thankful to Klaus-Peter Koepfli, Lisette Waits and Mehrdad Hajibabaei for providing a critical review and valuable comments on an early version of this paper. We thank Ana Carolina Escobar, Cristine Trinca, Manoel Rodrigues, Eunice Matte, Paulo Prates Jr., Taiana Haag and Cladinara Sarturi for their assistance in laboratory procedures. We are indebted to everyone listed in Table S2 for providing samples analyzed in this study, and Raquel Von Hohendorff (Parque Zoológico de Sapucaia do Sul/Fundação Zoobotânica do Rio Grande do Sul) for kindly assisting in sampling carnivores scats from the zoo. PBC, VGG and MBL were supported by fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

926 **Figure legends**

927 Figure 1: Graph depicting the number of scientific articles addressing the identification of carnivore
928 species using molecular methods. In a total of 112 articles, 69 used *cytb* (n=41) and/or D-loop (n=28)
929 segments of the mtDNA for sample identification. These segments were generally different among
930 studies (see figure 2 for some examples) or involved the design of a new set of primers (28 cases).
931 Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA
932 sequencing were the most used methods, being reported in 38 and 29 papers, respectively. In some
933 surveys, more than one method and/or marker was used. Eighty studies included faeces while 22
934 included hairs in their analyses. See Table S1 for a compilation of literature sources including the
935 investigated species, fragment size, primer references, types of samples and methods applied.

936

937 Figure 2: Schematic view of a linearized mitochondrial DNA molecule showing the relative positions of
938 most coding and non-coding portions (not drawn to scale) and large felid *numts*. Positions and lengths
939 (excluding primers) of some segments used for carnivore species identification are indicated in the
940 *cytb* scheme (A: Adams *et al*. 2000, B: Paxinos *et al*. 1997, C: Kocher *et al*. 1989, D: Farrell *et al*.
941 2000, E: Verma & Sing 2003, F: Foran *et al*. 1997 includes part of the adjacent control region). A
942 dashed box in the highlighted *ATP8-ATP6* segment illustrates the overlapping 42 bp portion shared by
943 these two genes. Short segments in black are those whose performances were evaluated in the
944 present study. Partially adapted from Kim *et al.* 2006.

945

946 Figure 3: ***a*** Sliding window graph of the *COI* barcode segment (658 bp), showing the distribution of
947 variable sites among carnivores across this mtDNA region. The graph indicates that the mean number
948 of variable sites in the standard barcode segment is higher on its 3' end. Each window size was 200
949 bp long, and was slid through the full segment 1 bp at a time. V_{184} shows the number of variable sites
950 in an alignment containing 184 carnivore sequences obtained from the BOLD Mammal database. V_{65}
951 shows the same analysis with only one individual per species (65 sequences) from the 184 alignment.
952 V_{min}=80/ V_{max}=101; ***b*** Sliding window graph comparing the number of variables sites among 50
953 carnivore species in the complete sequences of *ATP8-ATP6* (842 bp) and *cytb* (1140 bp), and the *COI*
954 barcode segment (658 bp). Arrows indicate the positions of the short segments analyzed here.
955 V_{min}=75/ V_{max}=140.

Figure 4: Neighbor-joining trees used for carnivore species identification, constructed on the basis of the *ATP6* (left) and *COI* (right) datasets. Highlighted boxes in green, blue, gray and magenta at the center of the figure present a more detailed view of the groups *Lycalopex*, *Conepatus*, *Leopardus pardalis*/*L. wiedii* and *Panthera*, respectively, which were targets of more in-depth analysis and discussion. These genera are illustrative examples where tree-based identification approaches can be problematic and species-specific characteristic attributes (CAs) help to unambiguously identify them (see text for details). Although in some cases both *ATP6* and *COI* segments have CAs (*e.g. L. pardalis*/*L. wiedii*), there are some in which only one segment provided reliable identification (*e.g. Conepatus chinga* was distinguished from *C. semistriatus* only with *COI*, while *Panthera onca* differed from its congeners at one site within *ATP6*). Sequences derived from faecal samples are printed in green font (those labeled with the species' common name [*e.g.* cougar, tiger] were sequenced within case study 1; those with a prefix "PN", "FAL" or "ES" are derived from case study 2; and those with a prefix "A" are part of case study 3) and a GenBank sequence printed in red in the *COI* tree ascribed to *Hydrurga leptonyx* (AY377134) is likely from *Omatophoca rossi*. Three-letter codes for species highlighted in boxes and CA tables are: *Lve*: *Lycalopex vetulus*, *Lgy*: *Lycalopex gymnocercus*, Lcu: *Lycalopex culpaeus*, Lfu: *Lycalopex fulvipes*, Lgr: *Lycalopex griseus*, Cch: *Conepatus chinga*, Cse: *Conepatus semistriatus*, Lpa: *Leopardus pardalis*, Lwi: *Leopardus wiedii*, Pon: *Panthera onca*, Ple: *Panthera leo*, Ppa: *Panthera pardus*, Pti: *Panthera tigris*. On-screen zoom can be used to visualize bootstrap support and other details in the complete trees.

997 **Tables**
998
999
1000 Table 1: Primers used to amplify the short *ATP6* and *COI* segments analyzed in this study.
1001

Name	3' Annealign position [d]	Sequence
ATP6-DF3 [a]	L7987	5'–AACGAAAATCTATTCGCCTCT–3'
ATP6-DR1 [b]	H8114	5'–CCAGTATTTGTTTTGATGTTAGTTG–3'
ATP6-DR2 [a]	H8122	5'–TGGATGGACAGTATTTGTTTTGAT–3'
BC-F2 [a]	L5867	5'–ATCACCACTATTGTTAATATAAAACCC–3'
HCO2198 [c]	H6054	5'–TAAACTTCAGGGTGACCAAAAAATCA–3'

1002 a: newly designed for this study; b: from Haag *et al*. (in prep.); c: from Folmer *et al*. (1994)
1003 d: referenced in the dog mtDNA genome (NC_002008)

Table 2: Main descriptive statistics (bootstrap, characteristic attributes [CA], maximum intra-specific [Intra] and minimum inter-specific [Inter] K2P distances [shown as percentages]) indicating the discriminatory power of the *ATP6* and *COI* markers for the identification of selected carnivore species.

Species	*ATP6*				*COI*			
	Bootstrap [a]	CA	Intra	Inter [b]	Bootstrap [a]	CA	Intra	Inter [b]
Felidae								
Leopardus pardalis	NM	1	5.0	2.4* Lwi	NM	5	3.9	3.6* Lwi
Leopardus wiedii	NM	1	2.5	2.4* Lpa	79	5	3.6	3.6* Lpa
Leopardus tigrinus	97	10	0.0	8.6 Lge	85	9	1.7	5.6 Lge
Leopardus geoffroyi	97	5	0.0	8.6 Lti-Lco	100	9	1.1	5.6 Lti
Leopardus colocolo	93	10	2.4	8.6 Lge	96	11	2.2	6.3 Lti
Felis catus	59	3	2.4	2.4* Fma	NM Fma	2	7.4	1.6* Fma
Panthera onca	NM	1	18.1	3.3* Ple	NM Ple	0	4.5	1.1* Ple
Panthera leo	28	0	0.8	2.5 Pti	NM	0	1.6	1.1* Pon
Panthera pardus	83	4	3.3	5.9 Ple	99	7	1.1	4.5 Ple
Panthera tigris	15	0	7.7	2.5* Ple	85	5	2.7	5.5 Ple
Puma concolor	73	9	0.0	7.2 Pya	100	18	0.5	10.6 Pya
Puma yagouaroundi	89	9	1.6	7.2 Pco	99	18	0.5	10.6 Pco
Otariidae								
Arctocephalus australis	NM Afo	1	0.8	0.8* Afo	53	3	1.7	2.2 Afo
Arctocephalus forsteri	64	1	0.0	0.8 Aau	NC	3	NC	2.2 Aau
Arctocephalus tropicalis	99	5	0.0	4.1 Apu	NS	NS	NC	NC
Arctocephalus gazella	75	7	0.8	5.9 Ato	NS	NS	NC	NC
Otaria flavescens	89	8	1.6	6.8 Aga	NC	4	NC	4.4 Ato-Nci
Phocidae								
Mirounga angustirostris	99	7	0.0	5.9 Mle	99	7	0.5	3.9 Mle
Mirounga leonina	90	7	1.6	5.9 Man	98	7	0.0	3.9 Man
Lobodon carcinophaga	97	14	1.6	12.4 Mmo	99	12	2.2	7.4 Hle
Mephitidae								
Conepatus chinga	NM Cse	0	2.6	0.8* Cse	99	5	0.5	2.7 Cse
Conepatus semistriatus	NM	0	11.3	0.8* Cch	NM	5	1.6	2.7 Cch
Procyonidae								
Nasua nasua	99	27	0.8	25.5 Bas	100	29	3.9	20.3 Bga
Procyon cancrivorus	99	10	0.8	8.6 Plo	100	11	0.6	6.8 Plo
Procyon lotor	99	10	0.8	8.6 Pca	99	11	1.6	6.8 Pca
Mustelidae								
Eira barbara	99	18	2.4	17.3 Mam-Mme	99	17	6.3	12.5 Ggu
Galictis cuja	99	23	1.7	22.8 Eba-Elu	100	25	2.7	15.0 Mml
Lontra longicaudis	99	17	3.3	17.3 Lcn	100	22	1.1	13.3 Lcn
Pteronura brasiliensis	99	21	0.0	19.6 Mvi	100	32	0.0	19.9 Mvi
Canidae								
Chrysocyon brachyurus	99	30	0.0	30.3 Sve	78	26	1.1	17.5 Sve
Speothos venaticus	99	30	2.4	30.3 Cbr	NC	26	NC	17.5 Cbr
Cerdocyon thous	96	9	3.3	7.8 Lgy	99	8	2.2	5.9 Lgy
Lycalopex vetulus	69	3	1.6	3.3 Lgy	82	3	1.6	2.2 Lfu-Lcu
Lycalopex gymnocercus	NM	0	2.4	0.0* Lcu	NM	0	3.0	0.0* Lgr
Lycalopex fulvipes	NM Lcu	0	0.8	0.0* Lcu	NM Lcu	0	1.6	0.0* Lcu
Lycalopex griseus	78	1	0.0	1.6 Lgy-Lcu	NM	0	3.9	0.0* Lgy
Lycalopex culpaeus	NM	0	2.4	0.0* Lgy-Lfu	NM	0	2.8	0.0* Lfu
Canis familiaris/C. lupus	91	5	1.6	5.0 Cla	97	6	1.1	3.3 Cla

[a] NM: non-monophyletic group; NC: not calculated; NS: no sequence available. A superscript three-letter code indicates that the species formed a monophyletic group that also contained one or more sequences belonging to a different taxon (identified by the three letters, see below); *i.e.* the species was paraphyletic with respect to that other taxon.

[b] Three-letter codes indicate the species exbiting the lowest sequence divergence relative to the focal taxon. This same species was used for the character-based analysis (CA column). * species whose interspecific distances were equal to or lower than the

Intra-specific diversity. **Species abbreviation code** – Aau: *Arctocephalus australis,* Afo: *Arctocephalus forsteri*, Aga: *Arctocephalus gazella*, Apu: *Arctocephalus pusillus*, Ato: *Arctocephalus towsendi,* Bas: *Bassariscus astutus*, Bga: *Bassaricyon gabbi*, Cla: *Canis latrans*, Cbr: *Chrysocyon brachyurus*, Cch: *Conepatus chinga*, Cse: *Conepatus semistriatus*, Eba: *Eira barbara*, Elu: *Enhydra lutris*, Fma: *Felis margarita,* Ggu: *Gulo gulo*, Hle: *Hydrurga leptonyx*, Lco: *Leopardus colocolo*, Lge: *Leopardus geoffroyi*, Lpa: *Leopardus pardalis*, Lti: *Leopardus tigrinus*, Lwi: *Leopardus tigrinus*, Lcn: *Lontra canadensis*, Lcu: *Lycalopex culpaeus*, Lfu: *Lycalopex fulvipes*, Lgr: *Lycalopex griseus,* Lgy: *Lycalopex gymnocercus*, Mam: *Martes americana*, Mme: *Martes melampus*, Mml: *Meles meles*, Man: *Mirounga angustirostris*, Mle: *Mirounga leonina*, Mmo: *Monachus monachus*, Mvi: *Mustela vison*, Nci: *Neophoca* cinerea, Ple: *Panthera leo*, Pon: *Panthera onca*, Pti: *Panthera tigris*, Pca: *Procyon* cancrivorus, Plo: *Procyon lotor*, Pco: *Puma concolor*, Pya: *Puma yagouarondi*, Sve: *Speothos venaticus*,

Figures

Figure 1

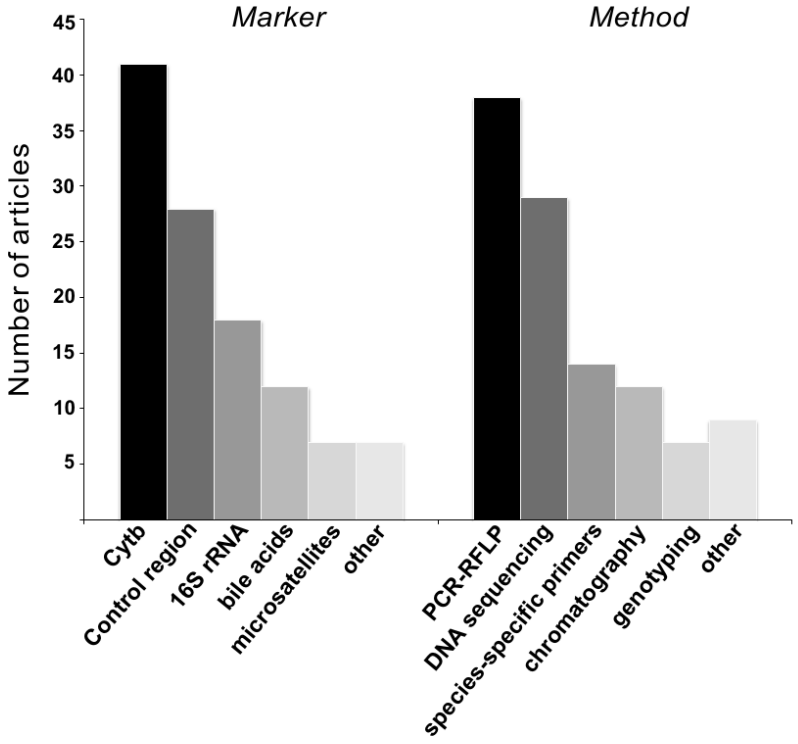
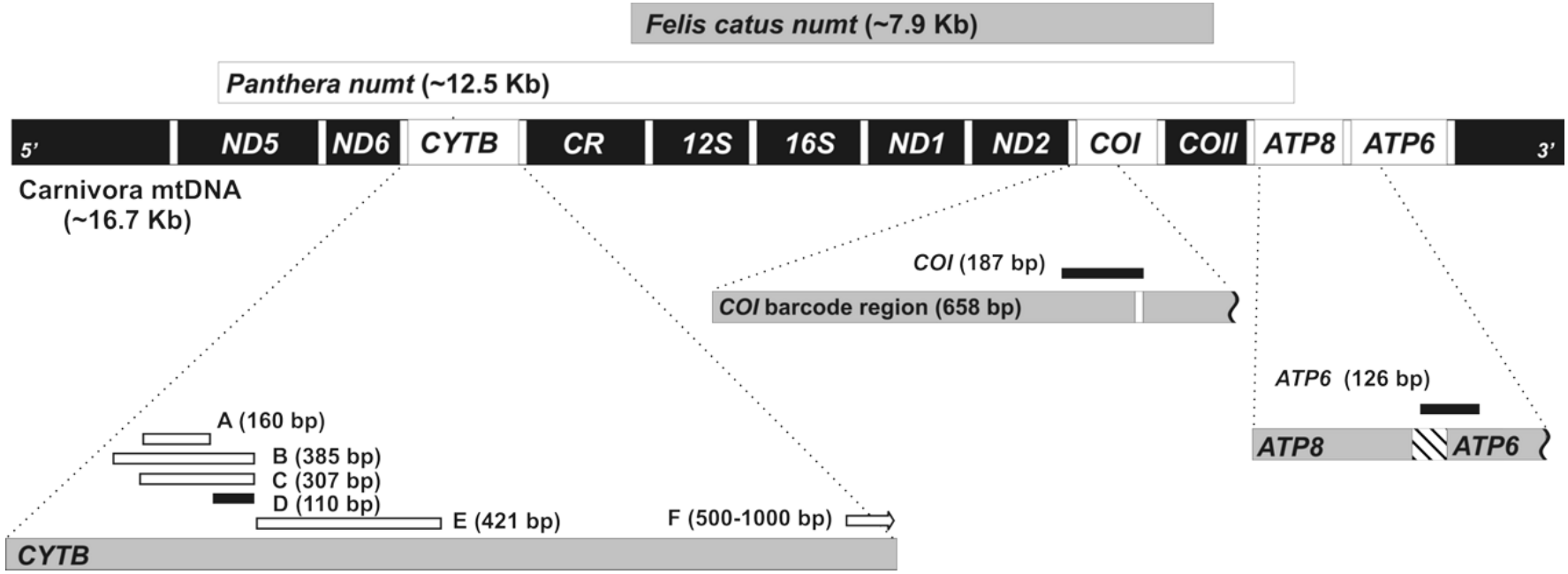
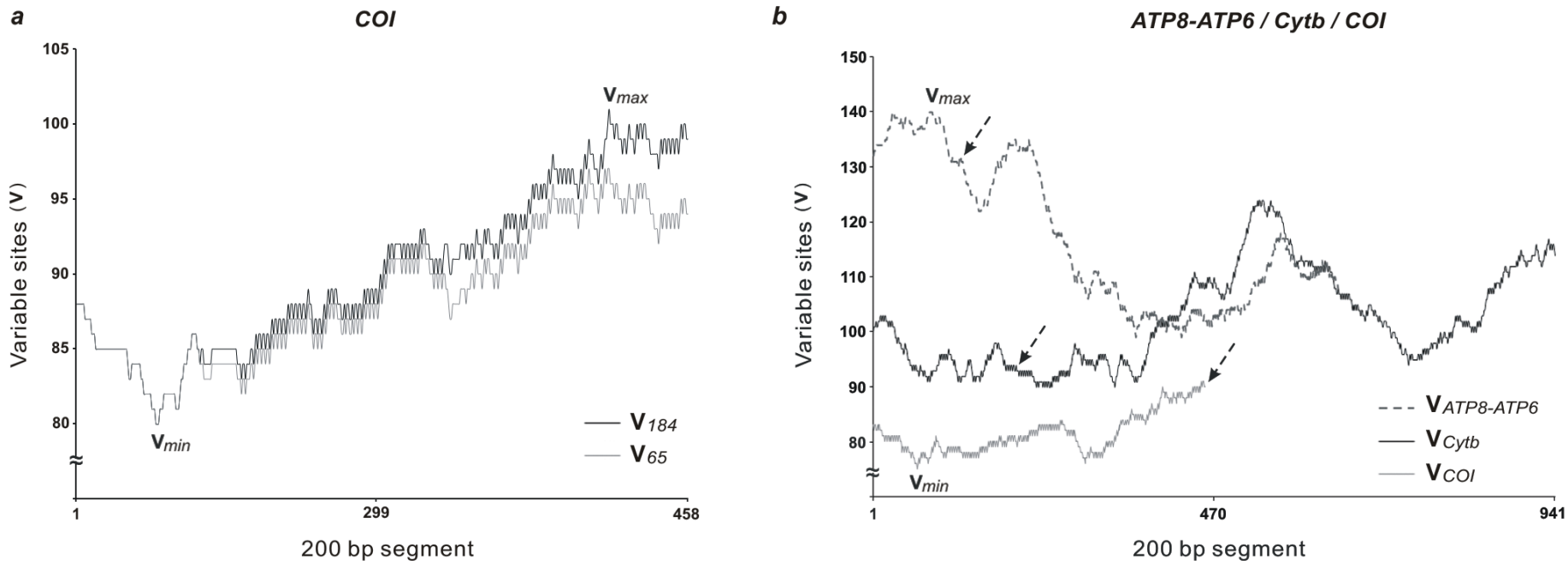


Figure 2

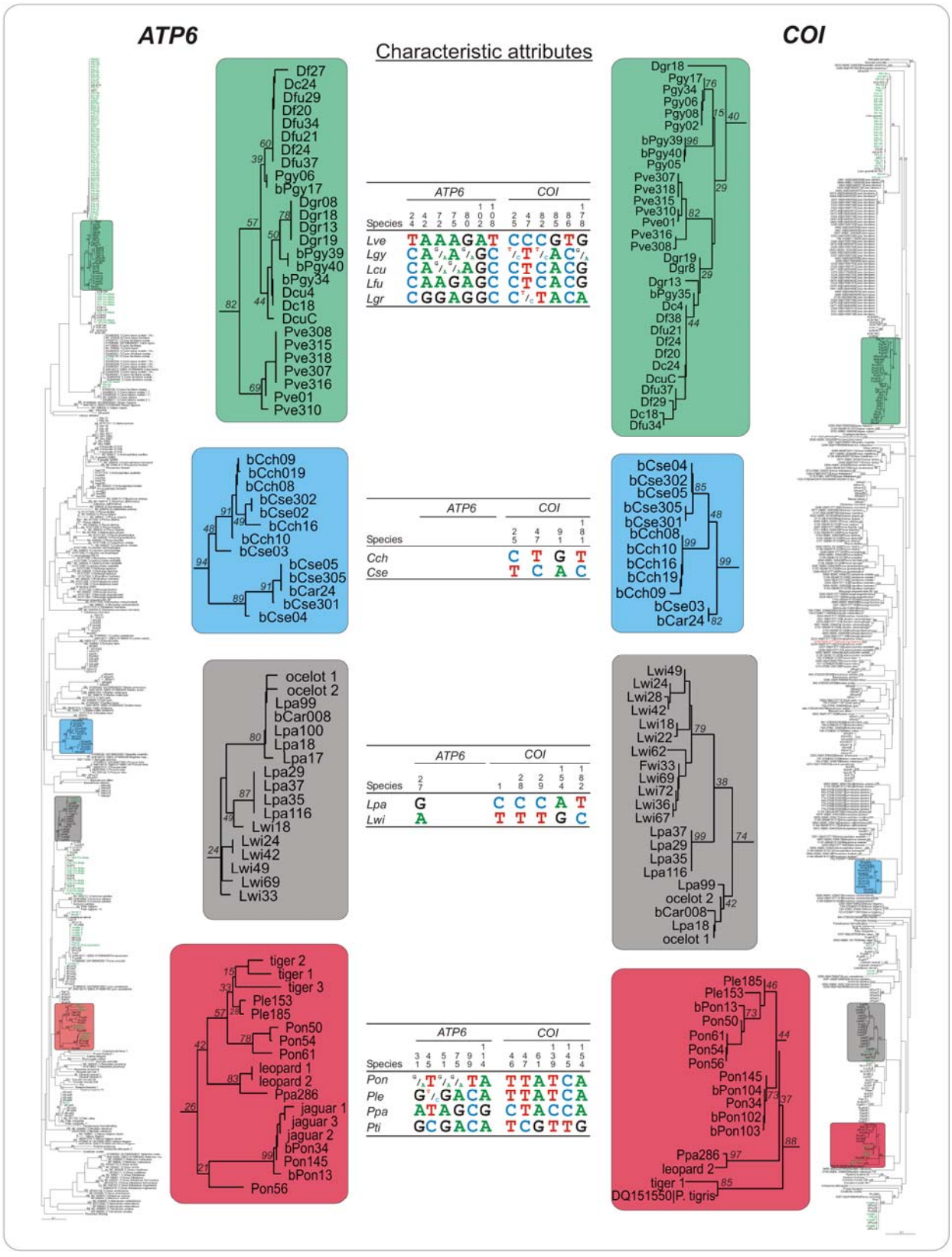

Felis catus numt (~7.9 Kb)
Panthera numt (~12.5 Kb)
5'
ND5
ND6
CYTB
CR
12S
16S
ND1
ND2
COI
COII
ATP8
ATP6
3'
Carnivora mtDNA
(~16.7 Kb)
COI (187 bp)
COI barcode region (658 bp)
ATP6 (126 bp)
ATP8
ATP6
A (160 bp)
B (385 bp)
C (307 bp)
D (110 bp)
E (421 bp)
F (500-1000 bp)
CYTB

1049 Figure 3
1050
1051



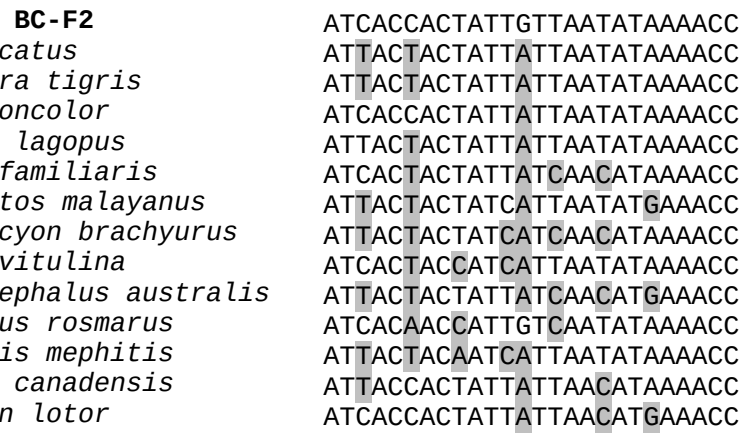
1052

Figure 4


ATP6
COI
Characteristic attributes
Df27
Dc24
Dfu29
Df20
Dfu34
Dfu21
Df24
Dfu37
Pgy06
bPgy17
Dgr08
Dgr18
Dgr13
Dgr19
bPgy39
bPgy40
bPgy34
Dcu4
Dc18
DcuC
Pve308
Pve315
Pve318
Pve307
Pve316
Pve01
Pve310
Species
Lve TAAAGAT CCCGTG
Lgy CA[G/A]A[G/A]GC [T/C][T/C]ACA
Lcu CA[G/A][G/A]AGC CTCACG
Lfu CAAGAGC CTCACG
Lgr CGGAGGC C[T/C]TACA
Dgr18
Pgy17
Pgy34
Pgy06
Pgy08
Pgy02
bPgy39
bPgy40
Pgy05
Pve307
Pve318
Pve315
Pve310
Pve01
Pve316
Pve308
Dgr19
Dgr8
Dgr13
bPgy35
Dc4
Df38
Dfu21
Df24
Df20
Dc24
DcuC
Dfu37
Df29
Dc18
Dfu34
bCch09
bCch019
bCch08
bCse302
bCse02
bCch16
bCch10
bCse03
bCse05
bCse305
bCar24
bCse301
bCse04
Species
Cch C T G T
Cse T C A C
bCse04
bCse302
bCse05
bCse305
bCse301
bCch08
bCch10
bCch16
bCch19
bCch09
bCse03
bCar24
ocelot 1
ocelot 2
Lpa99
bCar008
Lpa100
Lpa18
Lpa17
Lpa29
Lpa37
Lpa35
Lpa116
Lwi18
Lwi24
Lwi42
Lwi49
Lwi69
Lwi33
Species
Lpa G C C C A T
Lwi A T T T G C
Lwi49
Lwi24
Lwi28
Lwi42
Lwi18
Lwi22
Lwi62
Fwi33
Lwi69
Lwi72
Lwi36
Lwi67
Lpa37
Lpa29
Lpa35
Lpa116
Lpa99
ocelot 2
bCar008
Lpa18
ocelot 1
tiger 2
tiger 1
tiger 3
Ple153
Ple185
Pon50
Pon54
Pon61
leopard 1
leopard 2
Ppa286
jaguar 1
jaguar 3
jaguar 2
bPon34
Pon145
bPon13
Pon56
Species
Pon [G/A]T[G/A][C/A]TA TTATCA
Ple G[T/C]GACA TTATCA
Ppa ATAGCG CTACCA
Pti GCGACA TCGTTG
Ple185
Ple153
bPon13
Pon50
Pon61
Pon54
Pon56
Pon145
bPon104
Pon34
bPon102
bPon103
Ppa286
leopard 2
tiger 1
DQ151550|P. tigris

Supplementary material

Figure S1: Reference sequences used to design the internal forward primer BC-F2 for the amplification of a 239 bp *COI* segment. Shaded position show mismatches with respective sequences.



.

Table S1: Compilation of peer reviewed articles wherein identification of carnivore samples was undertaken via molecular methods. The search was started in Web of Science "topic" domain with terms "carnivores" or "carnivore" and "species identification". The 26 and 14 respective publications retrieved were then used as reference for an extensive cross-citation search over several internet sources. Because there is probably not an appropriate combination of terms that will result in a complete search of papers that address carnivore species identification, we acknowledge that some references might have been overlooked.

Reference	Marker (Method)	Carnivore species	Sample	Fragment size
Adams *et al*. 2003	Cytb, Control region (a,b)	Cru, Cla, Cfa, Vvu, Uci, Uam, Lcn, Lru	F, B, OS	200 bp, 360 bp np
Adams & Waits 2007	Cytb, Control region (a,b)	Cru, Cla	F, B	method from Adams *et al*. 2003
Adams & Waits 2007	Cytb, Control region (a,b)	Cru, Cla, Cfa, Vvu, Uci, Uam, Lru	F	method from Adams *et al*. 2003 and 170 bp Farrell *et al*. 2000
Barja *et al*. 2007	? (a)	Mma, Mfo	F	S. Ruiz, personal communication
Berry & Sarre 2007	tRNA/12S rRNA (c,f)	Cfa, Vvu, Fca	F, OT ?	157-176 bp, np
Berry *et al*. 2007	Cytb (b,c)	Vvu	F, B, R	134 bp np
Bhagavatula & Singh 2006	Cytb (c)	Pti	F, B	124 bp np; tested Farrell *et al*.'s primers
Bidlack *et al*. 2007	Cytb (a)	Cla, Lru, Uci, Vvu, Pco, Mmp, Plo	F, OS	196 bp, one new primer + Paxinos *et al*. 1997
Blejwas *et al*. 2006	Control region (a)	Cla	SV, OS	157 bp, primers from Pilgrim *et al*. 1998
Capurro *et al*. 1997	faecal bile acid (e)	Cth, Cfa, Lcu, Lgr, Lfu, Lgy, Lco, Lge, Cch	F	NA, method modified from Major *et al*. 1980
Colli *et al*. 2005	Cytb (a)	Mml, Mni, Mpu, Mfo, Mma	R	362 bp, primers from Kocher *et al*. 1989
Cossíos & Angers 2006	16S rRNA (a)	Lja, Lge, Lco, Pco, Fca, Lcu, Cfa	F, S, OS	257-263 bp np
Dalén *et al*. 2004a	Control region (c)	Ggu, Ala, Vvu	F, M	100, 242, 332 bp np
Dalén *et al*. 2004b	Control region (c)	Ala, Vvu	F	method from Dalén *et al*. 2004a
Davison *et al*. 2002	Control region (b)	Mma, Vvu	F	~ 200 bp np
Domingo-Roura 2001	nDNA microsatellite (d)	Mma, Mfo, Mml	B, M	128-232 bp np
Ernst *et al*. 2000	nDNA microsatellite (d)	Lru, Pco, Cfa, Cla	F, B, BS, H, M	105-209 bp, primers from Menotti-Raymond & O'Brien 1995, Menotti-Raymond *et al*. 1997
Farrell *et al*. 2000	Cytb (b)	Pon, Pco, Lpa, Cth	F, B	170 bp np
Fernandes *et al*. *in press*	Cytb (c)	Fsi, Hic, Gge, Clu, Vvu, Mml, Llu, Mfo, Mma, Mvi, Mer, Mni, Mpu, Mlu	F, M	< 250 bp np
Fernandez *et al*. 1997	faecal bile acid (e)	Pon, Pco	F	NA
Fernández *et al*. 2006	Control region (c)	Lpn	F	method from Palomares *et al*. 2002
Ferrando *et al*. 2008	Control region (b)	Mvi	F	265 bp, primers from Mucci *et al*. 1999
Foran *et al*. 1997a	Cytb/Control region (a)	Vvu, Uci, Clu, Cfa, Cla, Tta, Mam, Mpe, Fca, Lru, Lca, Pco, Plo, Bas	F, B, M	500-1000 bp, primers modified from Shields & Kocher 1991
Foran *et al*. 1997b	Cytb/Control region (a)	Ggu, Mam, Mpe, Lca	H	method from Foran *et al*. 1997a
Goméz-Moliner *et al*. 2004	Control region (a)	Mev, Mlu, Mvi, Mpu, Mma, Mfo, Llu	F, H	~240 bp, F primer from Daivison *et al*. 1999 + new H primer
Gompper *et al*. 2006	mtDNA (b,d)	Cla, Vvu, Mam, Mvi, Mpe, Uci, Plo, Cfa	F, OS	J. E. Maldonado, unpublished data

1100 Table S1: *Continued* (2/4)

Reference	Marker (Method)	Carnivore species	Sample	Fragment size
Guerrero *et al*. 2006	faecal bile acid (e)	Gcu, Lgr, Lcu, Pco, Lgu	F	NA, method from Major *et al*. 1980
Hagey *et al*. 1993	bile acid (e)	Uam, Uar, Uma, Hma, Mur, Tor, Ame, Afu, Plo, Mfu, Mmp, Gti, Ssu, Hbr, Pcr, Ple, Pti, Npr, Ome	F, GB	NA, method from Rossi *et al*. 1987
Hansen & Jacobsen 1999	Cytb (a)	Llu, Mvi, Mpu	F, M	189 bp np
Harrison *et al*. 2002		Vve	F	
Harrison 2006	16S rRNA (b)?	Cla, Cfa, Uci, Vve, Lru	F, H	379 bp, primers from Hoelzer & Green 1992
Jiménez *et al*. 1996	faecal bile acid (e)	Lcu, Lgr		NA, method form Major *et al*. 1980
Johnson *et al*. 1984	faecal bile acid (e)	Pco, Lru	F	NA
Johnson *et al*. 1998	16S, ATP8, ND5 (b)	Lja, Lpa, Lwi, Lti, Lco, Lge, Lgu, Fca, Pya	B, S	119-376 bp np + primers from Hoelzer & Green 1992
Kalz *et al*. 2006	Control region (b)	Llu, Mvi, Mpu, Mlu	F	360 bp np
Khorozyan *et al*. 2007	faecal bile acid (e)	Ppa, Lly, Clu	F	NA, method from Narvaez & Suhring 1999
Kohn *et al*. 1995		Uar	F	
Kohn *et al*. 1999	Control region (a)	Cla, Cfa, Uci	F, B	method from Pilgrim *et al*. 1998
Krausman *et al*. 2006	Control region (a)	Cla, Cfa		200 bp np
Kurose *et al*. 2005	Cytb (c)	Fbe, Mme, Msi, Fca	F, M	112-347 bp np
Leberg *et al*. 2004	16S rRNA (a,b)	Pco, Cfa	F	360 bp, primers from Hoelzel & Green 1992; method from Mills *et al*. 2000
Long *et al*. 2007	16S rRNA (b)?	Uam, Mpe, Lru		379 bp, primers from Hoelzel & Green 1992, amplified prey DNA
López-Giráldez *et al*. 2005	microsatellite (a,g)	Mlu, Mvi, Mpu, Mni, Mal, Mer, Mma, Mfo, Llu	H, RK, M, L, OS	436, 221 bp, primers from Domingo-Roura 2002
Lucentini *et al*. 2006	Cytb (a)	Mma, Mfo, Mpu, Vvu		365 bp np
Major *et al*. 1980	faecal bile acid (e)	Fca, Lru, Plo, Cfa, Cla, Vvu	F	NA
McKelvey *et al*. 2006	16S rRNA (a)	Lca	F, H	method from Mills *et al*. 2000
Mills *et al*. 2000	Cytb/Control region, 16S rRNA (a)	Lca, Lru, Fca, Pco	H, OS	500-1000 bp, primers modified from Shields & Kocher 1991; 360 bp, primers from Hoelzel & Green 1992
Miotto *et al*. 2007	Cytb (b)	Pco, Lpa	F, B	170bp, method from Farrell *et al*. 2000
Mukherjee *et al*. 2007	Cytb, Control region, ND5 (c)	Pti	F, B	164, 210, 225, 250 bp, new and From Luo *et al*. 2004
Murakami 2002	Cytb/tRNA/Control region (b)	Mzi, Mme, Mvi, Mni, Mit	F, L	521-524 bp, primers from Foran *et al*. 1997 and Shields & Kocher 1991
Murphy *et al*. 2000	Control region (g)	Uar, Uam	F, B	146-164 bp np, *apud* Murphy *et al*. 2007
Nagata *et al*. 2005	Cytb (a)	Pti, Ppa	F, B, L	280, 374 bp np
Napolitano *et al*. 2008	16S rRNA, ATP8, ND5 (b)	Lja, Lco, Pco, Cfa, Lcu	F, BN	342, 147, 280 bp, primers from Johnson *et al*. 1998 + primers from Hoelzer & Green 1992

1101

1102 Table S1: *Continued* (3/4)

Reference	Marker (Method)	Carnivore species	Sample	Fragment size
Narvaez & Sühring 1999	faecal bile acid (e)	Pon, Pco, Pya, Lge, Lwi, Lpa, Lco	F	NA
Novack *et al*. 2005	16S rRNA (b)	Pon, Pco	F	379 bp, primers from Hoelzer & Green 1992
Onorato *et al*. 2006	Control region, Cytb (b,g)	Pco, Cla, Clu, Mpe, Ggu, Uar, Uam	F, H	145-165 bp , primers from Murphy *et al*. 2000; ~770 bp primers from Shileds & Kocher 1991; 170 bp Farrell *et al*. 2000
O'Reilly *et al*. *in press*	Control region (f)	Mma, Vvu	F	60 bp np
Palomares *et al*. 2002	Control region (c)	Lpn	F, H, B , M, RK, FP, S, OS	< 130 bp np
Pandey *et al*. 2007	12S rRNA (b)	Ppa, Cfa	F	358 bp, primers from Kocher *et al*. 1989
Paxinos *et al*. 1997	Cytb (a)	Vma, Vvu, Uci, Cfa, Cla, Clu	F	412 bp, a new primer and one from Kocher *et al*. 1989
Perez *et al*. 2006	16S rRNA (b)	Ppa, Clu, Cca, Hhy	F	379 bp, primers from Hoelzel & Green 1992
Pilgrim *et al*. 1998	Control region (a,g)?	Cla, Clu		157 bp np
Pilot *et al*. 2007	nDNA microsatellite (d)	Mma, Mfo	F, H, M, S	128-200 bp, primers from Domingo-Roura (2002) + Davis & Strobeck 1998
Pires & Fernandes 2003	Control region/Cytb (c)	Lpn	F, M	130-161 bp, primers from Palomares *et al*. 2000
Polisar *et al*. 2003	Cytb (b)	Pco, Pon	F	170 bp, primers form Farrell *et al*. 2000
Posluszny *et al*. 2007	nDNA microsatellite (d)	Mma, Mfo, Mvi, Mpu	F	128-200 bp, primers from Domingo-Roura (2002) and Davis & Strobeck, 1998; method of Pilot *et al*. 2007
Quinn & Jackman 1994	faecal bile acid (e)	Cla	F	NA, method from Major *et al*. 1980
Prugh & Ritland 2005	Cytb (a)	Vvu, Cla, Cfa, Lru, Clu	F, BS, OS	~200 bp, method modified from Adams *et al*. 2003
Prugh *et al*. 2005	Cytb (a)	Vvu, Cla, Cfa, Lru, Clu	F	method from Prugh & Ritland 2005
Ray & Sunquist 2001	faecal bile acid (e)	Hna, Ppa, Apa, Gse, Cci, Bni, Pau, Nbi	F	NA, method from Major *et al*. 1980
Reed *et al*. 1997	nDNA microsatellite (d)	Hgr, Pvi, Man, Aau	F, B	85-350 bp
Reed *et al*. 2004	Control region (a)	Clu, Cla, Cfa	F, B, OS	method from Pilgrim *et al*. 1998
Riddle *et al*. 2003	Cytb (a)	Mpe, Ggu, Mam, Mvi, Mme, Uar, Uam, Clu, Plo, Lru, Mmp	H, OS	442 bp, primers from Kocher *et al*. 1989 and Paxinos *et al*. 1997
Ruell & Crooks 2007	16S rRNA , Cytb (a)	Lru, Pco, Fca, Cfa, Cla, Uci	F, H	methods from Mills *et al*. 2000 and Paxinos *et al*. 1997
Schwartz *et al*. 2004	nDNA microsatellite (d) 16S rRNA (a)	Lru, Lca	F, H, OS	primers from Carmichael *et al*. 2001/method of Mills *et al*. 2000
Schwartz *et al*. 2006	16S rRNA (a)	Uam, Uar	H	method from Mills *et al*. 2000
Smith *et al*. 2003	Cytb (a)	Vma, Cla, Uci, Cfa, Vvu	F	350 bp, a new primer + from Paxinos *et al*. 1997 + from Irwin *et al*. 1991
Smith *et al*. 2005	Cytb (a)	Vma	F	method from Paxinos *et al*. 1997
Smith *et al*. 2006	Cytb (a)	Vma, Vvu, Uci	F	350 bp, one primer from Paxinos *et al*. 1997 and one from Irwin *et al*. 1991/method modified from Paxinos *et al*. 1997

Table S1: *Continued* (4/4)

Reference	Marker (Method)	Carnivore species	Sample	Fragment size
Sugimoto *et al*. 2006	Cytb (c)	Pti, Ppa	F, H	156, 271 bp np
Taber *et al*. 1997	faecal bile acid (e)	Pon, Pco	F	NA, method from Johnson *et al*. 1984 and Capurro *et al*. 1997
Thornton *et al*. 2004	16S rRNA (b)	Lru, Cla	F	379 bp, primers from Hoelzel & Green 1992
Ulizio *et al*. 2006	Cytb (a,b)	Ggu, Vvu, Cla, Mam	F, H	method from Riddle *et al*. 2003
Vercillo *et al*. 2004	Cytb (a)	Mma, Mfo	F, H, M	218 bp np
Verma & Singh 2003	Cytb (b)	221 animal species, empirically tested in Cfa	M	472 bp np may amplify preys in faeces; empirically tested in 23 species
Verma *et al*. 2003	Cytb (b)	Pti, Ppa, Ple, Pun, Nne	F	method from Verma & Singh 2003
Walker *et al*. 2007	16S rRNA (b)	Lcu, Lco, Lja	F	379 bp, primers from Hoelzer & Green 1992
Wan & Fang 2003	Cytb (c)	Pti	F, H, M, S	408 and 582 bp np
Wan *et al*. 2003	nDNA VNTR (h)	Pti, Ppa, Nne	F, H, S	0.6-21.2 Kb
Wasser *et al*. 2004	Control region (g)	Uam, Uar	F	246 bp, primers from Wasser *et al*. 1997
Weckel *et al*. 2006a	16S rRNA (b)	Pon, Pco	F	379 bp, primers from Hoelzer & Green 1992
Weckel *et al*. 2006b	16S rRNA (b)	Pon, Pco	F	379 bp, primers from Hoelzer & Green 1992
Wetton *et al*. 2004	Cytb (c,f)	Pti	F, H, BN, B, OS	165 bp np
Williams *et al*. 2003	Control region (a)	Cla, Cfa, Uci, Lru	SV, B, OS	method from Pilgrim *et al*. 1998
Wilson *et al*. 2003	Control region (b)	Cla	S	230 bp, a new primer and primer F from Pilgrim *et al*. 1998
Zielinski *et al*. 2006	16S rRNA/Cytb (a)	Mpe, Mam, Uci, Uam, Bas	H	500-1000 bp, methods from Mills *et al*. 2000, 412 bp, methods from Paxinos *et al*. 1997 and Riddle *et al*. 2003
Zuercher *et al*. 2003	Cytb (a)	Pon, Pco, Pya, Sve, Cbr, Cth, Lgy, Lco, Lge, Lwi, Lti, Lpa	F	341, 338 and 276 bp np
Zuercher *et al*. 2005	Cytb (a)	Sve	F	method from Zuercher *et al*. 2003

Method key – (a) PCR-RFLP, (b) DNA sequencing, (c) species-specific primer, (d) genotyping, (e) chromatography, (f) real-time PCR, (g) species-specific amplicon size (h) Southern blot.
Carnivore species key – Ame: *Ailuropoda melanolecua*, Afu: *Ailurus fulgens*, Ala: *Alopex lagopus*, Aau: *Arctocephalus australis*, Apa: *Atilax paludinosus*, Bas: *Bassariscus astutus*, Bni: *Bdeogale nigripes*, Cfa: *Canis familiaris*, Clu: *Canis lupus*, Cru: *Canis rufus*, Cca: *Caracal caracal*, Cth: *Cerdocyon thous*, Cbr: *Chrysocyon brachyurus*, Cci: *Civettictis civetta*, Cch: *Conepatus chinga*, Fbe: *Felis bengalensis*, Fca: *Felis catus*, Fsi: *Felis silvestris*, Gcu: *Galictis cuja*, Gge: *Genetta genetta,* Gse: *Genetta servalina*, Gti: *Genetta tigrina*, Ggu: *Gulo gulo*, Hgr: *Halichoerus gripus*, Hma: *Helarctos malayanus*, Hic: *Herpestes ichneumon*, Hna: *Herpestes naso*, Hbr: *Hyaena brunnea*, Hhy: *Hyaena hyaena*, Lco: *Leopardus colocolo*, Lge: *Leopardus geoffroyi*, Lgu: *Leopardus guigna*, Lja: *Leopardus jacobitus*, Lti: *Leopardus tigrinus*, Lwi: *Leopardus wiedii*, Lcn: *Lontra canadensis*, Llo: *Lontra longicaudis*, Llu: *Lutra lutra*, Lcu: *Lycalopex culpaeus*, Lfu: *Lycalopex fulvipes*, Lgy: *Lycalopex gymnocercus*, Lgr: *Lycalopex griseus*, Lca: *Lynx canadensis*, Lly: *Lynx lynx*, Lpn: *Lynx pardinus*, Lru: *Lynx rufus*, Mam: *Martes americana*, Mfo: *Martes foina*, Mma: *Martes martes*, Mme: *Martes melampus*, Mpe: *Martes pennanti*, Mzi: *Martes zibellina*, Mml: *Meles meles*, Mur: *Melursus ursinus*, Mmp: *Mephitis mephitis*, Man: *Mirounga angustirostris*, Mal: *Mustela altaica*, Mer: *Mustela erminea*, Mev: *Mustela eversmanii*, Mfu: *Mustela furo*, Mit: *Mustela itatsi*, Mlu: *Mustela lutreola*, Mni: *Mustela nivalis*, Mpu: *Mustela putorius*, Msi: *Mustela sibirica*, Mvi: *Mustela vison*, Nbi: *Nandinia binotata,* Npr: *Nyctereutes procyonoides*, Ome: *Otocyon megalotis*, Ple: *Panthera leo*, Pon: *Panthera onca*, Ppa: *Panthera pardus*, Pti: *Panthera tigris*, Pun: *Panthera uncia*, Pvi: *Phoca vitulina*, Plo: *Procyon lotor*, Pau: *Profelis aurata*, Pcr: *Proteles cristata*, Pco: *Puma concolor*, Pya: *Puma yagouaroundi*, Sve: *Speothos venaticus*, Ssu: *Suricata suricatta*, Tta: *Taxidea taxus*, Tor: *Tremarctos ornatus*, Uci: *Urocyon cinereoargenteus*, Uam: *Ursus americanus*, Uar: *Ursus arctos*, Uma: *Ursus maritimus*, Vma: *Vulpes macrotis*, Vve: *Vulpes velox,* Vvu: *Vulpes vulpes*.
Sample key – B: blood, BN: bone, BS: buccal swab, F: faeces, FP: foot pad, GB: gallbladder bile, H: hair, M: Muscle, OS: other sources, RK: roadkill, S: skin, SV: saliva.
Fragment size key – np: new primers designed, NA: not applicable.
?: uncertain data (inferred whenever possible).

References from Table S1

Adams JR, Kelly BT, Waits LP (2003) Using faecal DNA sampling and GIS to monitor hybridization between red wolves (Canis rufus) and coyotes (Canis latrans). *Molecular Ecology* **12**, 2175-2186.

Adams JR, Lucash C, Schutte L, Waits LP (2007) Locating hybrid individuals in the red wolf (Canis rufus) experimental population area using a spatially targeted sampling strategy and faecal DNA genotyping. *Molecular Ecology* **16**, 1823-1834.

Adams JR, Waits LP (2007) An efficient method for screening faecal DNA genotypes and detecting new individuals and hybrids in the red wolf (Canis rufus) experimental population area. *Conservation Genetics* **8**, 123-131.

Banks SC, Horsup A, Wilton AN, Taylor AC (2003) Genetic marker investigation of the source and impact of predation on a highly endangered species. *Molecular Ecology* **12**, 1663-1667.

Barja I, Silvan G, Rosellini S*, et al.* (2007) Stress physiological responses to tourist pressure in a wild population of European pine marten. *Journal of Steroid Biochemistry and Molecular Biology* **104**, 136-142.

Berry O, Sarre SD (2007) Gel-free species identification using melt-curve analysis. *Molecular Ecology Notes* **7**, 1-4.

Berry O, Sarre SD, Farrington L, Aitken N (2007) Faecal DNA detection of invasive species: the case of feral foxes in Tasmania. *Wildlife Research* **34**, 1-7.

Bhagavatula J, Singh L (2006) Genotyping faecal samples of Bengal tiger Panthera tigris tigris for population estimation: A pilot study. *Bmc Genetics* **7**, 48.

Bidlack AL, Reed SE, Palsboll PJ, Getz WM (2007) Characterization of a western North American carnivore community using PCR-RFLP of cytochrome b obtained from fecal samples. *Conservation Genetics* **8**, 1511-1513.

Blejwas KM, Williams CL, Shin GT, McCullough DR, Jaeger MM (2006) Salivary DNA evidence convicts breeding male coyotes of killing sheep. *Journal of Wildlife Management* **70**, 1087-1093.

Capurro AF, Novaro AJ, Travaini A, Romero MS (1997) Improved bile-acid thin-layer chromatography to identify feces of neotropical carnivores. *Journal of Wildlife Management* **61**, 1424-1427.

Carmichael LE, Clark W, Strobeck C (2000) Development and characterization of microsatellite loci from lynx (Lynx canadensis), and their use in other felids. *Molecular Ecology* **9**, 2197-2198.

Colli L, Cannas R, Deiana AM, Gandolfi G, Tagliavini J (2005) Identification of mustelids (Carnivora : Mustelidae) by mitochondrial DNA markers. *Mammalian Biology* **70**, 384-389.

Cossios D, Angers B (2006) Identification of Andean felid faeces using PCR-RFLP. *Mastozoologia Neotropical* **13**, 239-244.

Dalen L, Elmhagen B, Angerbjorn A (2004) DNA analysis on fox faeces and competition induced niche shifts. *Molecular Ecology* **13**, 2389-2392.

Dalen L, Gotherstrom A, Angerbjorn A (2004) Identifying species from pieces of faeces. *Conservation Genetics* **5**, 109-111.

Davis CS, Strobeck C (1998) Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Molecular Ecology* **7**, 1776-1778.

Davison A, Birks JDS, Brookes RC, Braithwaite TC, Messenger JE (2002) On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. *Journal of Zoology* **257**, 141-143.

Deagle BE, Tollit DJ, Jarman SN*, et al.* (2005) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Molecular Ecology* **14**, 1831-1842.

Domingo-Roura X (2002) Genetic distinction of marten species by fixation of a microsatellite region. *Journal of Mammalogy* **83**, 907-912.

Eggert LS, Maldonado JE, Fleischer RC (2005) Nucleic acid isolation from ecological samples - Animal scat and other associated materials. In: *Molecular Evolution: Producing the Biochemical Data, Part B*, pp. 73-87.

Ernest HB, Penedo MCT, May BP, Syvanen M, Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology* **9**, 433-441.

Farrell LE, Romant J, Sunquist ME (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology* **9**, 1583-1590.

Fernandes CA, Ginja C, Pereira I *et al.* (*in press*) Species-specific mitochondrial DNA markers for identification of non-invasive samples from sympatric carnivores in the Iberian Peninsula. *Conservation Genetics*.

1179 Fernandez GJ, Corley JC, Capurro AF (1997) Identification of cougar and jaguar feces through bile
1180 acid chromatography. *Journal of Wildlife Management* **61**, 506-510.
1181 Fernandez N, Delibes M, Palomares F (2006) Landscape evaluation in conservation: Molecular
1182 sampling and habitat modeling for the Iberian lynx. *Ecological Applications* **16**, 1037-1049.
1183 Ferrando A, Lecis R, Domingo-Roura X, Ponsa M (2008) Genetic diversity and individual identification
1184 of reintroduced otters (Lutra lutra) in north-eastern Spain by DNA genotyping of spraints.
1185 *Conservation Genetics* **9**, 129-139.
1186 Foran DR, Crooks KR, Minta SC (1997) Species identification from scat: an unambiguous genetic
1187 method. *Wildlife Society Bulletin* **25**, 835-839.
1188 Foran DR, Minta SC, Heinemeyer KS (1997) DNA-based analysis of hair to identify species and
1189 individuals for population research and monitoring. *Wildlife Society Bulletin* **25**, 840-847.
1190 Gómez-Moliner BJ, Cabria MT, Rubines J *et al.* (2004) PCR-RFLP identification of mustelid species:
1191 European mink (*Mustela lutereola*), American mink (*Mustela vison*) and polecat (*Mustela*
1192 *putorius*) by analysis of excremental DNA. *Journal of Zoology* **262**, 311-316.
1193 Gompper ME, Kays RW, Ray JC*, et al.* (2006) A comparison of noninvasive techniques to survey
1194 carnivore communities in northeastern North America. *Wildlife Society Bulletin* **34**, 1142-1151.
1195 Guerrero C, Espinoza L, Niemeyer HM, Simonetti JA (2006) Using, fecal profiles of bile acids to
1196 assess habitat Use by threatened carnivores in the Maulino forest of central Chile. *Revista*
1197 *Chilena De Historia Natural* **79**, 89-95.
1198 Hagey LR, Crombie DL, Espinosa E*, et al.* (1993) Ursodeoxycholic acid in the ursidae - biliary bile-
1199 acids of bears, pandas, and related carnivores. *Journal of Lipid Research* **34**, 1911-1917.
1200 Hansen MM, Jacobsen L (1999) Identification of mustelid species: otter (Lutra lutra), American mink
1201 (Mustela vison) and polecat (Mustela putorius), by analysis of DNA from faecal samples.
1202 *Journal of Zoology* **247**, 177-181.
1203 Harrison RL (2006) A comparison of survey methods for detecting bobcats. *Wildlife Society Bulletin*
1204 **34**, 548-552.
1205 Hoelzel AR, Green A (1992) Analysis of populationlevel variation by sequencing PCR-amplified DNA.
1206 In:
1207 Molecular genetic analysis of populations: a practical approach (ed. Hoelzel AR), pp. 159–187.
1208 IRL Press, Oxford.
1209 Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome-b gene of mammals. *Journal of*
1210 *Molecular Evolution* **32**, 128-144.
1211 Jiménez JE, Yanez JL, Jaksic FM (1996) Inability of thin-layer chromatography to distinguish feces
1212 from congeneric foxes by their bile acid contents. *Acta Theriologica* **41**, 211-215.
1213 Johnson MK, Belden RC, Aldred DR (1984) Differentiating mountain lion and bobcat scats. *Journal of*
1214 *Wildlife Management* **48**, 239-244.
1215 Johnson WE, Culver M, Iriarte JA*, et al.* (1998) Tracking the evolution of the elusive Andean mountain
1216 cat (Oreailurus jacobita) from mitochondrial DNA. *Journal of Heredity* **89**, 227-232.
1217 Johnson WE, Obrien SJ (1997) Phylogenetic reconstruction of the Felidae using 16S rRNA and
1218 NADH-5 mitochondrial genes. *Journal of Molecular Evolution* **44**, S98-S116.
1219 Kalz B, Jewgenow K, Fickel J (2006) Structure of an otter (Lutra lutra) population in Germany - results
1220 of DNA and hormone analyses from faecal samples. *Mammalian Biology* **71**, 321-335.
1221 Khorozyan IG, Cazon A, Malkhasyan AG, Abramov AV (2007) Using thin-layer chromatography of
1222 fecal bile acids to study the leopard (Panthera pardus ciscaucasica) population. *Biology*
1223 *Bulletin* **34**, 361-366.
1224 Kocher TD, Thomas WK, Meyer A*, et al.* (1989) Dynamics of mitochondrial-dna evolution in animals -
1225 amplification and sequencing with conserved primers. *Proceedings of the National Academy*
1226 *of Sciences of the United States of America* **86**, 6196-6200.
1227 Kohn MH, York EC, Kamradt DA*, et al.* (1999) Estimating population size by genotyping faeces.
1228 *Proceedings of the Royal Society of London Series B-Biological Sciences* **266**, 657-663.
1229 Kovach AI, Litvaitis MK, Litvaitis JA (2003) Evaluation of fecal mtDNA analysis as a method to
1230 determine the geographic distribution of a rare lagomorph. *Wildlife Society Bulletin* **31**, 1061-
1231 1065.
1232 Krausman PR, Grinder MI, Gipson PS, Zuercher GL, Stewart GC (2006) Molecular identification of
1233 coyote feces in an urban environment. *Southwestern Naturalist* **51**, 122-126.
1234 Kurose N, Masuda R, Tatara M (2005) Fecal DNA analysis for identifying species and sex of sympatric
1235 carnivores: A noninvasive method for conservation on the Tsushima islands, Japan. *Journal of*
1236 *Heredity* **96**, 688-697.
1237 Leberg PL, Carloss MR, Dugas LJ*, et al.* (2004) Recent record of a cougar (Puma concolor) in
1238 Louisiana, with notes on diet, based on analysis of fecal materials. *Southeastern Naturalist* **3**,

653-658.
Long RA, Donovan TM, Mackay P, Zielinski WJ, Buzas JS (2007) Effectiveness of scat detection dogs for detecting forest carnivores. *Journal of Wildlife Management* **71**, 2007-2017.
López-Giráldez F, Gomez-Moliner BJ, Marmi J, Domingo-Roura X (2005) Genetic distinction of American and European mink (Mustela vison and M. lutreola) and European polecat (M. putorius) hair samples by detection of a species-specific SINE and a RFLP assay. *Journal of Zoology* **265**, 405-410.
Lucentini L, Vercillo F, Palomba A, Panara F, Ragni B (2007) A PCR-RFLP method on faecal samples to distinguish *Martes martes*, *Martes foina*, *Mustela putorius* and *Vulpes vulpes*. *Conservation Genetics* **8**,757-759.
Luo SJ, Kim JH, Johnson WE*, et al.* (2004) Phylogeography and genetic ancestry of tigers (Panthera tigris). *Plos Biology* **2**, 2275-2293.
Major M, Johnson MK, Davis WS, Kellogg TF (1980) Identifying scats by recovery of bile-acids. *Journal of Wildlife Management* **44**, 290-293.
McKelvey KS, Von Kienast J, Aubry KB*, et al.* (2006) DNA analysis of hair and scat collected along snow tracks to document the presence of Canada lynx. *Wildlife Society Bulletin* **34**, 451-455.
Menotti-Raymond M, David VA, Stephens JC, Lyons LA, Obrien SJ (1997) Genetic individualization of domestic cats using feline STR loci for forensic applications. *Journal of Forensic Sciences* **42**, 1039-1051.
Menotti-Raymond MA, Obrien SJ (1995) Evolutionary conservation of 10 microsatellite loci in 4 species of felidae. *Journal of Heredity* **86**, 319-322.
Mills LS, Pilgrim KL, Schwartz MK, McKelvey K (2000) Identifying lynx and other North American felids based on mtDNA analysis. *Conservation Genetics* **1**, 285-288.
Miotto RA, Rodrigues FP, Ciocheti G, Galetti PM (2007) Determination of the minimum population size of pumas (Puma concolor) through fecal DNA analysis in two protected cerrado areas in the Brazilian Southeast. *Biotropica* **39**, 647-654.
Mucci N, Pertoldi C, Madsen AB, Loeschcke V, Randi E (1999) Extremely low mitochondrial DNA control-region sequence variation in the otter Lutra lutra population of Denmark. *Hereditas* **130**, 331-336.
Mukherjee N, Mondol S, Andheria A, Ramakrishnan U (2007) Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. *Conservation Genetics* **8**, 1465-1470.
Murakami T (2002) Species identification of mustelids by comparing partial sequences on mitochondrial DNA from fecal samples. *Journal of Veterinary Medical Science* **64**, 321-323.
Murphy MA, Kendall KC, Robinson A, Waits LP (2007) The impact of time and field conditions on brown bear (Ursus arctos) faecal DNA amplification. *Conservation Genetics* **8**, 1219-1224.
Murphy MA, Waits LP, Kendall C (2000) Quantitative evaluation of fecal drying methods for brown bear DNA analysis. *Wildlife Society Bulletin* **28**, 951-957.
Nagata J, Aramilev VV, Belozor A, Sugimoto T, McCullough DR (2005) Fecal genetic analysis using PCR-RFLP of cytochrome b to identify sympatric carnivores, the tiger Panthera tigris and the leopard Panthera pardus, in far eastern Russia. *Conservation Genetics* **6**, 863-866.
Napolitano C, Bennett M, Johnson WE*, et al.* (2008) Ecological and biogeographical inferences on two sympatric and enigmatic Andean cat species using genetic identification of faecal samples. *Molecular Ecology* **17**, 678-690.
Narvaez AVC, Suhring SS (1999) A technique for extraction and Thin Layer Chromatography visualization of fecal bile acids applied to neotropical felid scats. *Revista De Biologia Tropical* **47**, 245-249.
Novack AJ, Main MB, Sunquist ME, Labisky RF (2005) Foraging ecology of jaguar (Panthera onca) and puma (Puma concolor) in hunted and non-hunted sites within the Maya Biosphere Reserve, Guatemala. *Journal of Zoology* **267**, 167-178.
Onorato D, White C, Zager P, Waits LP (2006) Detection of predator presence at elk mortality sites using mtDNA analysis of hair and scat samples. *Wildlife Society Bulletin* **34**, 815-820.
O'Reilly C, Statham M, Mullins J, Turner PD, O'Mahony D. (*in* press) Efficient species identification of pine marten (*Martes martes*) and red fox (*Vulpes vulpes*) scats using a 5′ nuclease real-time PCR assay. *Conservation Genetics*.
Palomares F, Godoy JA, Piriz A, O'Brien SJ, Johnson WE (2002) Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. *Molecular Ecology* **11**, 2171-2182.
Pandey PK, Dhotre DP, Dharne MS*, et al.* (2007) Evaluation of mitochondrial 12S rRNA gene in the identification of Panthera pardus fusca (Meyer, 1794) from field-collected scat samples in the Western Ghats, Maharashtra, India. *Current Science* **92**, 1129-1133.

Paxinos E, McIntosh C, Ralls K, Fleischer R (1997) A noninvasive method for distinguishing among canid species: Amplification and enzyme restriction of DNA from dung. *Molecular Ecology* **6**, 483-486.
Perez I, Geffen E, Mokady O (2006) Critically Endangered Arabian leopards Panthera pardus nimr in Israel: estimating population parameters using molecular scatology. *Oryx* **40**, 295-301.
Pilgrim KL, Boyd DK, Forbes SH (1998) Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *Journal of Wildlife Management* **62**, 683-689.
Pilot M, Gralak B, Goszczynski J, Posluszny M (2007) A method of genetic identification of pine marten (Martes martes) and stone marten (Martes foina) and its application to faecal samples. *Journal of Zoology* **271**, 140-147.
Pires AE, Fernandes ML (2003) Last lynxes in Portugal? Molecular approaches in a pre-extinction scenario. *Conservation Genetics* **4**, 525-532.
Polisar J, Maxit I, Scognamillo D*, et al.* (2003) Jaguars, pumas, their prey base, and cattle ranching: ecological interpretations of a management problem. *Biological Conservation* **109**, 297-310.
Posluszny M, Pilot M, Goszczynski J, Gralak B (2007) Diet of sympatric pine marten (Martes martes) and stone marten (Martes foina) identified by genotyping of DNA from faeces. *Annales Zoologici Fennici* **44**, 269-284.
Prugh LR, Ritland CE (2005) Molecular testing of observer identification of carnivore feces in the field. *Wildlife Society Bulletin* **33**, 189-194.
Prugh LR, Ritland CE, Arthur SM, Krebs CJ (2005) Monitoring coyote population dynamics by genotyping faeces. *Molecular Ecology* **14**, 1585-1596.
Quinn T, Jackman WR (1994) Influence of diet on detection of fecal bile-acids by thin-layer chromatography. *Journal of Wildlife Management* **58**, 295-299.
Ray JC, Sunquist ME (2001) Trophic relations in a community of African rainforest carnivores. *Oecologia* **127**, 395-408.
Reed JE, Baker RJ, Ballard WB, Kelly BT (2004) Differentiating Mexican gray wolf and coyote seats using DNA analysis. *Wildlife Society Bulletin* **32**, 685-692.
Reed JZ, Tollit DJ, Thompson PM, Amos W (1997) Molecular scatology: The use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology* **6**, 225-234.
Riddle AE, Pilgrim KL, Mills LS, McKelvey KS, Ruggiero LF (2003) Identification of mustelids using mitochondrial DNA and non-invasive sampling. *Conservation Genetics* **4**, 241-243.
Rossi SS, Converse JL, Hofmann AF (1987) High-pressure liquid-chromatographic analysis of conjugated bile-acids in human bile - simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile-acids. *Journal of Lipid Research* **28**, 589-595.
Ruell EW, Crooks KR (2007) Evaluation of noninvasive genetic sampling methods for felid and canid populations. *Journal of Wildlife Management* **71**, 1690-1694.
Schwartz MK, Pilgrim KL, McKelvey KS*, et al.* (2004) Hybridization between Canada lynx and bobcats: Genetic results and management implications. *Conservation Genetics* **5**, 349-355.
Schwartz MK, Cushman SA, McKelvey KS, Hayden J, Engkjer C (2006) Detecting genotyping errors and describing American black bear movement in northern Idaho. *Ursus* **17**, 138-148.
Shields GF, Kocher TD (1991) Phylogenetic-relationships of north-american ursids based on analysis of mitochondrial-dna. *Evolution* **45**, 218-221.
Smith DA, Ralls K, Cypher BL*, et al.* (2006) Relative abundance of endangered San Joaquin kit foxes (Vulpes macrotis mutica) based on scat-detection dog surveys. *Southwestern Naturalist* **51**, 210-219.
Smith DA, Ralls K, Cypher BL, Maldonado JE (2005) Assessment of scat-detection dog surveys to determine kit fox distribution. *Wildlife Society Bulletin* **33**, 897-904.
Smith DA, Ralls K, Hurt A*, et al.* (2003) Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (Vulpes macrotis mutica). *Animal Conservation* **6**, 339-346.
Sugimoto T, Nagata J, Aramilev VV*, et al.* (2006) Species and sex identification from faecal samples of sympatric carnivores, Amur leopard and Siberian tiger, in the Russian Far East. *Conservation Genetics* **7**, 799-802.
Taber AB, Novaro AJ, Neris N, Colman FH (1997) The food habits of sympatric jaguar and puma in the Paraguayan Chaco. *Biotropica* **29**, 204-213.
Thornton DH, Sunquist ME, Main MB (2004) Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. *Journal of Mammalogy* **85**, 973-982.
Ulizio TJ, Squires JR, Pletscher DH*, et al.* (2006) The efficacy of obtaining genetic-based identifications from putative wolverine snow tracks. *Wildlife Society Bulletin* **34**, 1326-1332.

1359 Vercillo F, Lucentini L, Mucci N *et al*. (2004) A simple and rapid PCR-RFLP method to distinguish
1360 *Martes martes* and *Martes foina*. *Conservation Genetics* **5**, 869-871.
1361 Verma SK, Prasad K, Nagesh N, Sultana M, Singh L (2003) Was elusive carnivore a panther? DNA
1362 typing of faeces reveals the mystery. *Forensic Science International* **137**, 16-20.
1363 Verma SK, Singh L (2003) Novel universal primers establish identity of an enormous number of animal
1364 species for forensic application. *Molecular Ecology Notes* **3**, 28-31.
1365 Walker RS, Novaro AJ, Perovic P*, et al.* (2007) Diets of three species of Andean carnivores in high-
1366 altitude deserts of Argentina. *Journal of Mammalogy* **88**, 519-525.
1367 Wan QH, Fang SG (2003) Application of species-specific polymerase chain reaction in the forensic
1368 identification of tiger species. *Forensic Science International* **131**, 75-78.
1369 Wan QH, Fang SG, Chen GF*, et al.* (2003) Use of oligonucleotide fingerprinting and faecal DNA in
1370 identifying the distribution of the Chinese tiger (Panthera tigris amoyensis Hilzheimer).
1371 *Biodiversity and Conservation* **12**, 1641-1648.
1372 Wasser SK, Davenport B, Ramage ER*, et al.* (2004) Scat detection dogs in wildlife research and
1373 management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta,
1374 Canada. *Canadian Journal of Zoology* **82**, 475-492.
1375 Wasser SK, Houston CS, Koehler GM, Cadd GG, Fain SR (1997) Techniques for application of faecal
1376 DNA methods to field studies of Ursids. *Molecular Ecology* **6**, 1091-1097.
1377 Weckel M, Giuliano W, Silver S (2006) Cockscomb revisited: Jaguar diet in the Cockscomb Basin
1378 Wildlife Sanctuary, Belize. *Biotropica* **38**, 687-690.
1379 Weckel M, Giuliano W, Silver S (2006) Jaguar (Panthera onca) feeding ecology: distribution of
1380 predator and prey through time and space. *Journal of Zoology* **270**, 25-30.
1381 Wetton JH, Tsang CSF, Roney CA, Spriggs AC (2004) An extremely sensitive species-specific ARMs
1382 PCR test for the presence of tiger bone DNA (vol 126, pg 137, 2002). *Forensic Science*
1383 *International* **140**, 137-+.
1384 Whittier CA, Horne W, Slenning B, Loomis M, Stoskopf MK (2004) Comparison of storage methods for
1385 reverse-transcriptase PCR amplification of rotavirus RNA from gorilla (Gorilla g. gorilla) fecal
1386 samples. *Journal of Virological Methods* **116**, 11-17.
1387 Williams CL, Blejwas K, Johnston JJ, Jaeger MM (2003) A coyote in sheep's clothing: predator
1388 identification from saliva. *Wildlife Society Bulletin* **31**, 926-932.
1389 Wilson PJ, Grewal S, McFadden T, Chambers RC, White BN (2003) Mitochondrial DNA extracted
1390 from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Canadian*
1391 *Journal of Zoology* **81**, 936-940.
1392 Zielinski WJ, Schlexer FV, Pilgrim KL, Schwartz MK (2006) The efficacy of wire and glue hair snares in
1393 identifying mesocarnivores. *Wildlife Society Bulletin* **34**, 1152-1161.
1394 Zuercher GL, Gipson PS, Carrillo O (2005) Diet and habitat associations of bush dogs Speothos
1395 venaticus in the Interior Atlantic Forest of eastern Paraguay. *Oryx* **39**, 86-89.
1396 Zuercher GL, Gipson PS, Stewart GC (2003) Identification of carnivore feces by local peoples and
1397 molecular analyses. *Wildlife Society Bulletin* **31**, 961-970.

1398 Table S2: Samples sequenced for assembling a reference database.

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
FELIDAE	*L. pardalis*	Lpa17 [c]	Panama		Summit Zoo
		Lpa18	Panama		Summit Zoo
		Lpa29	Guatemala		Autosafari Chapin
		Lpa35	South Mexico		Parque Zool. De Leon
		Lpa37	South Mexico		
		Lpa99	Bolivia		Santa Cruz Zoo
		Lpa100 [c]	Bolivia		Santa Cruz Zoo
		Lpa116	Central Mexico		Idaho State University/J. Landre
		bLpa138	Santarém, PA		G. Pontes
	L. wiedii	Lwi18	Amazonas, north of the Amazon		
		Lwi22 [a]	Costa Rica		
		Lwi24	Costa Rica		
		Lwi28 [a]			
		Lwi33			
		Lwi36 [a]	Guatemala		Autosafari Chapin
		Lwi42	South Mexico		
		Lwi49	North Mexico		Sonoran Ecological Center
		Lwi62 [a]	Pará, south of the Amazon		
		Lwi69	South Brazil		
		Lwi72 [a]			
	L. tigrinus	bLti04	South Brazil		Zoo Sapucaia
		Fti44	Curitibanos, SC	Fti #06	Zoo Curitiba
		bLti70	Sorocaba, SP		Zoo Sorocaba/Plano de Manejo Peq. Felinos Brasileiros
		bLti74	S. José do Rio Preto, SP		Plano de Manejo Peq. Felinos Brasileiros
		bLti75	probably from Bauru, SP		Plano de Manejo Peq. Felinos Brasileiros
		bLti76	Pedreira, SP		Plano de Manejo Peq. Felinos Brasileiros
		bLti85	probably from Goiânia, GO		Plano de Manejo Peq. Felinos Brasileiros
		bLti97 [a]	Domingos Martins, ES		Pró-Carnívoros/D. Sana
	L. geoffroyi	Fge12	Cachoeira do Sul, RS		E. Salomão
		Fge20	Uruguai	CA747	MCN/G. D'Elía
		Fge28 [a]	Tapes/Rambaré, RS		L. Veronese
		Fge29 [a]	Quaraí, RS		D. Sana, F. Michalski, C. Indrusiak, T. Trigo
		bLge31	Quaraí, RS		D. Sana, F. Michalski, C. Indrusiak, T. Trigo
		bLge33	Rosário/Alegrete, RS		G. Pontes and M. Martins
		bLge36	Taim, RS		T. Trigo
		Oge38 [a]	Santa Maria/São Sepé, RS		L. Cabral
		Oge63 [a]	Santa Cruz, Bolivia		Santa Cruz Zoo

1399 Table S2: *Continued* (2/6)

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
FELIDAE	*L. colocolo*	Lco4 [c]			
		Lco7 [a]	Argentina		Cordoba Zoological Park ?
		Lco8	Uruguay		
		Lco30 [a]	Chile		
		bLco303	Parque Nacional das Emas, GO		CENAP-IBAMA
		bLco315	Parque Nacional das Emas, GO		CENAP-IBAMA
	F. catus	bFca02	Porto Alegre, RS		A. C. Escobar
		bFca52	Porto Alegre, RS		A. C. Escobar
		bFca53	Porto Alegre, RS		A. C. Escobar
		bFca54	Porto Alegre, RS		A. C. Escobar
		bFca55	Porto Alegre, RS		A. C. Escobar
		bFca56 [c]	Porto Alegre, RS		A. C. Escobar
		bFca57	Porto Alegre, RS		A. C. Escobar
		bFca58 [c]	Porto Alegre, RS		A. C. Escobar
		bFca59	Porto Alegre, RS		A. C. Escobar
	P. onca	bPon13	Amazonas, north of the Amazon		CIGS, Manaus
		bPon34	French Guiana		B. de Thoisy
		Pon50	Chaco, Paraguay		Itaipu, Paraguay/W. Johnson
		Pon54	Amazonas State, Venezuela		Las Delicias
		Pon56	Falcon State, Venezuela		Las Delicias
		Pon61	unknown State, Venezuela		Barquisimento
		Pon145	Costa Rica		
	P. leo	Ple153			
		Ple185			
	P. pardus	Ppa286			
	P. yagouaroundi	bPya07 [a]	Barão, RS		
		bPya16	Iguaçu, PR		
		bPya18 [c]	Sapiranga, RS		
		bPya22 [c]	Rio Zoo, SP		Rio Zoo
		bPya26 [c]	Monte Alto, SP		
		bPya28 [c]	Sorocaba, SP		
		bPya31	Lajeado, RS		
		bPya34 [a]	MS		Zoo Cesp
		bPya35 [c]	Restinga Seca, RS		
		bPya47	CE		
		bPya67 [c]	Vila Velha/Gurapari, ES		RODOSOL/A. Kiekebusch

1400 Table S2: *Continued* (3/6)

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
FELIDAE	*P. concolor*	Pco07	Oregon, USA		
		bPco14	Cananéia, SP		F. Olmos
		bPco34	Parque Estadual Serra da Cantareira, RJ		Pró-Carnívoros/R. Morato
		bPco35	Jardim, MS		Pró-Carnívoros/R. Morato
		bPco37	Corumbá, MS		Pró-Carnívoros/R. Morato
		bPco42 [c]	Zoo Ilha Solteira, SP		Zoo Ilha Solteira-Pró-Carnívoros/D. Sana
		Pco544	Guanacaste, Costa Rica		
		Pco548	Costa Rica		
		Pco560 [c]	Argentina		
OTARIIDAE	*A. australis*	Aau01H [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Aau11H [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Aau20H [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Aau78G [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Aau586 [c]	Rio Grande do Sul, Brazil		GEMARS/L. R. Oliveira
		Aau587 [c]	Rio Grande do Sul, Brazil		GEMARS/L. R. Oliveira
		Aau687	Rio Grande do Sul, Brazil		GEMARS/L. R. Oliveira
		Aau694	Rio Grande do Sul, Brazil		GEMARS/L. R. Oliveira
	A. tropicalis	G1012 [c]	Rio Grande do Sul, Brazil	GEMARS1012	GEMARS/L. R. Oliveira
		G1018 [c]	Rio Grande do Sul, Brazil	GEMARS1018	GEMARS/L. R. Oliveira
		G1034 [c]	Rio Grande do Sul, Brazil	GEMARS1034	GEMARS/L. R. Oliveira
	A. gazella	G0862 [c]	Rio Grande do Sul, Brazil	GEMARS0862	GEMARS/L. R. Oliveira
		G0895 [c]	Rio Grande do Sul, Brazil	GEMARS0895	GEMARS/L. R. Oliveira
	O. flavescens	G0517 [c]	Rio Grande do Sul, Brazil	GEMARS0517	GEMARS/L. R. Oliveira
		G0822 [c]	Rio Grande do Sul, Brazil	GEMARS0822	GEMARS/L. R. Oliveira
		G0868 [c]	Rio Grande do Sul, Brazil	GEMARS0868	GEMARS/L. R. Oliveira
		G0967 [c]	Rio Grande do Sul, Brazil	GEMARS0967	GEMARS/L. R. Oliveira
		G0992 [c]	Rio Grande do Sul, Brazil	GEMARS0992	GEMARS/L. R. Oliveira
		Peru3 [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Peru1 [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Peru4 [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Peru5 [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
		Peru6 [c]	Punta San Juan, Peru		CSA-UPCH/P. Majluf
PHOCIDAE	*L. carcinophaga*	RS14 [c]	Tramandaí, RS		GEMARS/L. R. Oliveira
	M. leonina	G0885 [c]	Rio Grande do Sul, Brazil	GEMARS0885	GEMARS/L. R. Oliveira

1401 Table S2: *Continued* (4/6)

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
MEPHITIDAE	*C. chinga*	bCch08	São Francisco de Paula, RS		Pró-Carnívoros/F. Michalski
		bCch09	Tainhas, RS		Pró-Carnívoros/F. Michalski
		bCch10	BR285, RS		Pró-Carnívoros/F. Michalski
		bCch16	BR153, near Cachoeira do Sul, RS		E. Eizirik
		bCch19	BR 471/km 590, RS		P. Chaves
	C. semistriatus	bCse02 [c]	Serra da Canastra, near Formiga, MG		J. May
		bCse03	near Formiga/Piumhi, MG		F. Rodrigues
		bCse04	Piumhi, MG		J. May
		bCse05	near Três Marias, MG		F. Rodrigues
		bCse13	BR452 near Juliana and Nova Ponte, MG	LPC389	Coleção de Tecidos e DNA da UFES/L. Costa
		bCse301	Parque Nacional das Emas, GO		CENAP-IBAMA
		bCse302	Valença, PI		CENAP-IBAMA
		bCse305	Parque Nacional das Emas, GO		CENAP-IBAMA
PROCYONIDAE	*N. nasua*	bNna01 [a]	Serra da Mesa, GO	MN36755	MNRJ
		bNna02	Parque Nacional do Iguaçú, PR		Pró-Carnívoros/P. Crawshaw Jr.
		bNna03	Ibarama, RS	MPB183	UFSM
		bNna05 [a]	Machadinho, RS		Júlio César Menezes de Sá
		bNna14	Vila Velha/Gurapari, ES		RODOSOL/Andreas Kiekebusch
		bNna16	Corumbá, MS		CPAP-EMBRAPA/G. Mourão
		bNna21	Fortaleza, CE		M. R. Mattos
	P. cancrivorus	bPca10 [c]	Barão de Melgaço, MT		Pró-Carnívoros/R. Morato
		bPca21 [a]	Vila Velha/Gurapari, ES		RODOSOL/A. Kiekebusch
		bPca301	Corumbá, MS		CENAP-IBAMA
MUSTELIDAE	*E. barbara*	bEba02	Serra da Mesa, GO	MN36627	MNRJ
		bEba03	Serra da Mesa, GO	MN36726	MNRJ
		bEba06	Oriximiná/Rio Trombetas, PA		B. M. Costa
		bEba07	Zoo Sapucaia do Sul, RS	Entrada FZB: 170	FZB/M. Jardim
		bEba11	Corumbá, MS		CPAP-EMBRAPA/G. Mourão
	G. cuja	bGcu02 [c]	São Vicente Jaguari, RS	FZB 094	FZB/M. Jardim
		bGcu08	BR287, RS		Pró-Carnívoros/F. Michalski
		bGcu09	BR153, RS		Pró-Carnívoros/F. Michalski
		bGcu10 [c]	Não há localidade no banco	FZB 097	FZB/M. Jardim
		bGcu12	Itapuã/Viamão, RS	Entrada FZB: 284	FZB/A. Maciel e M. Jardim
		bGcu14	SC-438, Bom Jardim/Cruzeiro, SC		A. Garda e M. Lion

1402 Table S2: *Continued* (5/6)

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
MUSTELIDAE	*G. cuja*	bGvi01	SP 300 km 260+200m, Southwest SP		J. Griese
		bGvi02 [c]	Botucatu, SP 300 km 184+100m, SP		J. Griese
		bGvi03	DF-001, Brasília, DF		C. Campos
		bGvi04	SP-300, km 260 (+200m), Southeast SP		F. Lima
		bGvi302	Piumhi-São Roque, MG		CENAP-IBAMA
	L. longicaudis	bLlo06	RS 040 - Km 54, RS		P. H. Ott
		bLlo15	Guaratiba, RJ		H. Waldemarin
		bLlo17 [c]	Rio Negro/Rio Paraguai, MS		H. Waldemarin
		bLlo23 [c]	Antioquia, Colombia		Diego A. Arcila and H. Waldemarin
		bLlo30 [a]	Belo Horizonte, MG	Cad24852	São Paulo Zoo/K. Kassaro
		bLlo31 [a]	Corumbá, MS	Cad28861	São Paulo Zoo/K. Kassaro
		bLlo58	Osório, RS		P. Colombo, C. Zank and L. Volkmer
		bLlo67 [c]	Bolivia		
	P. brasiliensis	Pbr01 [c]	Corumbá, MS		H. Waldemarin
		Pbr02 [c]	Corumbá, MS		H. Waldemarin
CANIDAE	*C. brachyurus*	AE01	Est. Ecol. Águas Emendadas, Brasília		
		AELB75	Est. Ecol. Águas Emendadas, Brasília		
		Cbr05	Cuiabá, MT		
		bCbr11 [a]	Dourados, MS	LPC607	Coleção de Tecidos e DNA da UFES/L. Costa
		bCbr302 [a]	Parque Estadual da Serra da Canastra, MG		CENAP-IBAMA
		Extr1SC [a]	Lages, SC		
		Lobo-guará 3' [a]	Corrientes, Argentina		
	S. venaticus	bSve304 [c]	Nova Xavantina, MT		CENAP-IBAMA
		bSve305	Nova Xavantina, MT		CENAP-IBAMA
	C. thous	bCth05	Serra da Mesa, GO	MN37446	MNRJ
		bCth13	Cambará do Sul, RS		Pró-Carnívoros/F. Michalski
		bCth64	BR 277, Southwest PR		J. F. Cândido
		bCth142 [c]	Parque de Itapuã, RS		M. Correa
		bCth164	Anaurilândia, MS		Pró-Carnívoros/D. Sana
		bCth185	PE, Brazil		Zoo
		bCth194	CE, Brazil		Zoo/Luiz Carlos Diniz
		bCth225 [c]	Reserva Biológica do Gurupi, MA		UEMA/T. de Oliveira
		bCth269	Imperatriz, MA		IBAMA/L. Tchaicka

1403

Table S2: *Continued* (6/6)

Family	Species	Sample ID	Locality	Voucher #	Institution/Person (when available)
CANIDAE	*L. gymnocercus*	Pgy02 [c]	Sapucaia Zoo, RS		Zoo Sapucaia
		Pgy05 [c]	RS 153, Cachoeira do Sul, RS		A. Lorenz-Lemke and R. Schmitt
		Pgy06	BR116, Eldorado do Sul, RS		Pró-Carnívoros/F. Michalski
		Pgy08 [c]	BR116, Pedro Osório, RS		Pró-Carnívoros/F. Michalski
		Pgy17			
		Pgy34			
		bPgy35 [c]			
		bPgy39	Estação Ecológica do Taim, RS		
		bPgy40	Estação Ecológica do Taim, RS		
	L. vetulus	Pve01	Planaltina, DF		F. Rodrigues
		Pve307	Nova Xavantina, MG		J. Dalponte
		Pve308	Nova Xavantina, MG		J. Dalponte
		Pve310	near Pirenópolis, GO		A. Garda and F. Grazziottin
		Pve315	Nova Xavantina, MG		J. Dalponte
		Pve316	Nova Xavantina, MG		J. Dalponte
		Pve318	Nova Xavantina, MG		J. Dalponte
	L. fulvipes	Df20			
		Dfu21			
		Df24			
		Df27[C]			
		Df29			
		Dfu34			
		Dfu37 [c]			
		Df38 [a]			
	L. griseus	Dgr08			
		Dgr13			
		Dgr18			
		Dgr19			
	L. culpaeus	Dcu4			
		Dc18			
		Dc24			
		DcuC			

Superscript letters in the Sample ID column indicates that *ATP6* (a) or *COI* (c) segment was not sequenced for that particular sample.

Table S3: Additional PCR and sequencing trials shown by group of species within families.

Family	Species	*ATP6*	*COI*
Felidae	*Acinonyx jubatus*	S*	S
	Caracal caracal	S*	S
	Felis margarita	S*	S
	Felis nigripes	S*	S
	Profelis aurata	NP	NP
Hyaenidae	*Crocuta crocuta*	S*	S
	Proteles cristata	S*	NS
	Hyaena brunnea	S	S
	Hyaena hyaena	S	S
Viverridae	*Arctictis binturong*	S	S
	Civettictis civetta	S	S
	Galidia elegans	S	NP
	Paradoxurus hermafroditus	S*	S
	Prionodon linsang	S	S
Herpestidae	*Fossa fossana*	S	S
	Helogale parvula	S	S
	Herpestes javanicus	S	NS
	Ichneumia albicauda	S	S
	Rhyncogale melleri	S	NS
	Suricata suricatta	S	S
Eupleridae	*Cryptoprocta ferox*	S*	S
Nandiniidae	*Nandinia binotata*	NP	NP
Procyonidae	*Bassaricyon alleni*	S	S
	Bassariscus astutus	S	S
	Potos flavus	NS	NP
Mustelidae	*Ictonyx striatus*	S*	NS
Ursidae	*Ailuropoda melanoleuca*	NP	S
	Ursus arctos	NP	S
Ailuridae	*Ailurus fulgens*	S	S
Phocidae	*Mirounga angustirostris*	S*	S
	Phoca vitulina	S	S
Otariidae	*Arctocephalus forsteri*	S*	NP
	Phocarctos hookeri	S	NP
	Zalophus californianus	S*	NS
Odobenidae	*Odobenus rosmarus*	S*	S

S: sequenced
NP: no PCR
NS: no sequence
*primer ATP6-DR1 was used as alternative to ATP6-DR2

Table S4: Success of sequencing faecal DNA extracted from zoo carnivores that had been fed with rabbit prior to sample collection.

Sample ID	*ATP6*	*COI*
cougar 1	S	S
cougar 2	S	S
cougar 3	S	S
cougar 4	S	S
cougar 5	S	NP
jaguar 1	NP	S
jaguar 2	S	S
jaguar 3	S	S
jaguar 4	NP	NP
leopard 1	S	NS
leopard 2	S	S
ocelot 1	S	S
ocelot 2	S	S
serval 1	S	S
serval 2	NS	NP
serval 3	S	S
tiger 1	S	S
tiger 2	S	NS
tiger 3	S	NP
Total "S"	84%	68%

S: sequenced
NP: no PCR
NS: no sequence

Table S5: Putative maned wolf scats analyzed for interference of prey DNA in predator identification. The "PN" prefix codes for samples collected in the Parque Nacional de Brasília, "FAL" for Fazenda Águas Limpas and "ES" for Estaçao Ecológica de Águas Emendadas.

Sample ID	*ATP6*	*COI* [COV/IDENT]	Prey vestiges found in faeces
PN96 [g]	maned wolf	NS	fethers, hairs, bones, claws, two almost complete foots
PN97	maned wolf	maned wolf	a beak, feathers
PN98	NP	NP	hairs, a complete foot
PN100	maned wolf	NP	feathers, bones, a beak
PN101	maned wolf	NS	hairs, bones
PN102	maned wolf	*Metachirus nudicaudatus* 100/94	hairs, bones
PN106	maned wolf	*Metachirus nudicaudatus* 100/87	hairs, bone
PN108	maned wolf	NS	hairs, bone
PN110	maned wolf	maned wolf	hairs, bone
PN118	maned wolf	maned wolf	feathers, two beaks
PN119	maned wolf	maned wolf	feathers, bone, scales [?]
PN125	maned wolf	maned wolf	hairs, bones, self-cleaning hair [?]
PN126	NP	NP	feathers
PN131 [g]	maned wolf	maned wolf	hairs (mainly white)
PN132	maned wolf	maned wolf	hairs, armadillo shell bones
PN133	maned wolf	NP	hairs, bones, armadillo shell bones, scale [?]
PN145	dog	NA	
PN148	maned wolf	*Priolepis cincta* 94/85	feathers, bones
PN149	maned wolf	maned wolf	hairs (minly white), bones
PN150	dog	NA	
PN153	maned wolf	maned wolf	feathers, bones
PN155	maned wolf	maned wolf	hairs, bones, rodent teeth
PN156	NP	*Cormura brevirostris* 100/82 *Phyllostomus discolor* 100/82	hairs
PN160	maned wolf	maned wolf	hairs, bones, a foot (reptile)
FAL02	maned wolf	maned wolf	feathers, bone, armadillo shell bones
FAL05	maned wolf	maned wolf	hairs, bones, armadillo shell bones, scales [?]
FAL10	maned wolf	NS	hairs, bone, teeths
FAL11	NP	NP	feathers
FAL12	maned wolf	maned wolf	feathers
FAL15	maned wolf	maned wolf	hairs, bones, a tooth
FAL16	maned wolf	NP	hairs, bones, armadillo shell bones, scales [?]
FAL19	maned wolf	maned wolf	hairs, bones, armadillo shell bones, a foot
FAL20 [g]	maned wolf	maned wolf	feathers, bone
FAL24 [g]	maned wolf	maned wolf	hairs
FAL25	cougar	cougar	black and white banded hairs, bones
FAL26	NP	NP	feather
FAL31	maned wolf	maned wolf	bone, armadillo shell bones, a claw
FAL32	maned wolf	NP	hairs, bones, armadillo shell bones, a foot, scales [?]
FAL35	NP	NP	bones, a tooth, armadillo shell bones
FAL36	maned wolf	maned wolf	hairs, bones
FAL37	maned wolf	*Priolepis cincta* 94/85	none
FAL40	maned wolf	maned wolf	feathers
FAL41	maned wolf	NS	feathers, bones, hairs, rodent teeth
FAL42	maned wolf	NP	bone
FAL45	maned wolf	NP	bone, two feet
ES10	domestic cat	domestic cat	hairs
ES51 [g]	maned wolf	maned wolf	hairs, bones
ES54	maned wolf	NP	feathers
ES67	NP	NP	feathers, bones, scales [?]

Table S5: *Continued* (2/2)

Sample ID	*ATP6*	*COI* [COV/IDENT]	Prey vestiges found in faeces
ES87	NP	NP	feathers
ES92	NP	NP	bones, feathers
ES94	maned wolf	*Metachirus nudicaudatus* 99/91	feathers
ES98 [g]	NP	NP	feathers
ES99	NP	NP	none
ES104	maned wolf	NP	none
ES106	NP	NP	feathers
ES107	NP	NP	feathers
ES110	NP	NP	hairs
ES121	maned wolf	NS	feathers, two feet
ES122	maned wolf	NS	feather, two feet
ES129 [g]	maned wolf	NS	feathers, a beak
ES131 [g]	maned wolf	*Molossus* sp. 97/86	feathers
ES134	NP	NP	none
ES135	maned wolf	maned wolf	feathers
ES136	NP	NP	feathers, bones
ES147	maned wolf	*Spilogale putorius* 89/83	feathers, hairs
ES149	maned wolf	maned wolf	bones
ES150	maned wolf	maned wolf	feathers
ES151	maned wolf	*Molossus rufus* 93/81 *Thomomys umbrinus* 94/81	feathers
ES153	maned wolf	NP	feathers
ES163	maned wolf	NS	feathers
ES169	maned wolf	maned wolf	feathers, a foot
ES175	maned wolf	*Metachirus nudicaudatus* 100/94	feathers, hairs, bones
ES177	dog	NA	
ES186 [g]	NP	maned wolf	feathers
ES193 [g]	maned wolf	maned wolf	feather
ES195	maned wolf	NS	none
ES196	maned wolf	*Molossus sp.* 97/86	bone, feather
ES201	maned wolf	maned wolf	feathers
ES208 [g]	maned wolf	maned wolf	feathers, hairs
ES213	maned wolf	maned wolf	feather
ES216	maned wolf	NS	bones

[g] Samples genotyped for five canid microsatellites (M.B. Lion, unpublished data)
NP: no suitable PCR amplicon obtained for sequencing
NS: no suitable sequence obtained
NA: not amplified
[COV/IDENT] Max. coverage/Max. Identity of most similar BLAST search
?: uncertain identification

1458

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo