Diversidade Genética e Padrões Filogeográficos da

Lontra Neotropical (*Lontra longicaudis* [Olfers, 1818]);

(Mammalia: Mustelidae)

Cristine Silveira Trinca

Pontifícia Universidade Católica do Rio Grande do Sul

Faculdade de Biociências

Programa de Pós-Graduação em Biociências - Zoologia

Diversidade Genética e Padrões Filogeográficos da

Lontra Neotropical (*Lontra longicaudis* [Olfers, 1818]);

(Mammalia: Mustelidae)

Cristine Silveira Trinca

Orientador: Prof. Dr. Eduardo Eizirik

Dissertação de Mestrado

Porto Alegre – RS – Brasil

2007

Agradecimentos

Agradeço a todos os colegas do Genoma, por tornarem o ambiente tão agradável e descontraído para trabalhar e por sempre estarem dispostos a ajudar. Um agradecimento especial a Lari, pela ajuda nas análises dos microssatélites e a Cladi, por quebrar todos os galhos e fazer com muita eficiência o seu trabalho, deixando o nosso um pouco mais fácil.

Às minhas amigas queridas, colegas de trabalho e parceiras de futebol Taia e Aninha, que fazem do laboratório não só um local de trabalho, mas também um lugar para rir, chorar e fazer terapia de grupo. Muito obrigada por toda a ajuda e por serem pessoas tão especiais que eu tive a sorte de conhecer.

Ao Manoel, amigo e colega, por me ensinar as regras básicas do funcionamento do Genoma, por toda a ajuda e também pela amizade e carinho.

A todo o grupo de "carnivorólogos", um grupo formado há pouco tempo, mas bastante unido, que sempre contribuiu com discussões científicas permeadas por momentos de intensa descontração.

À Ana Paula Brandt e a Tatiane C. Trigo, à primeira por me apresentar às lontras, e à segunda pela paciência em ensinar extrair DNA e a fazer PCR; as duas, sem saber, mudaram todo o caminho que eu havia planejado.

A todos os pesquisadores e instituições que colaboraram com o projeto através do envio de amostras.

Aos amigos que trabalham em campo, e que se lembravam de mim ao encontrar uma lontra atropelada ou uma amostra de fezes e prontamente coletavam material.

Ao Professor Thales R. O. de Freitas por me aceitar em seu laboratório no começo da graduação, me dando a oportunidade de conhecer e trabalhar na área que me fascina, a genética da conservação.

Ao Duda, meu orientador e amigo, pela confiança, pelo grande aprendizado, por me ensinar a ser mais autônoma e confiante de minhas capacidades.

Sumário

Resumo

O conhecimento sobre a estruturação geográfica da diversidade genética em populações naturais permite inferir os processos históricos atuantes sobre as espécies e é fundamental para o planejamento de estratégias eficazes de conservação biológica. Neste contexto, o presente estudo é o primeiro a identificar e caracterizar a variabilidade genética, padrões de estruturação populacional e história demográfica de *Lontra longicaudis*. Para tanto, foram utilizados três segmentos do DNA mitocondrial (mtDNA; porção hipervariável I da região controladora, gene *ATP8* e gene *ND5*), bem como 12 locos de microssatélite, em indivíduos amostrados em diferentes regiões de sua distribuição geográfica. Ambos os marcadores revelaram moderados a altos níveis de variabilidade genética e padrões filogeográficos claros, os quais sugerem que as populações brasileiras desta espécie encontram-se geneticamente diferenciadas das outras regiões amostradas a Noroeste da América do Sul. As análises de mtDNA indicam a provável existência de quatro entidades filogeográficas: Colômbia, Bolívia, Guiana Francesa/Peru e Brasil. Colômbia e Bolívia foram representadas por apenas um indivíduo cada, os quais revelaram grande divergência genética dos outros indivíduos amostrados, sugerindo profunda subdivisão filogeográfica envolvendo estas regiões. A alopatria entre Guiana Francesa e Brasil é quase completa, sugerindo que a incongruência entre filogenia e geografia possa ser decorrente de um processo de colonização ancestral no sentido Norte-Sul. A inferência de diferenciação genética entre Brasil e as outras áreas amostradas na América do Sul são apoiadas pelas análises de microssatélite. Os resultados obtidos a partir das análises de mtDNA indicam ausência de estruturação genética no Brasil e são indicativos de um cenário de expansão populacional recente nesta região. Os padrões observados neste estudo têm implicações para a conservação de populações naturais de *Lontra longicaudis*. As quatro entidades filogeográficas reconhecidas demonstram-se suficientemente diferenciadas e deveriam, portanto, ser conservadas e manejadas independentemente. Estudos adicionais são necessários para melhorar o conhecimento sobre estas populações, bem como para investigar a existência de outras unidades demográficas ao longo da distribuição da lontra Neotropical.

Capítulo I – Introdução Geral
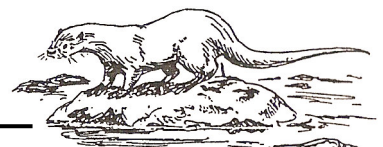
A Família Mustelidae

A Família Mustelidae compreende atualmente 25 gêneros e 67 espécies (Nowak 1999) colocadas em cinco subfamílias: Mustelinae, Melinae, Taxiidinae, Mellivorinae e Lutrinae (Dragoo & Honeycutt 1997; Koepfli & Wayne 2003; Flynn *et al*. 2005; Fulton & Strobeck 2006). Esta família compreende carnívoros de médio porte, dentre os quais se encontram os furões, texugos, lontras, martas e doninhas. Os membros desta família apresentam geralmente o corpo alongado, as patas relativamente curtas, e normalmente possuem uma cauda longa (Eisenberg & Redford 1999).

Mustelídeos habitam todos os continentes, exceto Austrália e Antártica, e não ocorrem em Madagascar nem em ilhas oceânicas. Membros desta família podem ser encontrados em ambiente terrestre, de água doce ou marinho. São principalmente carnívoros, e várias espécies incluem uma grande diversidade de vertebrados e invertebrados em sua dieta (Nowak 1999; Sato *et al*. 2003).

A Subfamília Lutrinae

A subfamília Lutrinae compreende 13 espécies de lontras, distribuídas em seis gêneros: *Aonyx, Enhydra, Lutrogale*, *Pteronura, Lutra* e *Lontra* (Kruuk 2006). Recentemente, Zyll de Jong (1987) forneceu evidências filogenéticas de que as lontras do Novo Mundo (exceto *Pteronura*), até então incluídas no gênero *Lutra*, deveriam passar a compor um novo gênero, *Lontra*, o qual inclui quatro espécies: *Lontra provocax*, *L. felina*, *L. canadensis* e *L. longicaudis* (Kruuk 2006).

As lontras encontram-se distribuídas por todos os continentes, exceto Austrália e Antártica (Foster-Turley *et al*. 1990). Na região Neotropical ocorrem quatro espécies de lontras: *Lontra provocax*, *L. felina*, *L. longicaudis* e *Pteronura brasiliensis* (Chehébar 1990). Duas destas espécies de lontras ocorrem no Brasil, a lontra neotropical (*Lontra longicaudis*) e a ariranha ou lontra gigante (*Pteronura brasiliensis*) (Chehébar 1990), sendo que em algumas regiões do país estas ocorrem em simpatria. Ambas foram intensamente caçadas para utilização da pele até a década de 1980 (Duarte & Rebelo 1985), apesar de a caça ter se tornado uma atividade proibida por lei desde 1967, em todo o território nacional.

A Lontra Neotropical

A lontra neotropical é um carnívoro de médio porte, de coloração marrom-pardacenta, quase preta, tendo apenas o lado ventral e o focinho amarelados. Possui grandes vibrissas que auxiliam na localização de presas embaixo d'água (Cimardi 1996). Suas patas apresentam

membranas interdigitais e sua cauda é longa e levemente achatada na extremidade, sendo ambas as estruturas adaptadas para a locomoção na água (Silva 1994).

É uma espécie de hábito semi-aquático, ocorrendo em uma grande variedade de habitats, em rios, lagos, pequenos canais, banhados e também ambientes marinhos associados a cursos d'água (p.ex. nos Estados de Santa Catarina e Rio de Janeiro) (Blacher 1987; Mason 1990; Fonseca *et al*. 1994); pode ser encontrada em uma ampla faixa de gradiente altitudinal (desde regiões litorâneas até cerca de 3.000 m de altitude) (Emmons 1990; IBAMA 1997). A dieta desta espécie, como a da maioria das espécies de lontras, consiste basicamente de peixes, crustáceos e moluscos, podendo ser complementada por aves, pequenos mamíferos, anfíbios e insetos (Chebez 1999).

Trata-se de uma das espécies de lontras menos conhecidas, sendo que as pesquisas realizadas até o momento são geograficamente restritas e direcionadas a estudos do hábito alimentar (Gallo 1986, 1989, 1997; Passamani & Camargo 1995; Spinola & Vaughan 1995; Parera 1996; Soldateli & Blacher 1996; Helder & De Andrade 1997; Pardini 1998; Colares & Waldemarin 2000; Quadros & Monteiro-Filho 2000, 2001; Utreras *et al.* 2002), distribuição local (Waldemarin 1997; Lacomba *et al.*, 2001), uso de abrigos como tocas e locais de descanso (Gallo 1989; Spinola & Vaughan 1995; Pardini 1999; Waldemarin & Colares 2000) e freqüência de marcação (Soldateli & Blacher 1996; Spinola & Vaughan 1995). Além disso, estudos sobre reprodução e fisiologia da espécie foram realizados apenas em cativeiro (Colares & Silva 1987; Colares & Best 1991; Parera 1996).

A distribuição geográfica atual da espécie é pouco conhecida, embora seja descrita como ocorrendo amplamente, ao longo de uma faixa contínua que inicia no México, cobrindo praticamente todo continente sul-americano e terminando no nordeste da Província de Buenos Aires (Argentina) e sul do Uruguai, alcançando também o norte do Peru (Chehébar 1990; Chebez 1999; Eisenberg & Redford 1999), e estando distribuída em todo o território brasileiro (Emmons 1990).

Em termos de *status* de conservação em nível internacional, *L. longicaudis* é considerada como tendo "Dados Insuficientes" pela IUCN (IUCN 2006), e está incluída no Apêndice I ("Espécies ameaçadas") da CITES ("Convention on International Trade in Endangered Species of Wild Fauna and Flora") (IUCN 2006). No Brasil, esta espécie é categorizada como "Quase Ameaçada" (IBAMA 2004), embora em Estados como Minas Gerais, São Paulo, Paraná e Rio Grande do Sul esteja classificada como "Vulnerável" (Paraná 1995; Machado *et al*. 1998; São Paulo 1998; Indrusiak & Eizirik 2003).

Dentre as principais ameaças a esta espécie encontram-se os conflitos com pescadores e proprietários de criadouros de peixes, o desmatamento e outras alterações antrópicas das

margens dos rios (Macdonald & Mason 1985). A poluição das águas também é uma grave ameaça à espécie, uma vez que pode ter influência indireta ou direta na estabilidade das populações de lontras. Danos indiretos referem-se à diminuição do estoque de alimento e contaminação do ambiente. Efeitos diretos causam impacto no animal, resultando em sua morte ou reduzindo seu sucesso reprodutivo (Macdonald & Mason 1990). Além disso, freqüentemente são mortas por atropelamentos em estradas (Macdonald & Mason 1990) e a alta densidade populacional humana também é citada como um fator que pode levar ao seu desaparecimento (Fonseca *et al.* 1994; IBAMA 1997).

Estruturação Geográfica de Populações Naturais – Inferências Evolutivas e Implicações para a Conservação

Uma das principais metas da Biologia da Conservação é a preservação da biodiversidade e a manutenção dos padrões e processos evolutivos que a geraram; para tal, é necessário elaborar programas adequados de conservação, nos quais deve ser decidido o que e como proteger (Johnson *et al.* 2001). Assim, uma área recente da Biologia, conhecida como Genética da Conservação, tem auxiliado na tomada destas decisões geralmente através da utilização de marcadores moleculares, os quais são em parte responsáveis pela possibilidade de estudar aspectos complexos de espécies e populações ameaçadas em condições naturais. A utilização de técnicas moleculares para este fim tem se expandido muito nas últimas duas décadas, sendo acompanhada também de uma crescente aceleração no desenvolvimento de métodos laboratoriais e analíticos empregados nestas abordagens (Frankham *et al.* 2002; Hey & Machado 2003; Luikart *et al.* 2003; DeSalle & Amato 2004; DeYoung & Honeycutt 2005; Kohn *et al.* 2006).

O conceito de filogeografia foi introduzido por Avise *et al.* (1987) para designar o estudo da distribuição da variabilidade genética de uma espécie em uma escala espacial e temporal. Os estudos filogeográficos têm por objetivo revelar a história evolutiva de uma linhagem, relacionando-a com sua distribuição geográfica, através, principalmente, das diferenças entre seqüências de DNA mitocondrial (mtDNA) (Avise 2000), utilizando as explicações geográficas históricas para interpretar as relações evolutivas entre os *taxa* (Stevens & Hogg 2003). Análises de padrões filogeográficos permitem a verificação de estruturação genética e a interpretação das possíveis barreiras ao fluxo gênico dentro e entre as espécies (p.ex. Eizirik *et al.* 2001), gerando um aumento do conhecimento sobre os processos históricos biogeográficos.

Além da análise dos níveis de diversidade genética, o acesso à distribuição geográfica desta variabilidade dentro de uma espécie é também de extrema importância para identificar e priorizar áreas nas quais programas de manejo e conservação devem ser elaborados (Moritz & Faith 1998). É fundamental avaliar se a espécie apresenta uma distribuição homogênea da

variabilidade genética ou algum grau de subdivisão, pois, no primeiro caso, qualquer área de sua distribuição é representativa da espécie enquanto que, havendo estruturação, a representatividade de cada sub-população deve ser preservada.

Assim, a partir de estudos de filogeografia podem-se identificar dois tipos de linhagens evolutivas para fins de manejo: (i) Unidades Evolutivamente Significativas ("Evolutionarily Significant Units" - ESUs) e (ii) Unidades de Manejo ("Management Units" - MUs). As primeiras são constituídas por unidades demográficas infra-específicas, distintas geograficamente e que se apresentam diferenciadas geneticamente (implicando isolamento histórico) de outras unidades semelhantes contidas na mesma espécie. As Unidades de Manejo estão contidas nas ESUs, e são formadas por populações regionais com restrita conexão demográfica entre si, mas não necessariamente com diferenciação genética profunda. Entretanto, em uma escala de tempo curta (uma ou poucas gerações), o contato entre estas populações através de migração ou re-colonização torna-se escasso, transformando-as em entidades ecológicas relativamente separadas, e que deveriam ser manejadas de forma independente ou coordenada (Ryder 1986; Moritz 1994; Eizirik 1996; Crandall *et al.* 2000; Fraser & Bernatchez 2001; Frankham *et al.* 2002)

Utilização de Marcadores Moleculares em Estudos Evolutivos e Populacionais

Entre os marcadores moleculares mais freqüentemente empregados em estudos populacionais, evolutivos e/ou voltados para conservação destaca-se o DNA mitocondrial (mtDNA), como marcador de diversidade genética, sendo também muito útil em investigações sobre relações filogenéticas entre diferentes *taxa* e identificação de subdivisão geográfica entre unidades populacionais (Avise *et al.* 1987; Bermingham & Moritz 1998; Avise 2000).

Duas razões justificam a ampla utilização do polimorfismo de mtDNA na reconstrução de filogenias moleculares: primeiro, o mtDNA representa a parte mais bem conhecida do genoma animal e, segundo, a taxa de evolução do mtDNA é 5 a 10 vezes mais rápida do que a dos locos nucleares de cópia única na maioria das espécies de mamíferos (Avise *et al.* 1992; Baker *et al.* 1993; Martin & Palumbi 1993; Ballard & Whitlock 2004).

De um modo geral, mtDNA animal apresenta duas características que podem ser vistas em alguns contextos como vantagens sobre os marcadores nucleares: (i) a relação filogenética do mtDNA reflete a história de linhagens maternas dentro de uma população ou espécie; (ii) o tamanho efetivo da população do genoma mitocondrial é de ¼ comparada aos genes autossômicos, levando a uma maior taxa de diferenciação local por deriva aleatória (Neigel & Avise 1986).

Além do mtDNA, o DNA nuclear tem se tornado cada vez mais utilizado em estudos evolutivos e populacionais. Dentre os marcadores nucleares mais amplamente empregados

encontram-se os microssatélites. Estes marcadores consistem de segmentos curtos de DNA (1-6 pares de bases), que se repetem em número variável em *tandem*, sendo em sua maioria, repetições de mono, tetra ou, principalmente, dinucleotídeos, apresentando-se como locos altamente polimórficos dispersos amplamente em genomas eucarióticos (Schlötterer 1998; Schlötterer 2004).

As características apresentadas pelos microssatélites (alto nível de polimorfismo, alta taxa de mutação, e tendência à neutralidade seletiva) permitem a sua utilização em estudos como comparação da variação genética entre espécies e populações (Johnson *et al.* 1999; Wisely *et al*. 2002), grau de estrutura das populações e migração (Ciofi & Bruford 1999; Waits *et al.* 2000; Cegelski *et al.* 2003) e determinação de parentesco e estrutura social (Nesje *et al.* 2000), os quais são extremamente necessários para o desenho de estratégias para a conservação de espécies ameaçadas. Além disso, permitem a utilização de DNA altamente fragmentado, em pequenas quantidades ou até mesmo de amostras antigas (Bruford & Wayne 1993).

Estudos Genéticos e Moleculares com Mustelídeos

O papel dos carnívoros na regulação de comunidades e manutenção da biodiversidade vem sendo amplamente discutido nas últimas décadas (Kitching 1986; Fonseca & Robinson 1988; Terborgh 1990, 1992; Wright *et al.* 1994; Asquith *et al.* 1997). Estudos realizados com a lontra marinha (*Enhydra lutris*), por exemplo, identificaram a importância desta espécie na manutenção da biodiversidade das comunidades marinhas onde vive (Kitching 1986). A lontra neotropical, por sua vez, além de ser um carnívoro, é uma das únicas espécies de maior porte que ocupa esta posição nas cadeias alimentares de ambientes aquáticos onde ocorre e, assim, pode desempenhar uma importante função na regulação das comunidades destes sistemas. Apesar de ter sofrido com a caça excessiva no passado, atualmente a lontra neotropical parece estar distribuída de forma ampla no Brasil, ocorrendo inclusive em áreas moderadamente urbanizadas e onde problemas ambientais como poluição orgânica e desmatamento da mata ciliar estão presentes. No entanto, os registros de extinções locais sofridas pela lontra euro-asiática (*Lutra lutra*) em vários países europeus, e pela lontra norte-americana (*Lontra canadensis*) em diversas localidades dos Estados Unidos, demonstram que mesmo espécies altamente adaptáveis e flexíveis podem sofrer declínios populacionais extremos perante modificações ambientais intensas (Foster-Turley *et al.* 1990; Swimley *et al.* 1998; Reuther *et al.* 2000). Embora a lontra neotropical seja uma espécie considerada como apresentando algum grau de tolerância à presença humana, pouco se sabe sobre os efeitos desta aproximação sobre as populações de *L. longicaudis.*

Estudos genéticos e moleculares sobre os mustelídeos realizados até o momento são principalmente direcionados à lontra euro-asiática (*Lutra lutra*) (Dallas & Piertney 1998; Dallas *et*

al. 1999; Mucci *et al*. 1999; Dallas *et al*. 2000; Dallas *et al.* 2002; Randi *et al*. 2003; Arrendal *et al*. 2004; Huang *et al*. 2005), lontra norte-americana (*L. canadensis*) (Serfass *et al*. 1998; Blundell *et al*. 2002; Beheler *et al*. 2004; Beheler *et al*. 2005), texugo euro-asiático (*Meles meles*) (Frantz *et al.* 2003; Pope *et al*. 2005), glutão ou carcaju (*Gulo gulo*) (Walker *et al*. 2001; Kyle & Strobeck 2001) e marta-americana (*Martes americana*) (Broquet *et al.* 2006).

Os únicos estudos genéticos incluindo *L. longicaudis* publicados até o momento são análises filogenéticas da família Mustelidae e da Subfamília Lutrinae como um todo, os quais não abordam quaisquer aspectos específicos desta lontra em particular (Zyll de Jong 1987; Koepfli & Wayne 1998; Marmi *et al*. 2004; Koepfli & Wayne 2003). Sendo assim, não existe até o momento qualquer estudo publicado voltado para a investigação de parâmetros genéticos das populações de *L. longicaudis*.

O presente estudo tem por objetivo, através da utilização da variação em seqüências do mtDNA e microssatélites, (1) identificar e caracterizar subdivisões geográficas em populações de *Lontra longicaudis*, e (2) a partir dos padrões observados inferir os processos históricos que atuaram sobre esta espécie, bem como comparar os padrões encontrados com aqueles descritos para outras espécies de vertebrados, além de avaliar o desempenho destes marcadores para estudos evolutivos e populacionais de mustelídeos.

Capítulo II - Artigo

"Phylogeographic Patterns and Evolutionary History of the Neotropical otter (*Lontra longicaudis*)"

CRISTINE S. TRINCA, HELEN F. WALDEMARIN, KLAUS-PETER KOEPFLI, BENOIT DE THOISY and EDUARDO EIZIRIK

1   **Phylogeographic Patterns and Evolutionary History of the Neotropical otter**

2   (***Lontra longicaudis***)

3   CRISTINE S. TRINCA [1], HELEN F. WALDEMARIN [2], KLAUS-PETER KOEPFLI [3], BENOIT

4   DE THOISY [4], EDUARDO EIZIRIK[1, 5]

5

6   [1] Faculdade de Biociências, PUCRS, Porto Alegre, Brazil.

7   [2] Projeto Ecolontras/Associação Ecológica Ecomarapendi, Rio de Janeiro, Brazil;

8   [3] Department of Organismic Biology, Ecology and Evolution, University of California, Los

9   Angeles, USA;

10   [4] Institute Pasteur de la Guyane, French Guiana;

11   [5] Instituto Pró-Carnívoros, Brazil.

12

13   Corresponding author:

14   Dr. Eduardo Eizirik

15   Faculdade de Biociências - PUCRS

16   Av. Ipiranga 6681, CEP 90619-900 Porto Alegre, RS, Brazil

17   Telephone number: 55 (51) 3320.3500 Ext. 4717

18   Fax number: 55 (51) 3320.3612

19   Email: eduardo.eizirik@pucrs.br

20

24   **Abstract**

25   The Neotropical otter (*Lontra longicaudis*) is a medium-sized carnivore with a broad
26   distribution in the Neotropical region. This species, as well as other otter species, suffered
27   with hunting in parts of its range until 1960s due to the high value of its pelts. Despite being
28   apparently common in many areas, it is one of the least known otters, and genetic studies on
29   this species are scarce. Here we have investigated its genetic diversity, population structure
30   and demographic history across part of its geographic range by analyzing 1472 base pairs
31   (bp) of mitochondrial DNA (mtDNA) and 12 microsatellite loci. *L. longicaudis* exhibits high
32   levels of mtDNA and microsatellite diversity. Both mitochondrial and nuclear data revealed a
33   consistent phylogeographic pattern, indicating that Brazilian populations are genetically
34   distinct from sampled populations distributed in Northwestern South America. The mtDNA
35   data indicates the probable existence of four phylogroups occurring in Brazil, French
36   Guiana/Peru, Bolivia and Colombia, respectively. The single Colombian haplotype was the
37   most basal relative to all other lineages, suggesting a substantial isolation of this phylogroup.
38   No substantial geographic substructure is observed within the Brazilian cluster, but a
39   demographic expansion is inferred. Inferences on the history of these phylogroups and
40   possible causes of such patterns are drawn, based on phylogenetic and population-genetic
41   approaches. Implications of the observed patterns for the conservation of *L. longicaudis* are
42   addressed, highlighting the finding that broad geographic regions contain differentiated
43   population segments that likely warrant independent management status.

44

Introduction

Phylogeographic studies of widely distributed semi-aquatic mammals can provide a natural link between the geological and biotic evolution of an area, since dispersal of these species is largely related to the direct connection among basins, and the history of basin interconnections is a result of geological processes (Bermingham & Martin 1998). In this context, mitochondrial DNA (mtDNA) genealogies have been used extensively to infer processes at population levels and the phylogenetic diversification of taxa in relation to their geographical distribution (Avise *et al*. 1987; Avise 2000). However, mtDNA reflects only a portion of the total historical record of a matriarchal component of the organismal pedigree (Avise 1994), and thus a more complete view can be obtained by additional investigation of nuclear loci. In this context, microsatellites have an interesting informative potential for demographic analysis, due to their rapid mutation rate and a biparental inheritance (Frankham *et al*. 2002).

Processes in the demographic history of populations, such as expansions or contractions, leave recognizable signatures in the pattern of molecular diversity (Harpending *et al*. 1998; Schneider & Excoffier 1999). Historical events can affect population size, and may result in low levels of genetic diversity in current populations even if these populations do not present small size. Moreover, climatic changes as occurred during the Pleistocene have been responsible for the current patterns of genetic variation in many species (Lessa *et al*. 2003).

So far, few studies have addressed the evolutionary history of Neotropical taxa from an intra-specific phylogeography perspective, so that most of the evolutionary aspects still need to be investigated. Some studies have investigated phylogeographic patterns in Neotropical vertebrates (Eizirik *et al*. 1998; Lovejoy & de Araújo 2000; Eizirik *et al*. 2001; Cantanhede *et al*. 2005; Vianna *et al*. 2006; Grazziotin *et al*. 2006; Marquéz *et al*. 2006; Tchaicka *et al*. 2007), but the region has still been poorly characterized with respect to widely distributed semi-aquatic mammals.

Lontra Longicaudis is a medium-size semi-aquatic carnivore, a relatively common species widely distributed in the Neotropical region that exhibits a fish-based diet, complemented by crustaceans and mollusks (Chebez 1999). In the past, and especially during the last two centuries, humans made heavy use of otters for skins. Otter pelts were very much in demand in the international market during the first half of the 20th century (Chehébar 1990). Although some illegal hunting continues, this species has been relatively free of exploitation since the 1960s, when the hunting pressure declined significantly, due to the growing concern for wildlife conservation and because of the Latin American countries

80 enforcing CITES regulations (Chehébar 1990). However, throughout this range, this mustelid
81 has been subjected to persecution because its supposed predation on fish stocks
82 (Macdonald & Mason 1990) and are also heavily killed on roads. Despite that, the species is
83 considered as "Data Deficient" by IUCN (2006), but in several localities of its distribution it is
84 categorized as "Near Threatened" or "Vulnerable" (Chehébar 1990).

85   Most otter species are poorly known and are important targets for conservation due to
86 be indicator of healthy aquatic environments. As otters are typical animals at the top of the
87 food chain, they are among the first species to refuse and disappear when the environment
88 is degraded (Foster-Turley *et al*. 1990). However, even basic information is difficult to obtain
89 because otters are rarely observed, and are very difficult to trap, mark and recapture (Dallas
90 & Piertney 1998). Some studies have addressed aspects of intra-specific genetic diversity
91 and population structure of basically three otter species (Bodkin *et al*. 1999; Blundell *et al*.
92 2002; Dallas *et al*. 2002; Larson *et al.* 2002; Pérez-Haro *et al*. 2005); however, no large-scale
93 study has yet been published on the Neotropical otter (*Lontra longicaudis*).

94   The present study aims to characterize the genetic structure and evolutionary history
95 of Neotropical otter populations on a broad geographic scale, based on the analysis of
96 independent loci that represent both the nuclear and mitochondrial genomes.
97 Phylogeographic partitions and estimated population genetic parameters are used to infer
98 the evolutionary history of this species. Since this is the first phylogeographic study of a
99 Neotropical semi-aquatic mammal, we aimed to test whether the observed patterns were
100 congruent with those observed in terrestrial taxa (e.g. other carnivores, small mammals -
101 Eizirik *et al*. 1998; Eizirik *et al*. 2001; Costa 2003; Tchaicka *et al*. 2007) or aquatic species
102 (e.g. manatees, fish, caimans – Lovejoy & de Araújo 2000; Sivasundar *et al*. 2001;
103 Cantanhede *et al*. 2005; Vianna *et al*. 2006; de Thoisy *et al*. 2006), or yet presented unique
104 features not yet detected in other organisms.
105

106 **Materials and Methods**
107 *Sample collection*
108   Biological samples were collected from 45 Neotropical otters across a large area of
109 the species' range (Fig. 1; Table 1). Blood samples were obtained from captive individuals
110 (with known geographic origin). Tissue samples were collected opportunistically by
111 collaborators from road-killed animals. Scat samples were collected by field researchers and
112 from captive individuals. A hair sample was obtained from one dead animal. Blood samples
113 were preserved in a salt saturated solution (100mM Tris, 100mM EDTA, 2% SDS) and
114 muscle and scat samples were preserved in ethanol 96%. All samples were stored at -20°C

115   prior to DNA extraction. Samples of *Lontra canadensis*, *Aonyx cinereus* and *Pteronura*
116   *brasiliensis* were also included to be used as outgroups in some of the analyses.

117

118   ***DNA extraction and molecular analyses***

119   Genomic DNA was extracted from blood and tissue samples using a standard
120   Proteinase-K digestion and phenol-chloroform-isoamyl alcohol protocol (Sambrook *et al.*
121   1989). DNA from hairs was extracted using the ChargeSwitch® Forensic DNA Purification Kit
122   (Invitrogen). DNA from scats was extracted using the QIAamp DNA Stool Mini Kit (Qiagen)
123   following the manufacturer's instructions. The scat DNA extractions were carried out in a
124   separate laboratory area, in a UV-sterilized laminar flow hood, dedicated to the DNA analysis
125   of non-invasive samples.

126   Three segments of the mitochondrial DNA were amplified by the Polymerase Chain
127   Reaction (PCR, Saiki *et al.* 1985): (I) the 5' portion of the mtDNA control region (CR),
128   containing the first hypervariable segment, using primers MTLPRO2 (5'-
129   CACTATCAGCACCCAAAGCTG-3') and CCR-DR1 (5'-CTGTGACCATTGACTGAATAGC-
130   3'); (II) a segment of the *ATP8* gene using primers ATP8-DF1 (5'-
131   AGAAGCTAAATAAGCATTACCTTTTA-3') and ATP8-DR1 (5'-
132   CCAGTATTTGTTTTGATGTTAGTTG-3'); and (III) a segment of the *ND5* gene using primers
133   ND5-DF1 (5'-TTGGTGCAACTCCAAATAAAAGT-3') and ND5-DR1 (5'-
134   AGGAGTTGGGCCTTCTATGG-3').

135   Since fecal samples tend to present degraded DNA, hindering the sequencing of long
136   fragments, we designed internal primers for the control region dividing it into three shorter,
137   overlapping fragments (each one with *ca.* 250 bp). Through this strategy it was possible to
138   obtain a complete sequence of the CR fragment from fecal samples. These primers are:
139   LonCR-R1 (used in combination with MTLPRO2) (5'-ATGGTTTCTCGAGGCATGGT-3'),
140   LonCR-F2 (used in combination with CCR-DR1) (5'-AACTATACCTGGCATCTGGTTCTT-3'),
141   and the internal pair LonCR-F1 (5'- GGTTTGCCCCATGCATATAA-3') + LonCR-R2 (5'-
142   TGTGTGATCATGGGCTGATT-3'). The scat samples were amplified only for the mtDNA
143   control region.

144   Each 20ul PCR reaction contained 1-2 ul of DNA, 1x PCR Buffer (Invitrogen), 1.5 -
145   2.0 mM MgCl$_2$, 200 uM dNTPs, 0.2uM of each primer and 0.5 unit of Taq DNA Polymerase
146   (Invitrogen). The PCR conditions were the same for the three mitochondrial segments, and
147   began with 10 cycles (*Touchdown*) of 94°C for 45s, 60-51°C for 45s, 72°C for 1.5 min; this
148   was followed by 30 cycles of 94°C for 45s, 50°C for 45s, 72°C for 1.5 min and final extension
149   of 72°C for 3 min. Products were visualized on a 1% agarose gel stained with ethidium

150 bromide, purified with PEG8000, sequenced using the *DYEnamic ET Dye Terminator*
151 *Sequencing Kit* (Amersham Biosciences), and analyzed in a MegaBACE 1000 automated
152 sequencer (Amersham Biosciences). Sequences were deposited in GenBank under
153 accession numbers XXXX-XXXX.

154   DNA extracts were also typed by PCR for 12 microsatellite loci (Lut453, Lut733,
155 Lut782, Lut701, Lut818, RIO06, RIO07, RIO11, RIO17, RIO18, RIO19 and RIO20),
156 developed for two other species of otters (*Lutra lutra* and *Lontra canadensis*) (Dallas *et al*.
157 1998; Beheler *et al*. 2004, 2005). Every forward primer was 5'-tailed with an M13 sequence
158 (5'- CACGACGTTGTAAAACGAC-3') (Boutin-Ganache *et al*. 2001), and used in combination
159 with an M13 primer that had the same sequence but was dye-labeled on its 5' end. The PCR
160 reactions were performed in 10ul reactions containing 0.5 – 1.5 ul of empirically diluted DNA,
161 1x PCR Buffer (Invitrogen), 1.5 - 2 mM MgCl$_2$, 200 uM dNTPs, 0.2 uM of the reverse and
162 M13-fluorescent primers, 0.0133 uM of the M13-tailed forward primer, and 0.5 unit of Taq
163 DNA Polymerase. The PCR conditions were the same as for the mitochondrial segments,
164 except for a 30min final extension at 72°C. PCR reactions were carried out for each locus
165 separately, and products from 1 to 3 loci were diluted and pooled together based on yield,
166 size range and fluorescent dye, and then analyzed in a MegaBACE 1000 automated
167 sequencer.

168

169 ***Sequence analysis***

170   Sequences were visually checked and manually corrected using CHROMAS 2.0
171 (http://www.thecnelysium.com.au/chromas.html) and aligned with the CLUSTALW algorithm
172 implemented in MEGA 3.1 (Kumar *et al*. 2004), with the resulting alignments edited by hand.
173 MEGA was also used to perform initial sequence comparisons and computations of
174 variability.

175   Phylogenetic analyses were performed separately for the mtDNA control region (CR),
176 coding fragments (*ATP8*+*ND5*), and full concatenation (CR+*ATP8*+*ND5*). The data sets were
177 assessed for the most appropriate model of nucleotide substitution using the Akaike
178 Information Criterion (AIC) as implemented in Modeltest 3.07 (Posada & Crandall 1998). We
179 inferred phylogenetic relationships among haplotypes using PAUP* 4.0b10 (Swofford 2002)
180 for three different optimality criteria: (i) maximum likelihood (ML) employing the selected
181 model and estimated parameters, with a heuristic search started from a neighbor-joining (NJ)
182 tree and using the nearest-neighbor interchange (NNI) branch-swapping method; (ii)
183 distance-based, using the (NJ) algorithm and ML genetic distances; and (iii) maximum
184 parsimony (MP) using heuristic searches with 50 replicates of random taxon addition and

tree-bisection-reconnection (TBR) branch-swapping. Group support for all of the above methods was evaluated with 100 nonparametric bootstrap replicates. Additionally, we performed Bayesian phylogenetic analyses (BI) with MrBayes 3.1 (Huelsenbeck & Ronquist 2001), with four Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains run for 1 million generations. Trees were sampled every 100 generations, discarding the first 2,500 trees as burn-in. Two independent runs were performed for each data set to evaluate convergence. We used *Lontra canadensis, Aonyx cinereus* and *Pteronura brasiliensis* as outgroups in the phylogenetic analyses.

A median-joining network (Bandelt *et al* 1999) was constructed using Network 4.1.1.2 (www.fluxus-engineering.com) to depict phylogenetic, geographic, and potential ancestor-descendent relationships among the sequences. Divergence times between inferred clades were estimated using a distance-based linearized tree method implemented in MEGA, employing a molecular calibration of 5.9 million years ago (MYA) (credibility interval (CI): 4 - 8.3 MYA) for the evolutionary separation between the *Aonyx* and *Lontra* lineages (E. Eizirik *et al*. unpublished).

Population structure analyses were performed assuming broad geographic units based on the observed phylogeographic pattern (see Results). The testing of additional, alternative scenarios of geographic subdivision could not be adequately performed with this approach due to limitations of sample size for some of the included areas. As measures of differentiation among populations, we estimated fixation indices (F_{ST}) (Wright 1965), using an Analysis of Molecular Variance (AMOVA) approach (Excoffier *et al.* 1992) implemented in ARLEQUIN 2.0 (Schneider *et al*. 2000).

A Mantel test (Mantel 1967) as implemented in AIS 1.0 (Miller 2005) was performed to test the null hypothesis of no correlation between geographic and genetic distances. Statistical significance was tested using 1000 random permutations. Statistics such as nucleotide (π) and haplotype (*h*) diversity, neutrality tests such as Tajima's D (Tajima 1989), Fu and Li's F* & D* (Fu & Li 1993), and Fu's F_s (Fu 1997), and Mismatch Distribution Analyses (Rogers & Harpending 1992) were computed using DnaSP (Rozas *et al*. 2003) and ARLEQUIN.

Microsatellite analyses

Microsatellite genotyping was performed using the software Genetic Profiler 2.2 (Amersham Biosciences). We calculated observed (H_O) and expected (H_E) heterozygosity for each locus and tested for evidence of deviation from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) using CERVUS 2.0 (Marshall *et al*. 1998) and ARLEQUIN. To

220   correct for multiple comparisons, Bonferroni adjustments (Rice 1989) with an original α level

221   of 0.05 were carried out for all tabulated results. Using the unlinked loci that were in HWE,

222   we performed a *G*-test to evaluate the null hypothesis that allelic frequencies were identical

223   across populations (Sokal & Rohlf 1981).

224   The degree of population genetic structure was estimated with the F_{ST} index using an

225   Analysis of Molecular Variance (AMOVA) approach implemented in ARLEQUIN. For

226   comparison, we also calculated R_{ST}, an analogous measure designed for microsatellite data

227   that incorporates a stepwise mutation model (Slatkin 1995). As an independent measure of

228   the partitioning of genetic variation among groups, the program STRUCTURE 2.0 (Pritchard

229   *et al.* 2000) was used to cluster individuals into subpopulations and to reveal patterns of

230   gene flow across the sampled area. STRUCTURE uses an iterative approach to cluster

231   microsatellite genotypes into *K* populations regardless of the geographic locations of

232   individuals. The approach is based on the assumptions of Hardy-Weinberg and linkage

233   equilibrium within the resulting clusters, so that the likelihood of K is estimated from the

234   genotype data alone. The highest likelihood value indicates the most likely number of

235   populations in the sample. Individuals can be assigned to one or more populations, including

236   the possibility of admixture. The first step of this analysis involved estimating the numbers of

237   populations (*K*). Five independent runs each of *K* = 1-5 were performed with $10^4 - 10^5$

238   MCMC iterations after a burn-in of $10^4 - 10^5$, using no prior information and assuming

239   uncorrelated allele frequencies and allowing admixture. In the second step of the analysis,

240   individuals were assigned to each original geographic sample group (using *K* = 2; see

241   Results). To evaluate the STRUCTURE results in determining how indicative an individual's

242   genotype was of the population from which it was sampled, we performed an assignment test

243   (Paetkau *et al.* 1995) as implemented in ARLEQUIN.

244   A Mantel test was performed with the program AIS to assess the significance of the

245   association between genetic and geographic distances. Statistical significance was tested

246   using 1000 random permutations.

247

248   **Results**

249   ***mtDNA sequences***

250   A 516 base-pair (bp) fragment of the control region (CR) was sequenced for 44 *L.*

251   *longicaudis* individuals. Due to a portion of ambiguous alignment, 25 sites from the control

252   region were excluded from all further analyses, totaling 491 bp. Sequences of the *ATP8* (329

253   bp) and *ND5* (651 bp) genes were obtained for 37 Neotropical otters each (Table 2).

254   Outgroups were sequenced for these three fragments yielding the same sequence length,

except for *Aonyx cinereus* whose control region segment was 1 bp longer than the remaining individuals (so that the total alignment was 517 bp-long). The three segments were concatenated totaling 1472 bp.

Moderate to high levels of genetic diversity were observed in all three segments (Table 2).The CR was the most variable segment when *L. longicaudis* samples were analyzed separately, but showed clear indications of saturation when other species were also included in the comparisons (see Table 2). Separate analyses were conducted for three different mtDNA data sets: (i) control region alone; (ii) coding segments (*ATP8 + ND5*); and (iii) full concatenation (CR + *ATP8 + ND5*).

Control region data set

Sequences generated for this segment defined 23 different haplotypes (Table 3). A relatively high level of nucleotide diversity was observed among individuals (Table 2). The Hasegawa-Kishino-Yano (Hasegawa *et al.* 1985) substitution model with a proportion of invariant sites and a gamma distribution of rate heterogeneity across sites (HKY+I+G) provided the best fit to this data set (ln = -1363.4178). The MP, ML, NJ and Bayesian phylogenetic analyses did not conflict with each other in the inferred tree topology, but were unable to resolve the relationships among the haplotypes (Fig. 2). The only supported grouping obtained with this segment was a clade containing most haplotypes from French Guiana and one sequence from Peru (subsequently named Clade 2 – see below). The haplotype network also failed to show a clear pattern, with a considerable amount of reticulation indicating the occurrence of homoplasy and likely saturation at variable sites (Fig. 3).

In spite of the apparent lack of discernible phylogenetic structure, the F_{ST} between two broadly defined geographic groups (Brazil *vs.* Northwestern South America; see below) was quite high (F_{ST} = 0.45; p = 0.000). Of the neutrality tests performed, only Fu's F* was significantly negative (Fu's F*= -6.546, p=0.001) when the Brazilian samples were analyzed separately, while all other tests were non-significant for separate or combined population samples. The mismatch distribution showed a multi-modal pattern for most data sets, except for the analysis of Brazilian individuals by themselves, which resulted in a roughly unimodal peak (Fig. 4A).

Combined data sets

Sequences from the concatenations of the *ATP8* and *ND5* segments (*n* = 36; data set II) and *ATP8 + ND5 +* CR (*n* = 35; data set III) led to the observation of 19 and 26 unique

290 haplotypes, respectively (Tables 4 and 5). Moderate to high levels of nucleotide and gene
291 diversity were observed for these data sets in Neotropical otter individuals (Table 6).

292   The TIM + I and the TIM + I + G models were selected for AIC as the best model of
293 nucleotide substitution to data sets II and III, respectively (ln = -3260.5420; ln = -
294 4453.25662), and were applied in all subsequent model-based analyses. All trees produced
295 with different phylogenetic methods were congruent, with mostly subtle differences in nodal
296 support (Figs. 5 and 6). With these data sets a clear phylogeographic pattern could be
297 observed, with all samples from Brazil clustering in a single, well-supported and very shallow
298 clade. The shape of its internal phylogeny, with very short branches, little structure and no
299 evidence of geographic differentiation (in spite of a broad sampling across Brazil), is
300 suggestive of a recent population expansion in this area (Lavery *et al*. 1996; Avise 2000).

301   The sister-group to this Brazilian clade was a single haplotype (Ll-AN15 and Ll-
302 ANC23 in the data sets II and III, respectively) found in two different individuals from French
303 Guiana (bLlo41 and bLlo66). Together, this haplotype and the Brazilian sequences formed a
304 well-supported phylogenetic group (indicated as Clade 1 in Figs. 5 and 6). A second well-
305 supported monophyletic cluster (Clade 2) included only haplotypes found in French Guiana
306 and Peru, with some indication of possible sub-structuring in this area.

307   The haplotype found in Bolivia (Ll-AN16 and Ll-ANC24 in data sets II and III,
308 respectively) was not contained in either Clade 1 or 2, but rather was similarly divergent from
309 both of them (Figs. 5 and 6). Its exact placement was not identical with the two data sets:
310 with *ATP8 + ND5*, it was positioned in a trichotomy with Clades 1 and 2, whereas in the full
311 concatenation (data set III), it was a sister-group to Clade 2, albeit with weak bootstrap
312 support. This suggests that Bolivia may contain a third phylogenetic lineage separate from
313 Clades 1 and 2, whose exact relationships should be further investigated with additional
314 sampling. Furthermore, the individual from Colombia (bLlo23) contained a very distinct
315 haplotype (Ll-AN14 and ANC21 in data sets II and III, respectively), which was consistently
316 placed as the most basal lineage of all *L. longicaudis*.

317   The haplotype network produced with *ATP8 + ND5* (Fig. 7) depicted a clear
318 phylogeographic pattern, with at least 6 mutational steps separating the samples belonging
319 to clades 1 and 2 recovered in the phylogenetic analyses. A star-shaped pattern with several
320 localized lineages connected by short branches to a more common, widespread haplotype, is
321 suggestive of a relatively recent population expansion in the Brazilian group. In contrast, the
322 median-joining network produced with data set III (Fig. 8) was not efficient at resolving the
323 relationships among the individuals, probably due to saturated mutation sites in the control
324 region; nevertheless, a similar star-shaped pattern could be observed in some Brazilian

haplotypes. Again, individuals bLlo41 and bLlo66 were positioned near the Brazilian group instead of being placed in association with other samples from their geographic region (French Guiana).

The AMOVA results indicated that most of the genetic variability in *L. longicaudis* mtDNA could be explained by a single Northwest *vs.* Southeast partition (*i.e.* Brazil [SE] *vs.* all other sampled locations [NW]. The F_{ST} between these two geographic groups was high (0.65, p < 0.001; and 0.54, p < 0.001, for data sets II and III, respectively), as expected due to their almost perfect allopatry. Given this result and the available sample, we focused several geography-based analyses on these two broad units, even though the NW group seems to contain a diverse assembly of non-monophyletic historical lineages (Figs. 5 and 6).

For the complete *L. longicaudis* sample, as well as and for the NW geographic group, all neutrality tests were non-significant. In contrast, significantly negative results were obtained for the Brazilian group (Fu's F* = -8.6625, p < 0.001, data set II; Fu's F* = -6.793, p < 0.001; Tajima's D = -5.872, p = 0.02, data set III), congruent with the inference of a recent population expansion in this region. The mismatch distribution analyses taking into account the whole species and the NW geographic group showed a multimodal distribution (not shown), which is expected given the observed phylogenetic structure in this area. On the other hand, the Brazilian sample revealed a unimodal pattern (Fig. 4B,C), which is again consistent with a historical demographic expansion.

The Mantel test was performed only with data set III (CR + *ATP8* + *ND5*) and revealed a weak but significant relationship between genetic and geographic distances when the entire sample was compared (r = 0.29; p = 0.02). However, when the two geographic groups of samples were analyzed individually, the correlation was very low and non-significant (SE: r = 0.054, p = 0.1, n = 29; NW: r = 0.034, p = 0.41, n = 7).

The divergence between *Lontra longicaudis* and *L. canadensis* was dated to *ca.* 4.16 MYA (confidence interval [CI]: 2-82 – 5.85 MYA). Within *L. longicaudis*, the divergence between the Colombian haplotype and the other lineages was estimated to have occurred around 0.644 MYA (CI: 0.437 – 0.906 MYA), while the three-way split among the Bolivian haplotype and Clades 1 and 2 was estimated to have occurred at *ca.* 0.450 MYA (CI: 0.303 – 0.629 MYA). The diversification within clades occurred soon afterwards. Of particular interest is the age of the base of Clade 1 (separation between the haplotype from French Guiana and the Brazilian group), estimated at 0.422 MYA (CI: 0.286 – 0.594), and the coalescence of the Brazilian clade itself (dated at 0.196 MYA; CI: 0.133 – 0.276).

Microsatellite data set

All twelve microsatellite loci analyzed were polymorphic for *L. longicaudis* (n = 36); however, two loci (RIO17 and Lut818) presented low amplification efficiency and were thus excluded from analyses. All individuals genotyped for the remaining ten loci presented unique multilocus composite genotypes. Moderate levels of genetic variability were found, with each locus yielding between 6 and 11 alleles and an average expected heterozygosity of 0.766 (Table 7). All loci were in linkage equilibrium in both populations after Bonferroni adjustments (α = 0.05). No significant departure from Hardy-Weinberg expectations was observed in NW geographic group after the Bonferroni correction (α = 0.05), but three departures from HWE were found in the Brazilian [SE] group (RIO07, RIO18 and RIO20). All departures from HWE were found to be heterozygote deficits, which can imply the presence of null alleles in this group. Comparative analyses excluding the loci that were out of equilibrium in any geographic group (uncorrected assessment) indicated no significant change in the results relative to our complete data set (data not shown). We have, therefore, used the complete data set of 10 loci throughout the analyses presented here.

As with the mtDNA data, the statistical analyses of population differentiation were performed assuming broad geographic units (Brazil [SE] and NW). Both F_{ST} and R_{ST} values were significant, indicating low to moderate structuring in this data set (F_{ST} = 0.058, p = 0.004; R_{ST} = 0.085, p = 0.026). These results are in agreement with mitochondrial analyses, showing a partition between Brazil and Northwestern South America. However, the magnitude of the differentiation is almost 10-fold lower in the microsatellite markers relative to the mtDNA data sets.

The *G*-test did not support a significant difference between these two major groups in their allele frequency distributions (p > 0.05). However, in the assignment test 88.9% (n = 32) of individuals were correctly allocated in their geographic group of origin. This result indicates that there is sufficient genetic distinction between these groups to allow mostly accurate assignments, in agreement with the significant results observed with the AMOVA.

Using the Bayesian clustering procedure, we initially evaluated the most likely subdivision scenario without using the information of the known geographic origin of each individual, and the probability of the observed data was maximum with two populations (k = 2; ln = -1182.93). We observed that 65% of individuals known to be from the same broad geographic area were assigned to the same genetic cluster. To better investigate the genetic composition of our data set, we performed a second set of analyses with STRUCTURE using population information. In this case, we considered the two geographic groups as separate populations, and the results were more compelling than in the first set of analysis, with

393 individuals that were *a priori* grouped in the SE and NW groups being correctly assigned with
394 97% and 89% accuracy, respectively (Fig. 9). An interesting case was that of individual
395 bLlo21, from French Guiana, which was estimated to have a 57% probability of belonging to
396 the Brazilian genetic cluster. Since this individual had 30% of its microsatellite data missing,
397 we repeated this analysis excluding the loci for which no genotypes had been obtained from
398 this animal. The proportion of its genomic ancestry in the Brazilian group decreased to 49%,
399 but remained too high to be easily dismissed, implying some level of recent gene flow
400 between the two groups.

401 The Mantel test revealed a weak but significant correlation between genetic and
402 geographic distances when the whole sample was analyzed ($r = 0.33$; $p = 0.001$). When
403 each group was analyzed separately, the Mantel test indicated a significant correlation for
404 the Brazilian group ($r = 0.47$, $p = 0.003$, $n = 29$), but not for the NW geographic group ($r = $
405 0.49, $p = 0.13$, $n = 7$).

406

407 **Discussion**

408 ***Genetic Diversity***

409 Observed levels of genetic variability in Neotropical otters (Tables 2, 6 and 7) were
410 considerably high when compared to other vertebrates. For example, analyses of mtDNA
411 control region data sets (overlapping with the segment employed here) from sympatric
412 mammals yielded nucleotide diversity (π) values of 0.0017 in marsh deer (*Blastocerus*
413 *dichotomus*) populations of the Río de la Plata basin (Márquez *et al*. 2006), and 0.00771 in
414 jaguars (*Panthera onca)* throughout their range (Eizirik *et al.* 2001), while in this study we
415 obtained values of 0.011 (Tables 2 and 6).

416 Tchaicka *et al.* (2007) used the same control region segment in phylogeographic
417 analyses of the crab-eating fox (*Cerdocyon thous*)*,* a genetically diverse Neotropical canid
418 sympatric with *L. longicaudis* throughout most of its range. The comparison between these
419 species indicates that haplotype diversity is higher in *L. longicaudis* ($h = 0.8911 \pm 0.0060$)
420 than in *Cerdocyon* ($h = 0.83 \pm 0.032$), while nucleotide diversity is lower ($\pi = 0.0113 \pm 0.0027$
421 *vs*. 0.019 ± 0.002).

422 Comparisons to other studies performed with mustelids suggest moderate to high
423 levels of mtDNA diversity in *Lontra longicaudis*. Both nucleotide and haplotype diversity found
424 in the Neotropical otter mtDNA CR ($\pi = 0.0113 \pm 0.0027$; $h = 0.8911 \pm 0.0060$) are similar to
425 or higher than estimates reported for *Mustela lutreola* ($\pi = 0.012 \pm 0.058$ to 0.0012 ± 0.088; *h*
426 $= 0.469$ to 0.939, Northeast and Southeast Europe populations, respectively; Michaux *et al*.
427 2005), *Enhydra lutris* ($\pi = 0.098 \pm 0.029$; $h = 0.412$; Larson *et al*. 2002) and *Lutra lutra* ($\pi = $

428 0.0006; h = 0.16 ± 0.06; Ferrando *et al*. 2004). Studies on European populations of *Lutra*

429 *lutra* based on 300bp of the 5' hypervariable sement of the mtDNA control region have so far

430 described only six haplotypes, all of which differ from each other by only one nucleotide,

431 indicating a low genetic variability for that species (Effenberger & Suchentrunk 1999; Mucci *et*

432 *al*. 1999; Cassens *et al*. 2000; Pérez-Haro *et al*. 2005). The absence of other genetic studies

433 on otter or any mustelid using the genes *ATP8* and *ND5* precludes direct comparisons of

434 these data to other species.

435   In spite of its high diversity relative to other mtDNA segments, our analyses indicate

436   that the control region may not be the best mitochondrial marker for phylogeographic studies

437   in *L. longicaudis* and perhaps other related species. Variable sites in this segment seem to

438   be saturated even at the intra-specific level, leading to a lower signal-to-noise ratio than

439   verified for the other two fragments. Conversely, the *ND5* segment used here seems to be a

440   very informative segment, less prone to saturation at recent levels, which has also been

441   observed for other carnivores studied by our group (unpublished data).

442   With respect to microsatellite diversity, Beheler *et al*. (2004) described 10 loci for *Lontra*

443 *canadensis*, two of which were used in this study. These markers exhibited more alleles in *L.*

444 *longicaudis* than reported for their original target species, whereas the observed

445 heterozygosity was higher in the latter. Beheler *et al*. (2005) described an additional 10

446 microsatellites for *L. canadensis*, four of which were analyzed here for *L. longicaudis*. Again,

447 more alleles were found for every locus in *L. longicaudis*, while the observed heterozygosity

448 was relatively lower in this species. The remaining microsatellite markers used here were

449 developed for *Lutra lutra* (Dallas & Piertney 1998); in these markers a similar number of

450 alleles and higher heterozygosity were observed for *L. longicaudis* relative to the original

451 target species. Other studies employing these markers include the following: Hung *et al*.

452 (2004) used three of these loci (Lut701, Lut733 e Lut782) on *Lutra lutra* scat samples in

453 Kinmen, and found fewer alleles than identified here; Dallas *et al.* (2002) also employed the

454 same three markers in *L. lutra* and found fewer alleles than reported here for two of the loci

455 (Lut701 e Lut733); finally, Randi *et al.* (2003) used four of these loci in European populations

456 of *L. lutra* and found a higher allelic diversity but observed heterozygosity generally lower than

457 we estimated for *L. longicaudis*. A practical implication of these results is that these markers

458 are quite variable in *L. longicaudis*, are thus informative for further investigations of population

459 genetics and molecular ecology in this species, and should provide useful comparisons to

460 other related species.

Phylogeography and Demographic History

461
462   The phylogenetic and network analyses of *Lontra longicaudis* mtDNA sequences
463 showed this species to be composed of at least four geographically structured phylogroups:
464 (i) Colombia; (ii) Bolivia; (iii) French Guiana; and (iii) Brazil. Colombia and Bolivia were
465 represented by a single sample each, whose genetic divergence from other individuals
466 indicates deep phylogeographic partitions involving these areas, which need to be further
467 ascertained by means of enhanced sampling.

468   The allopatry of the two better-sampled phylogroups (Brazil and French Guiana) is
469 almost complete, with only two individuals (bLlo41 and bLlo 66, which share the same
470 haplotype) found in a geographic region inconsistent with their phylogenetic placement. The
471 position of these French Guiana samples in Clade 1 is intriguing, as they are solidly placed at
472 the base of this clade. This suggests that this incongruence between geography and
473 phylogeny may be due to an ancestral colonization process, instead of secondary mtDNA
474 gene flow. In that case, it would imply that the Brazilian clade derives from a recent episode
475 of colonization from the north, so that northern populations are paraphyletic with respect to
476 more southerly ones. Another observation that is consistent with this inference is the position
477 of the single haplotype (sampled in two different individuals) from the Brazilian northeast,
478 which is the most basal of all lineages from Brazil (e.g. Ll-AN4B in Fig. 5 and Ll-ANC18 in
479 Fig. 6). This hypothesis needs to be further investigated on the basis of additional sampling
480 in northern Brazil and adjacent areas.

481   The mtDNA inference of a significant genetic partition between Brazil and the other
482 sampled areas in South America is supported by the microsatellite data set. However, the
483 magnitude of this difference is much lower with these fast-evolving nuclear markers than with
484 the matrilineal mitochondrial sequences. This may be due to slower effects of historical
485 genetic drift acting on two isolated groups, given the larger effective population size of
486 nuclear markers, or (non-exclusively) to the occurrence of ongoing male-biased gene flow
487 between these areas. Teasing apart these processes should prove to be an interesting
488 avenue for research in this species, for which data on individual dispersal and social
489 behavior is currently unavailable.

490   The pattern observed with the mtDNA phylogenies and haplotype networks is
491 consistent with a recent population expansion in Brazil, with most haplotypes from this region
492 differing from each other by only one or two mutational steps, often connected in a star-
493 shaped fashion (e.g. Figs. 5 - 8). This inference is also supported by the mtDNA mismatch
494 distribution analysis (Fig. 4) and the neutrality test results. The mismatch distribution results
495 of the *ATP8 + ND5* data set (seemingly "cleaner" in terms of phylogenetic signal, with less

496 homoplasy than inferred for the control region) fits particularly well the expected pattern
497 under a sudden demographic expansion (Rogers & Harpending 1992), with a single, smooth
498 prominent peak. The age of this expansion was estimated at *ca.* 200,000 years ago, possibly
499 following colonization of this area from northern South America.
500

501 ### *Implications for conservation*
502   The results presented here have implications for the conservation and management
503 of this species in the wild and in captivity. In spite of being represented by a single sample
504 each, the divergence observed in Colombia and Bolivia suggests that those areas may be
505 sufficiently differentiated to warrant recognition as separate Evolutionarily Significant Units
506 (ESU – Ryder 1986). As such, they should be conserved and managed as distinct entities.
507 Further studies are required to improve our knowledge about these populations and their
508 evolutionary relationships to others.
509   French Guiana otters are not monophyletic in their mtDNA lineages trees, but should
510 be viewed as separate conservation unit. This area seems to contain a very high level of
511 genetic diversity, and may hold important clues to better understand the evolutionary history
512 of this species. It should therefore be viewed as an important focus for additional research
513 and conservation efforts. Other demographic units probably exist in the Neotropical otter,
514 which should be investigated in more detailed population genetic studies involving an
515 expanded geographic sampling of the species range.
516

517 **References**
518 Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific Phylogeography: the mithocondrial
519   DNA bridge between populations genetics and systematics. *Annual Review of Ecology*
520   *and Systematics*, **18**, 489:522.
521 Avise JC (1994) *Molecular markers, natural history e evolution*. Chapman & Hall, New York.
522 Avise JC (2000) *Phylogeography – The History and Formation of Species*. Harvard
523   University Press, Cambridge, Massachusetts.
524 Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific
525   phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

526   Beheler AS, Fike JA, Murfitt LM, Rhodes Jr. OE, Serfass TS (2004) Development of
527   polimorphic microsatellite loci for North American river otters (*Lontra canadensis*) and
528   amplification in related Mustelids. *Molecular Ecology Notes*, **4**, 56-58.

529   Beheler AS, Fike JA, Murfitt LM *et al.* (2005) Ten new polymorphic microsatellite loci for
530   North American river otters (*Lontra canadensis*) and their utility in related mustelids.
531   *Molecular Ecology Notes*, **5**, 602-604.

532   Bermingham E, Martin AP (1998) Comparative mtDNA phylogeography of neotropical
533   freshwater fishes: testing shared history to infer the evolutionary landscape of lower
534   Central America. *Molecular Ecology*, **7**, 499-517.

535   Bodkin JL, Ballachey BE, Cronin MA, Scribner KT (1999) Population demographics and
536   genetic diversity and remnant and translocated populations of sea otters. *Conservation
537   Biology*, **13**, 1378-1385.

538   Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers
539   improve the readability and usability of microsatellite analyses performed with two
540   different allele-sizing methods. *Biothecniques*, **31**, 25-28.

541   Blundell GM, Ben-David M, Groves P, Bowyer RT, Geffen E (2002) Characteristics of sex-
542   biased dispersal and gene flow in coastal river otters: implications for natural
543   recolonization of extirpated populations. *Molecular Ecology*, **13**, 289-303.

544   Cantanhede AM, da Silva VMF, Farias IP *et al.* (2005) Phylogeography and population
545   genetics of the endangered Amazonian manatee, *Trichechus inunguis* Natterer, 1883
546   (Mammalia, Sirenia). *Molecular Ecology*, **14**, 401-413.

547   Cassens I, Tiedemann R, Suchentrunk F, Hartl GB (2000) Mitochondrial DNA variation in the
548   European otter (*Lutra lutra*) and the use of spatial autocorrelation analysis in
549   conservation. *The Journal of Heredity*, **91**, 31-41.

550   Chebez JC (1999) *Los que se van – Espécies argentinas em peligro*. Buenos Aires, Editorial
551   Albatroz Saci.

552 Chehébar CE (1990) Action Plan from Latin American Otters. In: *Otters: A Plan for their*
553   *Conservation* (eds. Foster-Turley P, Macdonald S, Mason C), pp. 64-73. IUCN Otter
554   Specialist Group.

555 Costa LP (2003) The historical bridge between the Amazon and the Atlantic Forest of Brazil:
556   a study of molecular phylogeography with small mammals. *Journal of Biogeography*,
557   **30**, 71-86.

558 Dallas JF, Piertney SB (1998) Microsatellite primers for the Eurasian otter. *Molecular*
559   *Ecology*, **7**, 1248-1251.

560 Dallas JF, Marshall F, Piertney SB, Bacon PJ, Racey P (2002) Spatially restricted gene flow
561   and reduced microsatellite polymorphism in the Eurasian otter *Lutra lutra* in Britain.
562   *Conservation Genetics*, **3**, 15-29.

563 de Thoisy B, Hrbek T, Farias IP, Vasconcelos WR, Larvegne A (2006) Genetic structure,
564   population dynamics, and conservation of Black caiman (*Melanosuchus niger*).
565   *Biological Conservation*, **133**, 474-482.

566 Effenberger S, Suchentrunk F (1999) RFLP analyses of the mitochondrial DNA of otters
567   (*Lutra lutra*) from Europe – implications for conservation of a flagship species.
568   *Biological Conservation*, **90**, 229-234.

569 Eizirik E, Bonatto SL, Johnson WE *et al.* (1998) Phylogeographic patterns and mitochondrial
570   DNA control region evolution in two Neotropical cats (Mammalia, Felidae). *Journal of*
571   *Molecular Evolution*, **47**, 613-624.

572 Eizirik E, Kim J, Menotti-Raymond M *et al.* (2001) Phylogeography, population history e
573   conservation of jaguars (*Panthera onca*, Mammalia, Felidae). *Molecular Ecology*, **10**,
574   65-79.

575 Excoffier L, Smouse P, Quattro J (1992) Analysis of Molecular Variance inferred from metric
576   distances among DNA haplotypes: application to human mitochondrial DNA restriction
577   data. *Genetics*, **131**, 479-491.

578 Ferrando A, Ponsà M, Marmi J, Domingo-Roura X (2004) Eurasian otters, *Lutra lutra*, have a

579   dominant mtDNA haplotype from the Iberian Peninsula to Scandinavia. *The Journal of*

580   *Heredity*, **95**, 430-435.

581 Foster-Turley P, Macdonald S, Mason CF (1990) *Otters: An action plane for their*

582   *conservation*. IUCN/SSC Otter Specialist Group.

583 Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*.

584   Cambridge University Press, Cambridge.

585 Fu YX (1997) Statistical test of neutrality of mutations against population growth, hitchhiking

586   and background selection. *Genetics*, **147**, 915-925.

587 Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693-709.

588 Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL (2006) Phylogeography of the

589   *Bothrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island

590   colonization in the Brazilian Atlantic Forest. *Molecular Ecology*, **15**, 3969-3982.

591 Harpending HC, Batzer MA, Gurven M *et al.* (1998) Genetic traces of ancient demography.

592   *Proceedings of Natural Academy of Sciences*, **95**, 1961-1967.

593 Hasegawa M, Kishino H, Yano T (1985) Data of the human-ape splitting by a molecular clock

594   of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

595 Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny.

596   *Bioinformatics*, **17**, 754-755.

597 Hung C, Li S, Lee L (2004) Faecal DNA typing to determine the abundance and spatial

598   organisation of otters (*Lutra lutra*) along two stream systems in Kinmen. *Animal*

599   *Conservation*, **7**, 301-311.

600 IUCN (2006) *IUCN Red List of Threatened Species.* <www.iucnredlist.org> Downloaded in

601   27 February 2007.

602 Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary

603   genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 2.

604   Larson SE, Jameson RJ, Bodkin JL, Staedler M, Bentzen P (2002) Microsatellite DNA and
605   mtDNA variation within and among remnant and translocated sea otter, *Enhydra lutris*,
606   populations. *Journal of Mammalogy*, **83**, 893-906.

607   Lavery S, Moritz C, Fielder DR (1996) Genetic patterns suggest exponential population
608   growth in a declining species. *Molecular Biology and Evolution*, **13**, 1106-1113.

609   Lessa EP, Cook AC, Patton JL (2003) Genetic footprints of expansion in North America, but
610   not Amazonia, during the late Quaternary. *Proceedings of National Academy of*
611   *Sciences USA*, **100**, 10331–10334.

612   Lovejoy NR, de Araújo MLG (2000) Molecular systematics, biogeography and population
613   structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*.
614   *Molecular Ecology*, **9**, 259-268.

615   Macdonald SM, Mason CF (1990) Threats. In*: Otters: An Action Plan for their Conservation*
616   (eds. Foster-Turley P, Macdonald S, Mason C), pp. 11-14. IUCN Otter Specialist
617   Group.

618   Mantel N (1967) The detection of disease clustering and a generalized regression approach.
619   *Cancer Research*, **27**, 209-220.

620   Marquéz A, Maldonado JE, Gonzáles S *et al.* (2006) Phylogeography and Pleistocene
621   demographic history of the endangered marsh deer (*Blastocerus dichotomus*) from the
622   Río de la Plata Basin. *Molecular Ecology*, **7**, 563-575.

623   Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-
624   based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

625   Michaux JR, Hardy OJ, Justy F *et al.* (2005) Conservation genetics and population history of
626   the threatened European mink *Mustela lutreola*, with an emphasis on the West
627   European populations. *Molecular Ecology*, **14**, 2373-2388.

628   Miller MP (2005) ALLELES IN SPACE: Computer software for the joint analysis of
629   interindividual spatial and genetic information. *Journal of Heredity*, **96**, 711-724.

630 Mucci N, Pertoldi C, Madsen AB, Loeschcke V, Randi E (1999) Extremely low mitochondrial
631   DNA control-region sequence variation in the otter *Lutra lutra* population of Denmark.
632   *Hereditas*, **130**, 331-336.

633 Pérez-Haro M, Viñas J, Mañas F *et al.* (2005) Genetic variability in the complete
634   mitochondrial control region of the Eurasian Otter (*Lutra lutra*) in the Iberian Peninsula.
635   *Biological Journal of the Linnean Society*, **86**, 397-403.

636 Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution.
637   *Bioinformatics Applications Notes*, **14**, 817-818.

638 Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population
639   structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

640 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
641   multilocus genotype data. *Genetics*, **155**, 945–959.

642 Randi E, Davoli F, Pierpaoli M *et al.* (2003) Genetic structure in otter (*Lutra lutra*) populations
643   in Europe: implications for conservation. *Animal Conservation*, **6**, 93-100.

644 Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

645 Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of
646   pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552-569.

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

649 Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends*
650   *in Ecology and Evolution*, **1**, 9-10.

651 Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning, 2nd edn. Cold Spring Harbor
652   Laboratory Press, New York.

653 Saiki RK, Scharf S, Faloona F *et al.* (1985) Enzymatic amplification of beta-globin genomic
654   sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*,
655   **230**, 1350-1354.

656   Schneider S, Excoffier L (1999) Estimation of past demography parameters from the

657   distribution of pairwise differences when the mutation rates vary among sites:

658   Application to human mitochondrial DNA. *Genetics*, **152**, 1079-1089.

659   Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: A Software for Population Genetic*

660   *Data Analysis*, Version 2.0. Genetics and Biometry Laboratory, Department of

661   Anthorpology, University of Geneva, Geneva, Switzerland.

662   Sivasundar A, Bermingham E, Ortí G (2001) Population structure and biogeography of

663   migratory freshwater fishes (*Prochilodus*: Characiformes) in major South America

664   rivers. *Molecular Ecology*, **10**, 407-417.

665   Slatkin M (1995) A measure of population subdivision based on microsatellite allele

666   frequencies. *Genetics*, **139**, 457–462.

667   Sokal RR, Rohlf FJ (1981) Biometry, the principals and practice of statistics in biological

668   research, 3rd edn. W.H. Freeman & Co., New York.

669   Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Others Methods),*

670   Version 4. Sinauer Associates, Suderland, Massachusetts.

671   Tajima F (1989) Statistical methods to test for nucleotide mutation hypothesis by DNA

672   polymorphism. *Genetics*, **123**, 585-595.

673   Tchaicka L, Eizirik E, De Oliveira TG, Cândido Jr JF, Freitas TRO (2007)

674   Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*)

675   *Molecular Ecology*, **16**, 819–838.

676   Vianna JA, Bonde RK, Caballero S *et al.* (2006) Phylogeography, phylogeny and

677   hibridization in trichechid sirenians: implications for manatee conservation. *Molecular*

678   *Ecology*, **15**, 433-447.

679   Wright S (1965) The interpretation of population structure by *F*-statistics with special regard

680   to systems of mating. *Evolution,* **19**, 395-420.

681

682

Figure Legends

Fig 1. Map depicting currently assumed geographic distribution (*shaded area*) of the Neotropical otter (modified from http://www.otterspecialistgroup.org/Species/Lontra_longicaudis.html), with approximate sample collection sites. Black circles represent individuals from Brazil and black squares indicate individuals from Northwestern South America (French Guiana, Colombia, Bolivia, Peru). Numbers next to the collection sites are sample identification labels (number after "bLlo" in Table 1) of *L. longicaudis* individuals in each area. Boxes indicate individuals from the same region.

Fig. 2. Maximum likelihood tree of *L. longicaudis* mtDNA CR haplotypes identified in this study, based on 492 bp. Labels are haplotype identification numbers (see Table 3). Values above branches indicate support for the adjacent node based on ML / MP / NJ / BI. Given the evidence for saturation in the control region data set (see text), only *L. canadensis* was used as outgroup in these analyses (see Figs. 5 and 6).

Fig. 3. Median-joining network of haplotypes of *L. longicaudis* mtDNA control region (using 438 bp, excluding all sites containing indels or missing information). Circle size indicates the frequency of each haplotype, which is identified as listed in Table 3. Bars on branches indicate nucleotide substitutions inferred to have occurred between the connected haplotypes. Vertically hatched circles are haplotypes sampled in Brazil; horizontally hatched symbols are haplotypes sampled in French Guiana; the light grey haplotype (Ll-C18) was sampled in Colombia; the dark grey haplotype (Ll-C21) was sampled in Bolivia and the white one (Ll-C22) was sampled in Peru.

Fig. 4. Mismatch distribution analysis for the Brazilian samples. (A) mtDNA control region; (B) *ATP8 + ND5* data set; (C) CR + *ATP8 + ND5* data set. The dotted line indicates the

718 observed frequency of pairwise differences among haplotypes, while the continuous lines
719 depicts the expected frequency under the sudden population expansion model.
720
721 Fig. 5. Maximum likelihood tree of *L. longicaudis* mtDNA *ATP8 + ND5* haplotypes identified in
722 this study. Labels are haplotype identification numbers (see Table 4). Values above
723 branches indicate support for the adjacent node based on ML / MP / NJ / BI.
724
725 Fig. 6. Maximum likelihood tree of *L. longicaudis* mtDNA CR + *ATP8 + ND5* lineages
726 identified in this study, based on 1472 bp. Labels are haplotype identification numbers (see
727 Table 5). Values above branches indicate support for the adjacent node based on ML / MP /
728 NJ / BI.
729
730 Fig. 7. Median-joining network of *L. longicaudis* mtDNA *ATP8 + ND5* haplotypes (using 681
731 bp; all sites containing indels or missing information were excluded). The crossed marks are
732 nucleotide substitutions inferred in that branch. Bars on branches indicate nucleotide
733 substitutions inferred to have occurred between the connected haplotypes. Vertically hatched
734 circles are haplotypes sampled in Brazil; horizontally hatched symbols are haplotypes
735 sampled in French Guiana and Peru; the light grey haplotype (Ll-AN14) was sampled in
736 Colombia; the dark grey one (Ll-AN16) was sampled in Bolivia.
737
738 Fig 8. Median-joining network of *L. longicaudis* mtDNA CR + *ATP8 + ND5* haplotypes (using
739 1164 bp; all sites containing indels or missing information were excluded). The crossed
740 marks are nucleotide substitutions inferred in that branch. Bars on branches indicate
741 nucleotide substitutions inferred to have occurred between the connected haplotypes.
742 Vertically hatched circles are haplotypes sampled in Brazil; horizontally hatched symbols are
743 haplotypes sampled in French Guiana; the light grey haplotype (Ll-ANC21) was sampled in
744 Colombia; the dark grey haplotype (Ll-ANC24) was sampled in Bolivia and the white one (Ll-
745 ANC25) was sampled in Peru.
746
747 Fig. 9. Best population clustering result (k = 2 clusters) in a Bayesian analysis of
748 microsatellite data. Each otter individual is represented by a vertical bar partitioned into dark
749 grey and light grey segments, the lengths of which indicate the probability of membership in
750 each population cluster (parenthesis numbers: (1) Brazil; (2) Northwestern South America).
751 Numbers below to the horizontal axe are sample identification labels (number after "bLlo" in
752 Table 1) of *L. longicaudis* individuals in each area.

Table 1. Samples of the Neotropical otter analyzed in the present study.

ID	Sample	Geographic origin	Source Institution / Contact
bLlo01	muscle	Rio Grande do Sul State, Brazil	G. Bencke and J. M. Reppold
bLlo02	muscle	Santa Catarina State, Brazil	Sapucaia do Sul Zoo
bLlo03	muscle	Rio Grande do Sul State, Brazil	Carnívoros do RS Project
bLlo04[1]	footpad	Rio Grande do Sul State, Brazil	Carnívoros do RS Project
bLlo05	muscle	Gravataí, Rio Grande do Sul State, Brazil	G. L. Gonçalves
bLlo06	muscle	Rio Grande do Sul State, Brazil	P. H. Ott
bLlo07, bLlo08	muscle	Torres, Rio Grande do Sul State, Brazil	P. Colombo
bLlo09	muscle	Jataí, Goiás State, Brazil	CENAP / IBAMA
bLlo10	kidney	Moji-Mirim, São Paulo State, Brazil	CENAP / IBAMA
bLlo11	muscle	Rio Grande do Sul State, Brazil	A. P. Brandt
bLlo12	muscle	Dois Irmãos, Rio Grande do Sul State, Brazil	Prefeitura de Dois Irmãos / I. Fick
bLlo14	muscle	Angra dos Reis, Rio de Janeiro State, Brazil	H. F. Waldemarin
bLlo15	muscle	Guaratiba, Rio de Janeiro State, Brazil	H. F. Waldemarin
bLlo16	muscle	Barra, Rio de Janeiro State, Brazil	H. F. Waldemarin
bLlo17, bLlo18[2]	muscle	Pantanal, Mato Grosso do Sul State, Brazil	H. F. Waldemarin
bLlo19, bLlo20, bLlo21, bLlo22[2], bLlo25, bLlo41	muscle	French Guiana	B. de Thoisy
bLlo23	skin	Maceo, Antioquia, Colombia	D. Arcila
bLlo24	muscle	Rio Grande do Sul State, Brazil	T. R. O. de Freitas
bLlo26[2]	faeces	Eldorado do Sul, Rio Grande do Sul State, Brazil	Sapucaia do Sul Zoo / R. von Hohendorff
bLlo28[2]	hair	Pantanal, Mato Grosso State, Brazil	I. C. Pfeifer
bLlo29	blood	Foz do Iguaçú, Paraná State, Brazil	São Paulo Zoo / K. Kassaro
bLlo30	blood	Belo Horizonte, Minas Gerais State, Brazil	São Paulo Zoo / K. Kassaro
bLlo31	blood	Corumbá, Mato Grosso do Sul State, Brazil	São Paulo Zoo / K. Kassaro
bLlo34	muscle	Blumenau, Santa Catarina State, Brazil	FURB / S. Althoff
bLlo36[2]	faeces	Sumaré, São Paulo State, Brazil	Campinas Zoo / E. Ferraz
bLlo37[2]	faeces	Nova Santa Rita, Rio Grande do Sul State, Brazil	Canoas Mini Zoo / M. Martins
bLlo38	muscle	P.N. Ilha Grande, Paraná State, Brazil	L. Koprowski
bLlo39	skin	Ribeirão Preto, São Paulo State, Brazil	Ribeirão Preto Zoo / M. dos Santos
bLlo42	muscle	Capela de Santana, Rio Grande do Sul State, Brazil	Canoas Mini Zoo / M. Martins
bLlo51[2]	faeces	P.E. da Ilha do Cardoso, São Paulo State, Brazil	E. Nakano
bLlo57	blood	Paranapanema River, Paraná State, Brazil	L. Koprowski
bLlo58	muscle	Osório, Rio Grande do Sul State, Brazil	P. Colombo, C. Zank and G. Volkmer
bLlo60, bLlo61	blood	Recife, Pernambuco State, Brazil	Dois Irmãos Park / A. L. Brito
bLlo64[2]	faeces	Vermelho River, Corumbá, Mato Grosso do Sul State, Brazil	Embrapa-Pantanal / G. Mourão
bLlo66[3]	DNA	French Guiana	K.P. Koepfli
bLlo67[3]	DNA	Bolivia	K.P. Koepfli
bLlo68[3]	DNA	Peru	K.P. Koepfli

[1] samples typed only for microsatellite loci

[2] samples typed only for the mtDNA control region.

[3] samples typed only for mtDNA segments.

XL

Table 2. mtDNA diversity estimates for the Neotropical river otter and related species.

Segment [a]	Length (bp) [b]	N	No. of haplotypes	S [c]	PI [d]	δ [e,g]	d_{xy} (*Lontra canadensis*) [f,g]	d_{xy} (*Amblonyx cinereus*) [f,g]	d_{xy} (*Pteronura brasiliensis*) [f,g]
CR	492 (438)	44	23	27 / 40	15 /19	0.01133 ± 0.00266	0.05173 ± 0.00953	0.06761 ± 0.01135	0.06834 ± 0.01103
ATP8	329 (295)	37	11	16 / 66	1 / 7	0.00394 ± 0.00128	0.18369 ± 0.02257	0.20953 ± 0.02329	0.20412 ± 0.02343
ND5	651 (547)	37	17	36 / 99	11 / 21	0.00732 ± 0.00198	0.10591 ± 0.01478	0.17378 ± 0.01599	0.15148 ± 0.01696
ATP8 + ND5	980 (681)	36	19	49 / 162	14 / 30	0.00587 ± 0.00130	0.14187 ± 0.01302	0.18971 ± 0.015011	0.17344 ± 0.01367
CR + *ATP8 + ND5*	1472 (1164)	35	26	76 / 206	31 / 50	0.00809 ± 0.00127	0.10857 ± 0.00851	0.14691 ± 0.00940	0.13940 ± 0.00904

[a] CR= mtDNA control region; *ATP8* : *ATPase subunit 8* ; *ND5* : *NADH dehydrogenase subunit 5* .

[b] values in parentheses are segment lengths after exclusion of all sites containing gaps or missing information.

[c] S = Segregating (polymorphic) sites; values are given for the *L. longicaudis* data set / data set including *L. longicaudis + L. canadensis* .

[d] PI = Parsimony-informative sites; values are given for the *L. longicaudis* data set / data set including *L. longicaudis + L. canadensis* .

[e] δ = nucleotide diversity per site.

[f] dxy= mean nucleotide divergence (p-distance) between *L. longicaudis* haplotypes and selected outgroups.

[g] all sites containing indels or missing information were excluded from this analysis.

Table 3. List of individuals that bear each mtDNA control region haplotype. Also indicated are the absolute frequency in the total sample (Fr) and the geographic distribution of each haplotype.

Haplotype[a]	Individuals	Fr	Country of haplotype occurrence
Ll-C1	bLlo01, bLlo03, bLlo11, bLlo24	4	Brazil
Ll-C2A	bLlo02, bLlo06, bLlo08, bLlo09, bLlo12, bLlo14, bLlo15, bLlo18, bLlo26, bLlo34, bLlo38, bLlo58, bLlo64	13	Brazil
Ll-C2B	bLlo30	1	Brazil
Ll-C3	bLlo05, bLlo39	2	Brazil
Ll-C4	bLlo07	1	Brazil
Ll-C5	bLlo10	1	Brazil
Ll-C6	bLlo16	1	Brazil
Ll-C7	bLlo17	1	Brazil
Ll-C8	bLlo28	1	Brazil
Ll-C9	bLlo29, bLlo37	2	Brazil
Ll-C10	bLlo31	1	Brazil
Ll-C11	bLlo36	1	Brazil
Ll-C12	bLlo42	1	Brazil
Ll-C13	bLlo51	1	Brazil
Ll-C14	bLlo57	1	Brazil
Ll-C15	bLlo60, bLlo61	2	Brazil
Ll-C16	bLlo19, bLlo20	2	French Guiana
Ll-C17	bLlo21, bLlo22	2	French Guiana
Ll-C18	bLlo23	1	Colombia
Ll-C19	bLlo25	1	French Guiana
Ll-C20	bLlo41, bLlo66	2	French Guiana
Ll-C21	bLlo67	1	Bolivia
Ll-C22	bLlo68	1	Peru

[a] Haplotypes with the same number and different letters (e.g. Ll-C2A, 2B) are collapsed into a single haplotype when all sites with missing information or indels are excluded (e.g. Fig.3).

Table 4. List of individuals that bear each mitochondrial DNA *ATP8 + ND5* haplotype. Also indicated are the absolute frequency on the total sample (Fr) and the geographic distribution of each haplotype.

Haplotype [a]	Individuals	Fr	Country(ies) of haplotype occurrence
Ll-AN1	bLlo01	1	Brazil
Ll-AN2	bLlo02, bLlo14, bLlo15	3	Brazil
Ll-AN3	bLlo03, bLlo24, bLlo31	3	Brazil
Ll-AN4A	bLlo05, bLlo06, bLlo08, bLlo12, bLlo16, bLlo29, bLlo30, bLlo34, bLlo57, bLlo58	10	Brazil
Ll-AN4B	bLlo60, bLlo61	2	Brazil
Ll-AN5	bLlo07	1	Brazil
Ll-AN6	bLlo09, bLlo39	2	Brazil
Ll-AN7	bLlo10	1	Brazil
Ll-AN8	bLlo11	1	Brazil
Ll-AN9	bLlo17	1	Brazil
Ll-AN10	bLlo42	1	Brazil
Ll-AN11A	bLlo19	1	French Guiana
Ll-AN11B	bLlo20	1	French Guiana
Ll-AN12A	bLlo21, bLlo68	2	French Guiana, Peru
Ll-AN12B	bLlo25	1	French Guiana
Ll-AN13	bLlo22	1	French Guiana
Ll-AN14	bLlo23	1	Colombia
Ll-AN15	bLlo41, bLlo66	2	French Guiana
Ll-AN16	bLlo67	1	Bolivia

[a] Haplotypes with the same number and different letters (e.g. Ll-AN4A, 4B) are collapsed into a single haplotype when all sites with missing information or indels are excluded (e.g. Fig.5).

Table 5. List of individuals that bear each mitochondrial DNA CR + *ATP8* + *ND5* haplotype. Also indicated are the absolute frequency on the total sample (Fr) and the geographic distribution of each haplotype.

Haplotype [a]	Individuals	Fr	Country of haplotype occurrence
Ll-ANC1	bLlo01	1	Brazil
Ll-ANC2	bLlo02, bLlo14, bLlo15	3	Brazil
Ll-ANC3	bLlo03, bLlo24	2	Brazil
Ll-ANC4	bLlo05	1	Brazil
Ll-ANC5	bLlo06, bLlo08, bLlo12, bLlo34, bLlo58	5	Brazil
Ll-ANC6	bLlo07	1	Brazil
Ll-ANC7	bLlo09	1	Brazil
Ll-ANC8	bLlo10	1	Brazil
Ll-ANC9	bLlo11	1	Brazil
Ll-ANC10	bLlo16	1	Brazil
Ll-ANC11	bLlo17	1	Brazil
Ll-ANC12	bLlo29	1	Brazil
Ll-ANC13	bLlo30	1	Brazil
Ll-ANC14	bLlo31	1	Brazil
Ll-ANC15	bLlo39	1	Brazil
Ll-ANC16	bLlo42	1	Brazil
Ll-ANC17	bLlo57	1	Brazil
Ll-ANC18	bLlo60, bLlo61	2	Brazil
Ll-ANC19A	bLlo19	1	French Guiana
Ll-ANC19B	bLlo20	1	French Guiana
Ll-ANC20	bLlo21	1	French Guiana
Ll-ANC21	bLlo23	1	Colombia
Ll-ANC22	bLlo25	1	French Guiana
Ll-ANC23	bLlo41, bLlo66	2	French Guiana
Ll-ANC24	bLlo67	1	Bolivia
Ll-ANC25	bLlo68	1	Peru

[a] Haplotypes with the same number and different letters (e.g. Ll-ANC19A, 19B) are collapsed into a single haplotype when all sites with missing information or indels are excluded (e.g. Fig.7).

Table 6. Nucleotide and haplotype diversity observed in the *Lontra longicaudis* mtDNA segments, specified separately for different geographically defined populations.

Segment[a]	Group[b]	Nucleotide diversity (SE)[c]	Haplotype diversity (SE)[c]
CR	Brazil	0.0062 ± 0.0019	0.8217 ± 0.0106
	FG	0.0165 ± 0.0039	0.8571 ± 0.0386
	NW	0.0178 ± 0.0040	0.9333 ± 0.0196
	Total	0.0113 ± 0.0027	0.8911 ± 0.0060
ATP8 + ND5	Brazil	0.0019 ± 0.0006	0.7754 ± 0.0153
	FG	0.0058 ± 0.0016	0.9048 ± 0.0389
	NW	0.0094 ± 0.0019	0.9333 ± 0.0196
	Total	0.0059 ± 0.0013	0.8762 ± 0.0077
CR + *ATP8 + ND5*	Brazil	0.0038 ± 0.0009	0.9538 ± 0.0053
	FG	0.0100 ± 0.0018	0.8667 ± 0.0527
	NW	0.0124 ± 0.0018	0.9444 ± 0.0233
	Total	0.0081 ± 0.0012	0.9714 ± 0.0027

[a] CR: mtDNA control region; *ATP8*: *ATPase subunit 8*; *ND5*: *NADH dehydrogenase subunit 5*.

[b] FG: French Guiana; NW: Northwestern South America (including French Guiana, Peru, Bolivia and Colombia).

[c] All sites containing indels or missing information were excluded from this analysis. SE: standard error.

Table 7. Measures of microsatellite diversity in the two geographic groups of the Neotropical otter investigated in this study.

| | Brazil | | | | | | Northwestern South America | | | | | |
Locus	N[a]	NEA[b]	SR (bp)[c]	H_e[d]	H_o[e]	HWE[f] (P)	N[a]	NEA[b]	SR (bp)[c]	H_e[d]	H_o[e]	HWE[f] (P)
Lut453	6	3	136-146	0.756	0.704	ns	3	0	138-144	0.648	0.429	ns
Lut701	6	1	180-198	0.680	0.826	ns	7	2	172-198	0.924	0.833	ns
Lut733	7	2	154-178	0.756	0.690	ns	5	0	154-170	0.758	0.714	ns
Lut782	6	2	178-198	0.672	0.517	ns	5	1	182-206	0.803	0.500	ns
RIO06	8	5	261-289	0.729	0.630	ns	3	0	275-281	0.711	0.000	ns
RIO07	8	5	176-194	0.791	0.379	*	3	0	182-186	0.604	0.286	ns
RIO11	11	7	168-188	0.895	0.862	ns	4	0	168-182	0.758	0.429	ns
RIO18	11	7	142-174	0.827	0.462	*	5	1	142-154	0.670	0.429	ns
RIO19	9	5	286-306	0.712	0.571	ns	6	2	290-304	0.747	0.571	ns
RIO20	8	5	261-279	0.845	0.250	*	3	0	265-269	0.545	0.000	ns
Mean	8	4.2	-	0.766	0.589	-	4.4	0.6	-	0.717	0.419	-

[a] Number of alleles

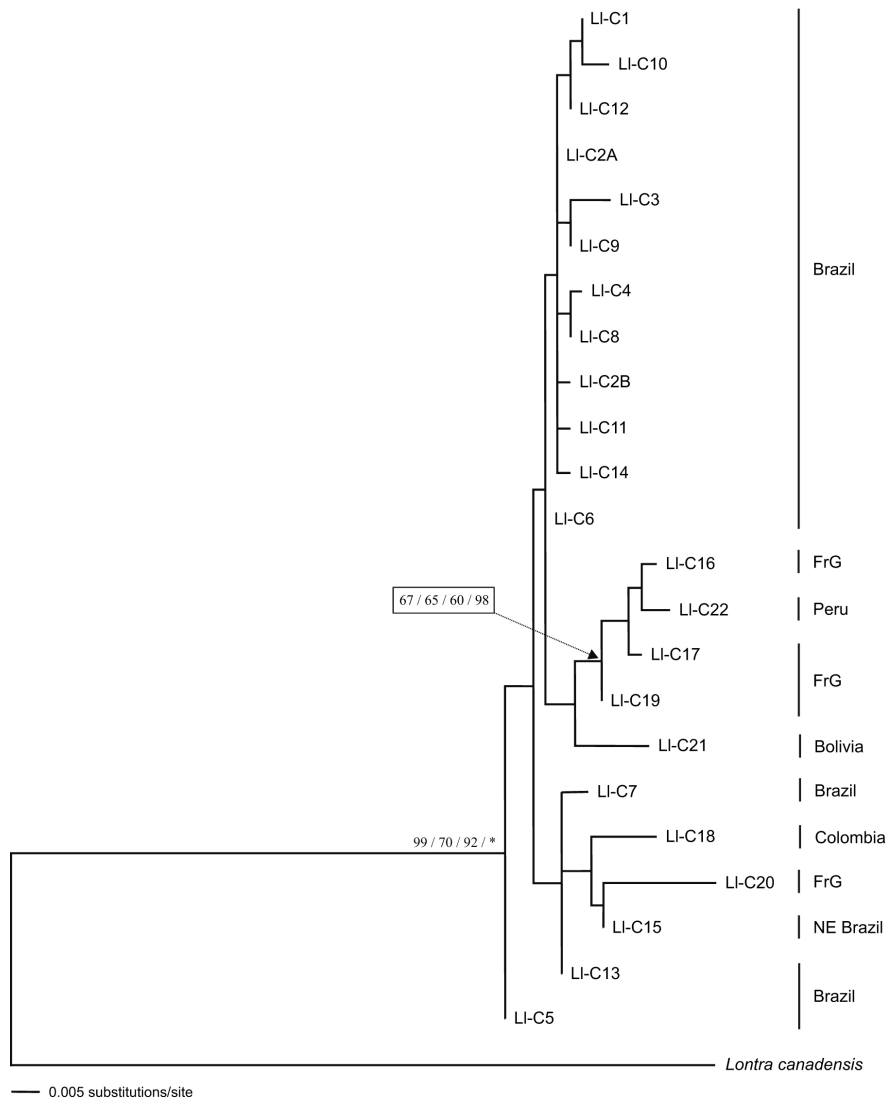[b] Number of exclusive alleles

[c] Size range
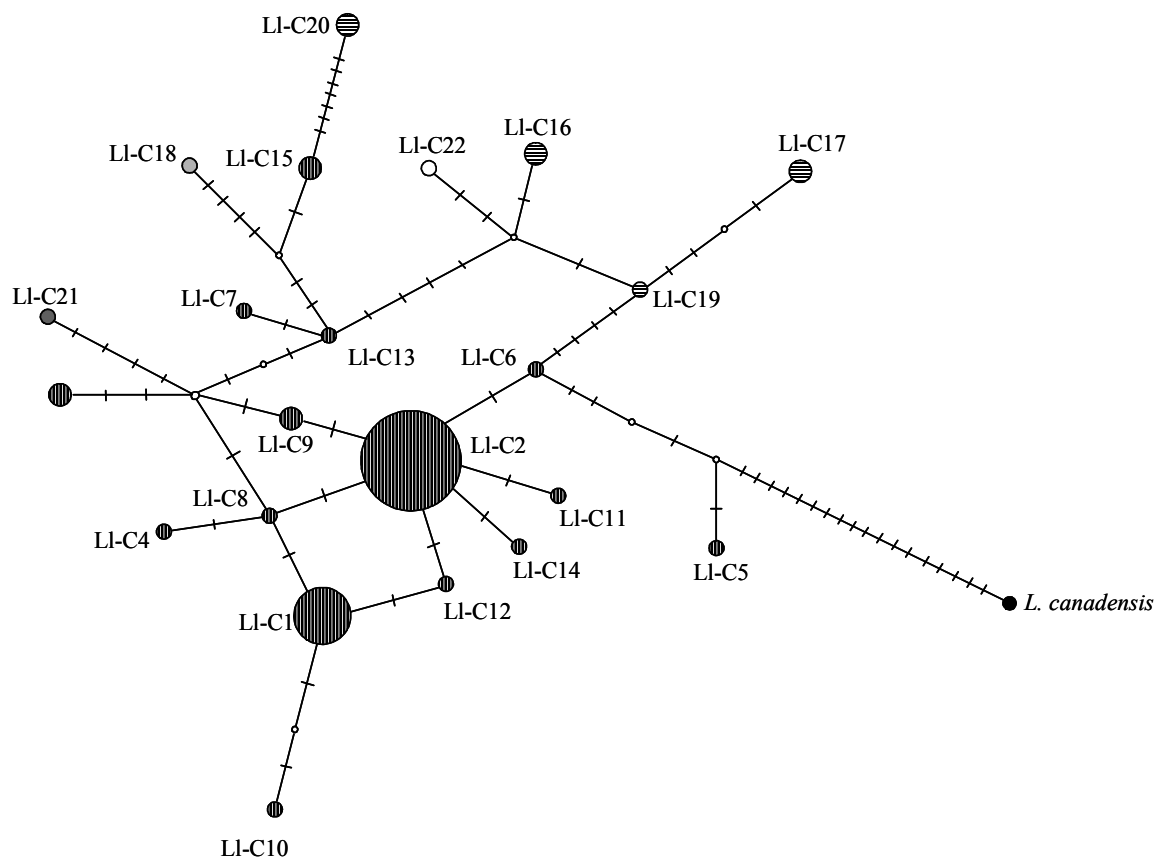
[d] Expected heterozygosity

[e] Observed heterozygosity

[f] Deviation from Hardy-Weinberg Equilibrium
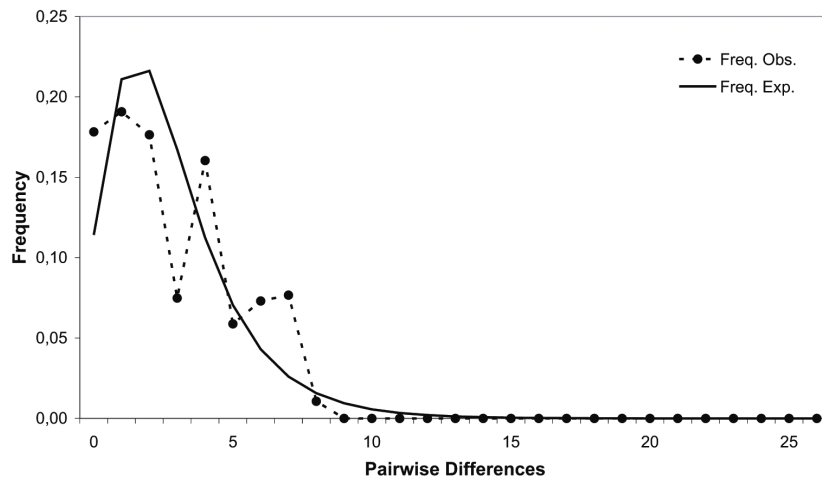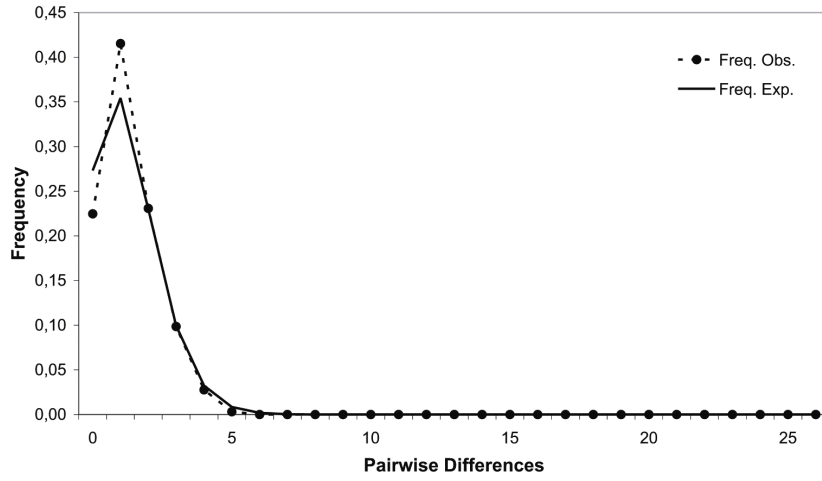
* Significant after Bonferroni adjustment (p < 0.001)

N

Brazil

NW

Species range

23

19, 20, 21
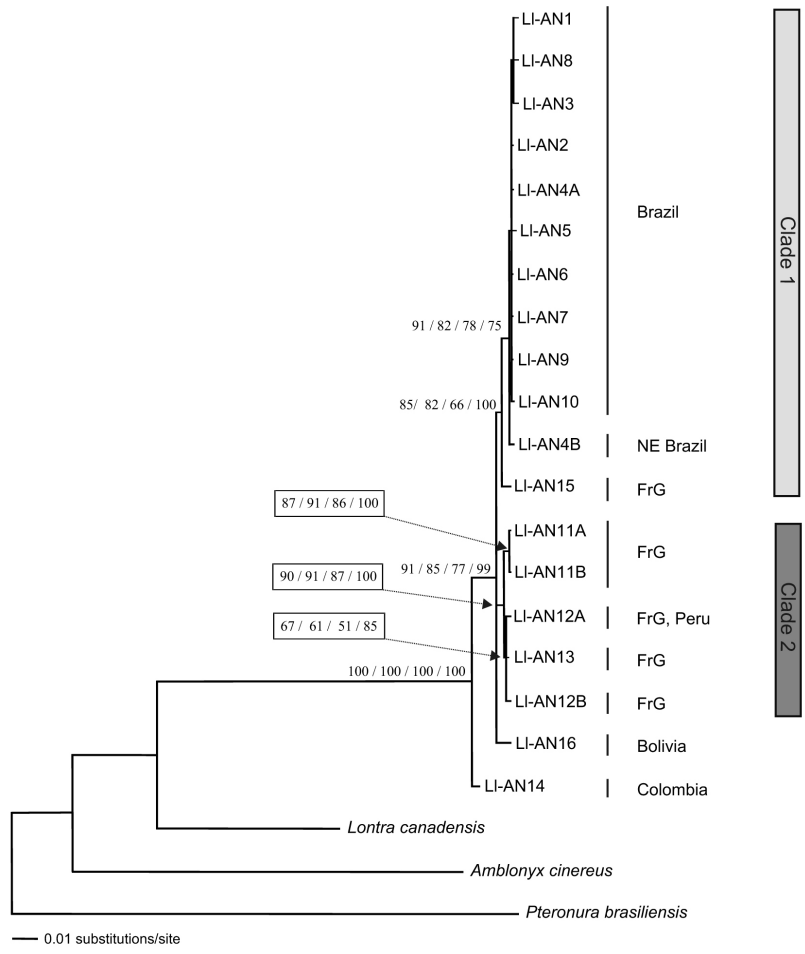22, 25, 41
66

60, 61

68

67

28

09

30   64

31

17   18

39

16

57

10

38

36

15

29

51

14

04

34

03

01

02, 05, 06
07, 08, 11
12, 24, 26
37, 42, 58

0   200   400 mi

0   200 400 km

LI-C1
LI-C10
LI-C12
LI-C2A
LI-C3
LI-C9
LI-C4
LI-C8
LI-C2B
LI-C11
LI-C14

Brazil

LI-C6
LI-C16   FrG
LI-C22   Peru
LI-C17
67 / 65 / 60 / 98
LI-C19   FrG
LI-C21   Bolivia
LI-C7   Brazil
LI-C18   Colombia
99 / 70 / 92 / *
LI-C20   FrG
LI-C15   NE Brazil
LI-C13
Brazil
LI-C5

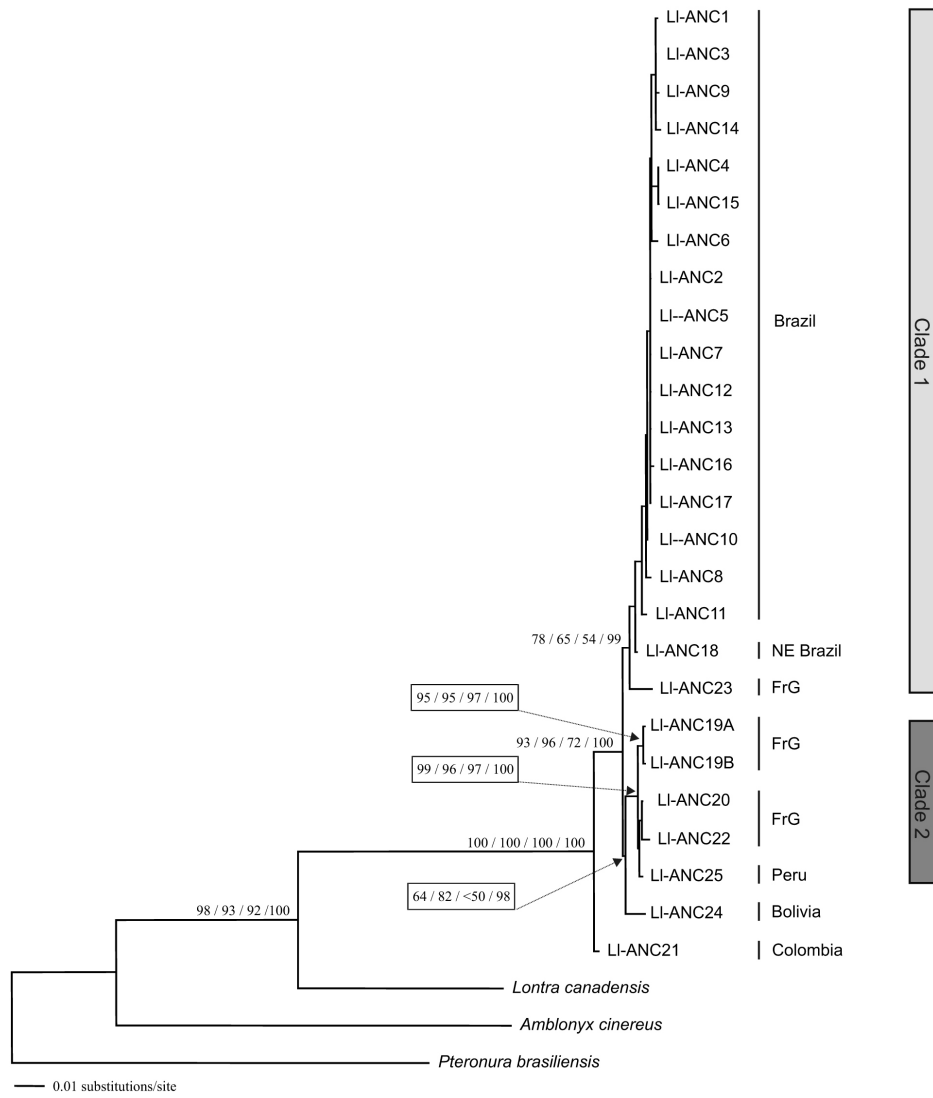*Lontra canadensis*

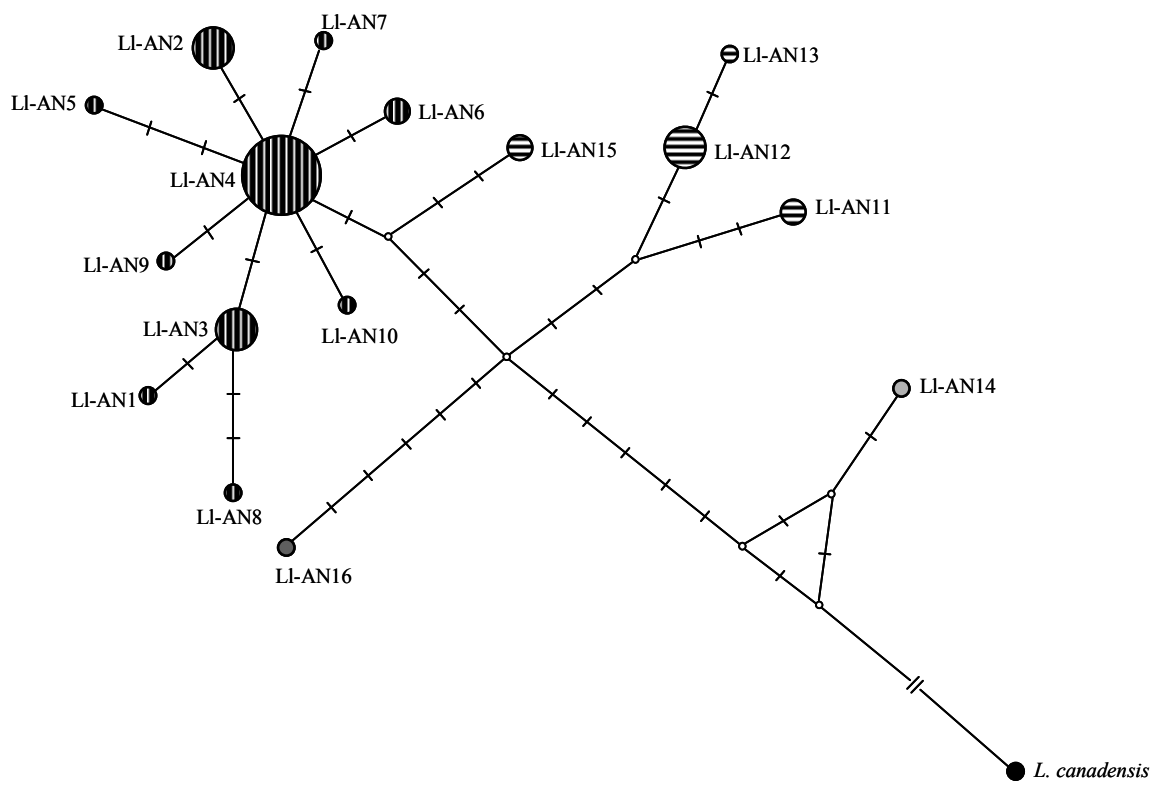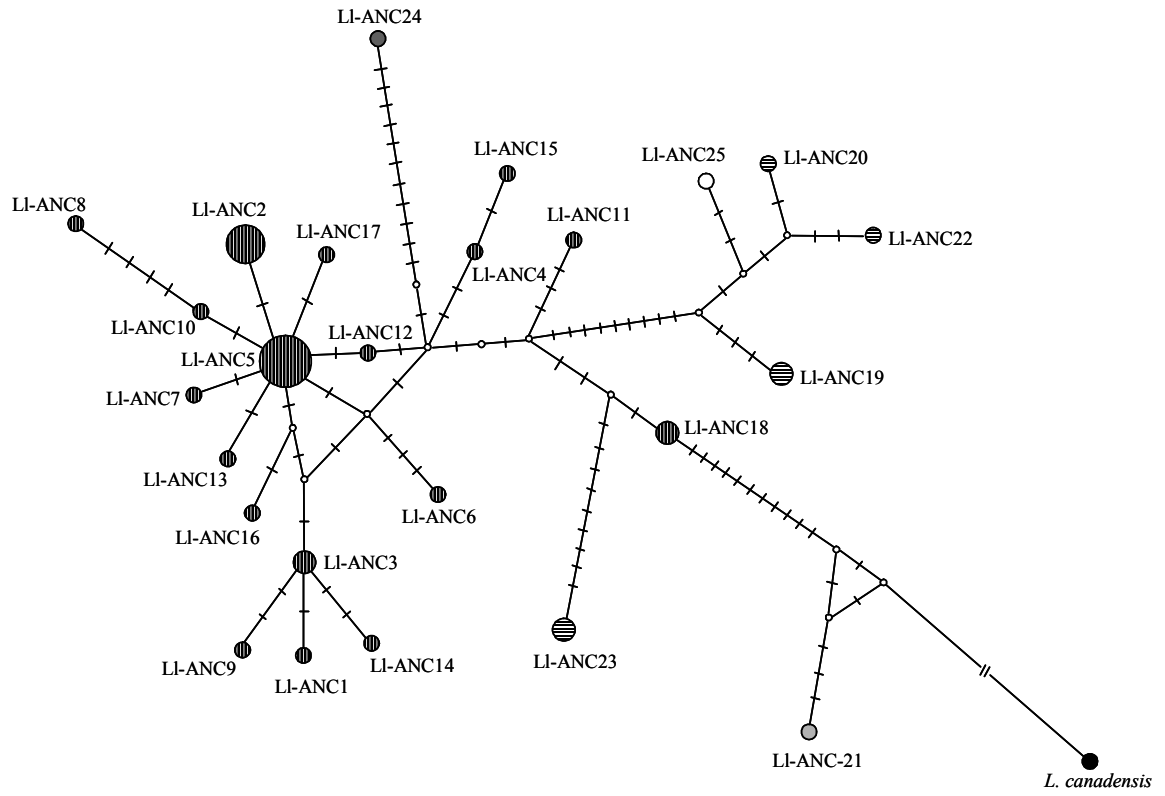—— 0.005 substitutions/site
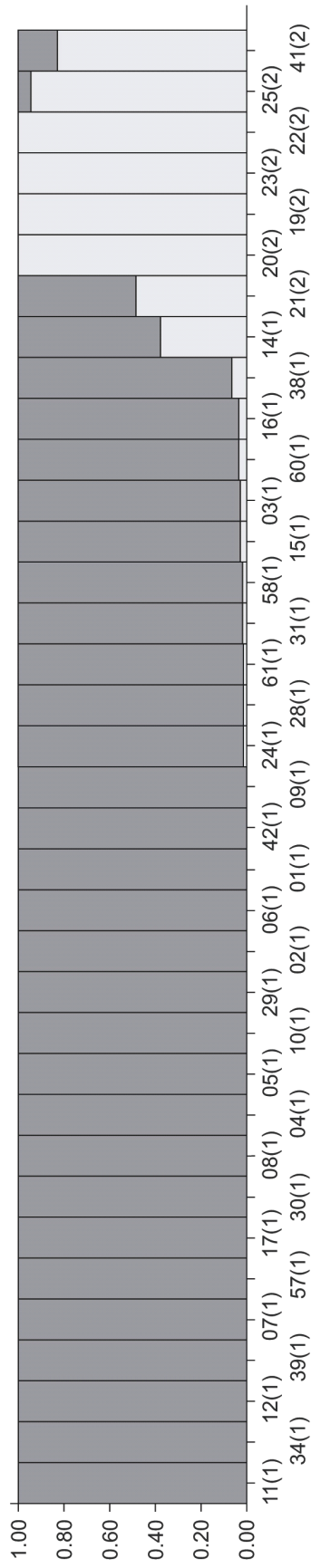
XLVIII

(A)

(B)

(C)

L

LIV

LV

Author Information Box

This project is part of Cristine S. Trinca's M. Sc. thesis at the Graduate Program in Zoology of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil, where she is advised by Dr. Eduardo Eizirik. Her research interests are focused on population genetics, ecology, phylogeography and conservation of Neotropical otters. Dr. Helen Waldemarin is an otter biologist interested in ecology, behavior and conservation of Neotropical populations of this group. Dr. Klaus-Peter Koepfli works on carnivore phylogenetics, evolutionary biology and biogeography. Dr. Benoit de Thoisy is a conservation biologist interested in diverse issues pertaining to genetics and ecology of South American taxa. Dr. Eizirik is an evolutionary biologist focusing most of his research on phylogenetics, conservation genetics and molecular ecology of Neotropical carnivores.

Capítulo III – Discussão Geral

Os resultados obtidos no presente trabalho evidenciam a existência de padrões filogeográficos claros em *Lontra longicaudis*. A partir das análises filogenéticas e populacionais realizadas, são discutidas algumas hipóteses a respeito da história demográfica desta espécie, bem como sugestões para incorporar estas informações no planejamento de estratégias adequadas para a conservação e manejo da mesma. Inferências sobre os padrões de variabilidade nos segmentos de mtDNA utilizados no estudo foram realizadas, avaliando-se seu desempenho como marcadores para estudos evolutivos recentes em mustelídeos. Estes aspectos foram abordados na discussão do artigo precedente. Sendo assim, neste espaço, será feita uma breve revisão dos mesmos, salientando alguns aspectos e implicações adicionais.

O presente trabalho é o primeiro a caracterizar a diversidade genética e a estruturação geográfica observada em populações naturais de *L. longicaudis*, uma vez que os únicos estudos até o momento incluindo esta espécie são análises filogenéticas da família Mustelidae e da Subfamília Lutrinae em geral (Van Zyll de Jong 1987; Koepfli & Wayne 1998, 2003; Marmi *et al.* 2004), não abordando qualquer aspecto genético e/ou populacional da lontra Neotropical em particular. Alguns estudos desta natureza foram realizados até o momento para outras espécies de mustelídeos (p.ex. Mucci *et al.* 1999; Kyle & Strobeck 2001; Fleming & Cook 2002; Blundell *et al.* 2002; Broquet *et al.* 2006; Pertoldi *et al.* 2006), o que permite comparações iniciais com os resultados aqui obtidos. Estas comparações indicam que a lontra Neotropical apresenta níveis relativamente altos de diversidade genética nos segmentos mitocondriais empregados, bem como alta variabilidade nos locos de microssatélite analisados.

A região controladora têm sido bastante empregada em estudos evolutivos e demográficos de diversas espécies de mamíferos, revelando padrões filogeográficos claros em diversas espécies de vertebrados (Sivasundar *et al.* 2001; Márquez *et al.* 2006; Tchaicka *et al.* 2007). Entretanto, este segmento, apesar de bastante variável, não apresentou boa resolução na identificação de padrões de estruturação geográfica e história evolutiva de *Lontra longicaudis*. Nota-se que apesar de relativamente variável em nível intra-específico, quando utilizado para comparações interespecíficas, sua variabilidade é bastante reduzida, dando claras indicações de saturação. Entretanto, ainda assim, este segmento mostrou-se mais variável do que em *Lutra lutra,* em que diversos estudos encontraram uma diversidade nucleotídica e haplotípica bastante reduzida em diversas regiões da Europa (Mucci *et al.* 1999; Effenberger & Suchentrunk 1999; Cassens *et al.* 2000; Pérez-Haro *et al.* 2005).

Comparações com outros estudos realizados com diversas espécies de carnívoros sugerem moderados níveis de variabilidade genética no DNA mitocondrial de *Lontra longicaudis*. A diversidade haplotípica geral encontrada para a região controladora do mtDNA é relativamente alta (h_{Conc}= 0.8911 ± 0.0060), em comparação, por exemplo, com *Mustela lutreola* (h = 0.469 e 0.939,

para duas populações da Europa; Michaux *et al*. 2005), *Enhydra lutris* (*h* = 0.412; Larson *et al*. 2002) e *Lutra lutra* (*h* = 0.16 ± 0.06; Ferrando *et al*. 2004). A diversidade nucleotídica encontrada neste conjunto de dados é moderada (π = 0.0113 ± 0.0027) comparada com estimativas obtidas para outros mustelídeos, como por exemplo, *Lutra lutra* (π = 0.0006; Ferrando *et al*. 2004), e *Mustela lutreola* (π = 0.012 ± 0.058 e 0.0012 ± 0.088 para cada uma das duas populações analisadas). Em contrapartida, uma menor diversidade é observada quando comparada a *Enhydra lutris* (π = 0.098 ± 0.029; Larson *et al*. 2002).

Johnson *et al*. (1999) utilizaram os genes *ATP8* e *ND5* na investigação de padrões filogeográficos de quatro espécies de pequenos felinos neotropicais. A diversidade nucleotídica (π) encontrada variou de 0.0029 (*Oncifelis guigna*) a 0.0126 apresentando grande número de sítios polimórficos (em média 36, exceto para *O. guigna*). Os valores encontrados para *L. longicaudis* podem ser considerados altos (36 sítios polimórficos e π = 0.0080 ± 0.0015).

Todavia, comparações com outras espécies de carnívoros, especialmente mustelídeos, sobre a variabilidade dos segmentos dos genes *ATP8* e *ND5* não puderam ser realizadas, pois os estudos realizados até o momento não utilizaram estas regiões do mtDNA.

Quando a diversidade genética é comparada entre os segmentos empregados, a região controladora é mais variável do que os genes *ATP8* e *ND5*, entretanto, em comparações interespecíficas, estes dois apresentam-se muito mais informativos. Isto também fica evidente na análise das árvores filogenéticas, as quais foram claras em demonstrar sinais filogeográficos, com exceção daquela construída somente com a porção hipervariável I da região controladora (Fig. 2). Isto sugere que este segmento esteja saturado de mutações, perdendo assim poder informativo. Este resultado é interessante do ponto de vista experimental e analítico, uma vez que a região controladora tem sido muito utilizada para investigar padrões filogeográficos com diversas espécies de vertebrados. Caso o padrão da região controladora observado para *Lontra longicaudis* venha a ser corroborado em outras espécies, é possível que a escolha preferencial deste segmento como portador de sinais filogeográficos seja questionada.

Em contrapartida, os genes *ATP8* e *ND5* apresentaram alta diversidade intra- e interespecífica, sendo que devido ao tamanho do segmento e número de sítios polimórficos e informativos para as análises dentro e entre espécies, o gene *ND5* parece, neste caso, ser o mais indicado para análises filogeográficas.

Com relação aos locos de microssatélite, estes se mostraram bastante variáveis, com grande número de alelos, comparados às espécies para os quais foram descritos (*Lontra canadensis*, Beheler *et al*. 2004, 2005; *Lutra lutra*, Dallas & Piertney 1998). Beheler *et al*. (2004, 2005) encontraram, em geral, menor número de alelos e maior heterozigosidade em *L. canadensis* em relação ao observado para *L. longicaudis*. Este fato, porém, pode ser um efeito do tamanho

amostral analisado para *L. longicaudis*, o qual pode não ter sido totalmente representativo da variabilidade nestes locos na espécie em questão. Dallas & Piertney (1998) encontraram números semelhantes de alelos, todavia, em menor nível de heterozigosidade do que o observado em *L. longicaudis*.

Estudos direcionados à análise e estruturação da variabilidade genética de populações naturais de *Lutra lutra* foram realizados em diversas regiões da Europa e Ásia (Dallas *et al.* 2002; Randi *et al.* 2003; Hung *et al.* 2004), os quais utilizaram alguns dos mesmos locos de microssatélite empregados neste estudo. De um modo geral, estes locos demonstraram-se tão ou mais variáveis em *Lontra longicaudis* do que em *Lutra lutra*, indicando que os marcadores selecionados são eficientes para a investigação de parâmetros populacionais naquela espécie.

As análises de mtDNA revelaram a existência de um padrão filogeográfico claro, sem compartilhamento de haplótipos entre as regiões. Este padrão é necessariamente decorrente de um extenso período de isolamento entre as populações ancestrais (Avise *et al*. 1987). Os resultados obtidos indicam a existência de quatro entidades filogeográficas, duas delas sustentadas por um conjunto amostral robusto e as outras duas representadas por apenas um indivíduo cada. Os dois maiores grupos de linhagens de mtDNA são representados pelo Clado 1, o qual compreende todas as linhagens brasileiras e uma proveniente da Guiana Francesa, e o Clado 2 incluindo todos os outros haplótipos encontrados na Guiana Francesa e Peru.

As duas outras linhagens de mtDNA são provenientes da Bolívia e da Colômbia. Coletado na Cordilheira central dos Andes, o indivíduo proveniente da Colômbia revelou conter a linhagem mais divergente, sugerindo que a Cordilheira dos Andes pode ser uma barreira ao fluxo gênico entre os indivíduos localizados a Leste e Oeste da mesma. Contudo, o tempo de divergência entre esta linhagem e o restante, parece bastante recente (644 mil anos atrás), indicando que o episódio de isolamento deste grupo foi posterior ao surgimento desta provável barreira.

O tempo de divergência entre *L. longicaudis* e *L. canadensis* datado foi cerca de 4.16 Ma. A divergência média entre o haplótipo Colombiano e as outras linhagens foi estimada em 0.644 Ma, enquanto que a separação da linhagem encontrada na Bolívia e dos Clados 1 e 2 foi datada em aproximadamente 450 mil anos. Estes dois clados apresentaram uma alopatria quase completa, exceto por um haplótipo geograficamente proveniente da Guiana Francesa incluído no Clado 1, predominantemente brasileiro. Este dado indica haver alguma conectividade (ao menos ancestral) entre as populações destas duas regiões, onde o sentido de colonização seria Norte–Sul, tendo em vista as posições dos haplótipos da Guiana Francesa e do Nordeste brasileiro (basal entre todas as seqüências do Brasil) no Clado 1. De especial interesse é a datação do tempo de divergência na base do Clado 1 (separação entre o haplótipo da Guiana e o grupo brasileiro de linhagens), a qual foi estimada em 0.422 Ma. A coalescência das linhagens brasileiras foi datada em cerca de 196

Ma. Além disso, os haplótipos da Guiana Francesa demonstram algum grau de sub-estruturação indicado pelas análises filogenéticas, com tempo de divergência datado em aproximadamente 176 mil anos atrás.

Apesar de Colômbia e Bolívia serem, cada uma, representadas por apenas um indivíduo, sua posição consistente nas várias análises e o alto grau de apoio aos principais ramos das árvores filogenéticas indica que há uma alta probabilidade de haver entidades demográficas distintas nestas regiões.

Resultados obtidos através das análises dos locos de microssatélite concordam com aqueles revelados pelo mtDNA, indicando haver clara separação entre o Brasil e o Noroeste da América do Sul (apoiado pelo alto valor de F_{ST} e pelos testes de associação). Entretanto, devido ao pequeno tamanho amostral, não foi possível testar cenários mais refinados de estruturação geográfica da variabilidade genética destes marcadores. Fluxo gênico no sentido Norte-Sul também pode ser inferido, revelado pelos testes de associação, os quais indicaram haver um indivíduo com localização geográfica na Guiana Francesa e que apresentou maior similaridade de alelos com as amostras brasileiras.

Entretanto, estudos mais aprofundados e com maior tamanho amostral devem ser conduzidos em diferentes escalas geográficas, a fim de desvendar padrões ainda desconhecidos e também confirmar se estas subdivisões devem ser interpretadas como Unidades de Manejo (MUs) ou Unidades Evolutivamente Significativas (ESUs), merecendo considerações próprias em iniciativas de conservação.

É importante definir quais populações devem ser consideradas separadamente para fins de conservação, de modo a não interferir na distribuição geográfica da diversidade genética formada através dos processos históricos. Esforços isolados voltados para a conservação da espécie estão em desenvolvimento, abordando especialmente aspectos ecológicos, enquanto persiste uma carência significativa de informações sobre a composição genética das populações naturais de *Lontra longicaudis*. A identificação detalhada de unidades populacionais com diferenciação genética e/ou demográfica ao longo da distribuição da espécie é extremamente importante para uma avaliação precisa da representatividade das áreas protegidas, e o desenho adequado de estratégias de conservação.
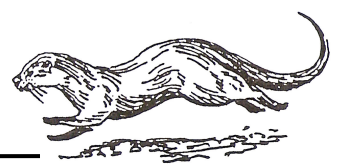
Arrendal J, Walker CW, Sundqvist A, Hellborg L, Vilà C (2004) Genetic evaluation of a translocation program. *Conservation Genetics*, **5**, 79-88.

Asquith NM, Wright SJ, Clauss MJ (1997) Does mammal community composition control recruitment in neotropical forests? Evidence from Panama. *Ecology*, **78**, 941-946.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific Phylogeography: the mithocondrial DNA bridge between populations genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489-522.

Avise JC, Bowen B, Lamb T, Meylan AB, Berminghom E (1992) Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in Testudines. *Molecular Biology and Evolution*, **9**, 457-473.

Avise JC (2000) *Phylogeography – The history and formation the species*. Harvard University Press, Cambridge, Massachusetts.

Baker CS, Pery A, Bannister JL, Weinrich MT, Abernethy RB, Calambokidis K, Lien J, Lambertsen RH, Urban-Ramirez J, Vasquez O, Clapham PJ, Alling A, O'Brien SJ, Palumbi SR (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences*, **90**, 8239-8243.

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology*, **13**, 729-744.

Beheler AS, Fike JA, Murfitt LM, Rhodes Jr OE, Serfass TS (2004) Development of polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and amplification in related Mustelids. *Molecular Ecology Notes*, **4**, 56-58.

Beheler AS, Fike JA, Murfitt LM, Dharmarajan G, Rhodes Jr OE, Serfass TS (2005) Ten new polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and their utility in related mustelids. *Molecular Ecology Notes*, **5**, 602-604.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology*, **7**, 367-369.

Blacher C (1987) Ocorrência e preservação de *Lutra longicaudis* (Mammalia: Mustelidae) no Litoral de Santa Catarina. *Boletim da Fundação Brasileira para Conservação da Natureza*, **22**, 105-117.

Blundell GM, Ben-David M, Groves P, Bowyer RT, Geffen E (2002) Characteristics of sex-biased dispersal and gene flow in coastal river otters: implications for natural recolonization of extirpated populations. *Molecular Ecology*, **13**, 289-303.

Broquet T, Johnson CA, Petit E, Thompson I, Burel F, Fryxell M (2006) Dispersal and genetic structure in the American marten, *Martes americana. Molecular Ecology*, **15**, 1689-1697.

Bruford MW, Wayne RK (1993) Microsatellite and their application to population genetic studies. *Current Opinion in Genetics & Development*, **3**, 939-943.

Cassens I, Tiedemann R, Suchentrunk F, Hartl GB (2000) Mitochondrial DNA Variation in the Europian Otter (*Lutra lutra*) and the Use of Spatial Autocorrelation Analysis in Conservation. *The Journal of Heredity*, **91**, 31-41.

Cegelski CC, Waits LP, Anderson NJ (2003) Assessing population structure e gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology*, **12**, 2907-2918.

Chebez JC (1999) *Los que se van – Espécies argentinas em peligro*. Buenos Aires, Editorial Albatroz Saci.

Chehébar CE (1990) Action Plan from Latin American Otters In*: Otters: An Action Plan for their Conservation* (eds. Foster-Turley P, Macdonald S, Mason C), pp. 64-73. IUCN Otter Specialist Group.

Cimardi AV (1996) *Mamíferos de Santa Catarina*, 1a ed. Florianópolis: FATMA.

Ciofi C, Bruford MW (1999) Genetic structure e gene flow among Komodo dragon populations inferred by microsatellite loci analysis. *Molecular Ecology*, **8**, 17-30.

Colares EP, Best RC (1991) Blood parameters of amazon otters (*Lutra longicaudis*, *Pteronura brasiliensis*) (Carnivora, Mustelidae). *Comparative Biochemistry and Physiology*, **99A**, 513-515.

Colares EP, Silva MNF (1987) Efeito da redução da alimentação na digestibilidade em lontras *Lutra longicaudis* (Mammalia: Mustelidae). In: *II Reunião de trabalhos de especialistas em mamíferos aquáticos da América do Sul*.

Colares EP, Waldemarin HF (2000) Feeding of the neotropical river otter (*Lontra longicaudis*) in the coastal region of the Rio Grande do Sul State, Southern Brazil. *IUCN Otter Specialist Group Bulletin*, **17**, 6–13.

Crandall KA, Bininda-Emond ORP, Mace GM, Wayne RK (2000) Considering evolutionary process in conservation biology. *Trends in Ecology and Evolution*, **15**, 290-295.

Dallas JF, Piertney SB (1998) Microsatellite primers for the Eurasian otter. *Molecular Ecology*, **7**, 1248-1251.

Dallas JF, Bacon PJ, Carss DN, Conroy JWH, Green R, Jefferies DJ, Kruuk H, Marshall F, Piertney SB, Racey PA (1999) Genetic diversity in the Eurasian otter, *Lutra lutra*, in Scotland. Evidence from microsatellite polymorphism. *Biological Journal of the Linnean Society*, **68**, 71-86.

Dallas JF, Carss DN, Marshall F, Koeplfi KP, Kruuk H, Piertney SB, Bacon PJ (2000) Sex identification of the Eurasian otter *Lutra lutra* by PCR typing of spraints. *Conservation Genetics*, **1**, 181-183.

Dallas JF, Marshall F, Piertney SB, Bacon PJ, Racey P (2002) Spatially restricted gene flow and reduced microsatellite polymorphism in the Eurasian otter *Lutra lutra* in Britain. *Conservation Genetics*, **3**, 15-29.

DeSalle R, Amato G (2004) The expansion of conservation genetics. *Nature Reviews Genetics*, **5**, 702-712.

DeYoung RW, Honeycutt RL (2005) The molecular toolbox: genetic techniques in wildlife ecology and management. *Journal of Wildlife Management*, **69**, 1362-1384.

Dragoo JM, Honeycutt R (1997) Systematics of Mustelid-like Carnivores. *Journal of Mammalogy*, **78**, 426-443.

Duarte JCS, Rebêlo GH (1985) Carnivore skins held in Brazil. *Trafic bulletin*, **7**, 16-17.

Effenberger S, Suchentrunk F (1999) RFLP analyses of the mitochondrial DNA of otters (*Lutra lutra*) from Europe – implications for conservation of a flagship species. *Biological Conservation*, **90**, 229-234.

Eisenberg JF, Redford KH (1999) *Mammals of the Neotropics*, Vol. 3. The Central Tropics: Ecuador, Peru, Bolivia, Brazil. Chicago, London, University of Chicago Press.

Eizirik E (1996) Ecologia molecular, genética da conservação, e o conceito de Unidades Evolutivamente Significativas. *Revista Brasileira de Genética*, **19**, 23-29.

Eizirik E, Kim J, Menotti-Raymond M, Crawshaw Jr PG, O'Brien SJ, Johnson WE (2001) Phylogeography, population history e conservation of jaguars (*Panthera onca*, Mammalia, Felidae). *Molecular Ecology*, **10**, 65-79.

Emmons LH (1990) *Neotropical Rainforest Mammals: A field guide*. Chicago, London, University of Chicago Press.

Ferrando A, Ponsà M, Marmi J, Domingo-Roura X (2004) Eurasian otters, *Lutra lutra*, have a dominant mtDNA haplotype from the Iberian Península to Scandinavia. *The Journal of Heredity*, **95**, 430-435.

Fleming MA, Cook JA (2002) Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Molecular Ecology*, **11**, 795–807.

Flynn JJ, Finarelli JÁ, Zehr S, Hsu J, Nedbal MA (2005) Molecular phylogeny of the Carnivora (Mammalia): assessing the impact of increased sampling on resolving enigmatic relationships. *Systematic Biology*, **54**, 317-337.

Fonseca GAB, Robinson J (1988) Forest size and structure: competitive and predatory effects on small mammal communities. *Biological Conservancy*, **53**, 265-294.

Fonseca GA, Rylands AB, Costa CMR, Machado RB, Leite YLR (1994) *Livro vermelho dos mamíferos ameaçados de extinção*. Belo Horizonte, Fundação Biodiversitas.

Foster-Turley P, Macdonald S, Mason CF (1990) *Otters: An action plan for their conservation*. IUCN/SSC Otter Specialist Group.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.

Frantz AC, Pope LC, Carpenter PJ, Roper TJ, Wilson GJ, Delahay RJ, Burke T (2003) Reliable microssatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. *Molecular Ecology*, **12**, 1649-1661.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752.

Fulton TR, Strobeck C (2006) Molecular phylogeny of the Arctoidea (Carnivora): Effects of missing data on supertree and supermatrix analyses of multiple gene data sets. *Molecular Phylogenetics and Evolution*, **41**, 1665-181.

Gallo JP (1986) Otters in México. *Journal of the Otter Trust*, **1**, 19-24.

Gallo JP (1989) *Distribución y estado actual de la nutria o perro de agua (Lutra longicaudis annectens Major, 1897) en la Sierra Madre del Sur, México*. Tese de Mestrado, Universidad Nacional Autónoma de México.

Gallo JP (1997) Situación y distribución de las nutrias en México, con énfasis en *Lontra longicaudis annectens* (Major, 1897). *Revista Mexicana de Mastozoología*, **2**, 10-32.

Helder J, De Andrade HK (1997) Food and feeding habitats of the neotropical river otter *Lontra longicaudis* (Carnivora, Mustelidae). *Mammalia*, **61**, 193-203.

Hey J, Machado CA (2003) The study of structured populations — new hope for a difficult and divided science. *Nature Reviews Genetics*, **4**, 535-543.

Huang C, Hsu C, Lee L, Li S (2005) Isolation and characterization of tetramicrosatellite DNA markers in the Eurasian otter (*Lutra lutra*). *Molecular Ecology Notes*, **5**, 314-316.

Hung C, Li S, Lee L (2004) Faecal DNA typing to determine the abundance and spatial organisation of otters (*Lutra lutra*) along two stream systems in Kinmen. *Animal Conservation*, **7**, 301-311.

IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (1997) *Mamíferos aquáticos do Brasil: Plano de Ação*. Brasília.

IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (2004) *Plano de ação: Pesquisa e Conservação de mamíferos carnívoros do Brasil*. Centro Nacional de Pesquisa e Conservação dos Predadores Naturais – CENAP, São Paulo.

Indrusiak CB, Eizirik E (2003) Carnívoros. In: *Livro vermelho da fauna ameaçada de extinção no Rio Grande do Sul*. Edipucrs, Porto Alegre.

IUCN (2006) *IUCN Red List of Threatened Species.* <www.iucnredlist.org> Downloaded in 27 February 2007.

Johnson WE, Pecon-Slattery J, Eizirik E, Kim JH, Menotti-Raymond M, Bonacic C, Cambre R, Crawshaw P, Nunes A, Seuánez HN, Seymour KL, Simon F, Swanson W, O'Brien SJ (1999) Disparate phylogeography patterns of molecular genetic variation in four closely related South American small cat species. *Molecular Ecology*, **8**, 79-92.

Johnson WE, Eizirik E, Roelke-Parker M, O'Brien SJ (2001) Applications of genetic concepts e molecular methods to carnivore conservation. In: *Carnivore Conservation* (eds. Gittleman JL, Funk SM, MacDonald D, Wayne RK), pp. 335-358. Cambridge University Press, Cambridge.

Kitching RL (1986) Prey-Predator Interactions. In*: Community Ecology: Patterns and Process* (eds. Kikkawa J, Anderson DJ), pp. 214-240. Blackell Scientific Publications.

Koepfli KP, Wayne RK (1998) Phylogenetic relationships of otters (Carnivora: Mustelidae) based on mitochondrial cytochrome *b* sequences. *Journal of Zoology*, **246**, 401-406.

Koepfli KP, Wayne RK (2003) Type ITS markers are more informatives than cytocrome *b* in phylogenetic reconstruction of the Mustelidae (Mammalia: Carnivora). *Systematic Biology*, **52**, 571-593.

Kohn MH, Murphy WJ, Ostrander EA, Wayne RK (2006) Genomics and conservation genetics. *Trend in Ecology and Evolution*, **21**, 629-637.

Kruuk R (2006) Otters. Ecology, behavior and conservation. 265pp. Oxford University Press.

Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 337-347.

Lacomba I, Soutullo A, Prigioni CM (2001) Distribution and conservation status of the Neotropical river otter (*Lontra longicaudis*) in the coastal lagoons of the Uruguayan Atlantic basin and their main tributaries. *IUCN Otter Specialist Group Bulletin*, **18**, 20-24.

Larson SE, Jameson RJ, Bodkin JL, Staedler M, Bentzen P (2002) Microsatellite DNA and mtDNA variation within and among remnant and translocated sea otter, *Enhydra lutris*, populations. *Journal of Mammalogy*, **83**, 893-906.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4,** 981-994.

Macdonald SM, Mason CF (1985) Otters, their habitat and conservation in Northeast Greece. *Biological Conservation,* **31**, 191-210.

Macdonald SM, Mason CF (1990) Threats. In*: Otters: An Action Plan for their Conservation* (eds. Foster-Turley P, Macdonald S, Mason C), pp. 11-14. IUCN Otter Specialist Group.

Machado ABM, Fonseca GAB, Machado RB, Aguiar LMS, Lins LV (1998) *Livro vermelho das espécies ameaçadas de extinção da fauna de Minas Gerais*. Fundação Biodiversitas, Belo Horizonte.

Marmi J, López-Giraldéz F, Domingo-Moura X (2004) Phylogeny, evolutionary history and taxonomy of the Mustelidae based on sequences of the cytochome *b* gene and a complex repetitive flanking region. *Zoologica Scripta*, **33**, 481-499.

Márquez A, Maldonado JE, González S, Beccaceci MD, Garcia JE, Duarte JMB (2006) Phylogeography and Pleistocene demographic history of the endangered marsh deer (*Blastocerus dichotomus*) from the Río de La Plata Basin. *Conservation Genetics*, **7**, 563-575.

Martin A & Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences*, **90**, 4087-4091.

Mason C (1990) An Introduction to the Otters. In: *Otters: An Action Plan for their Conservation* (eds. Foster-Turley P, Macdonald S, Mason C), pp. 4-7. IUCN Otter Specialist Group.

Michaux JR, Hardy OJ, Justy F, Fournier P, Kranz A, Cabria M, Davison A, Rosoux R, Libois R (2005) Conservation genetics and population history of the threatened Europian mink *Mustela lutreola*, with an emphasis on the West European populations. *Molecular Ecology*, **14**, 2373-2388.

Moritz C (1994) Defining "Evolutionary Significant Units" for conservation. *Trends in Ecology and Evolution*, **9**, 373-375.

Moritz C, Faith D (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*, **7**, 419-429.

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.

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary processes and theory* (eds. Nevo E, Karlin S), pp. 513-534. Academy Press, New York.

Nesje M, Roed KH, Lifjeld JT, Lindberg P, Steens OF (2000) Genetic relationship in the falcon (*Falco peregrinus*) analyzed by microsatellite DNA markers. *Molecular Ecology*, **9**, 53-60.

Nowak RM (1999) *Walker's mammals of the world,* 6th edn. Johns Hopkins University Press, Baltimore.

Paraná (Secretaria do Estado do Meio Ambiente) (1995) *Lista vermelha de animais ameaçados de extinção no Estado do Paraná.* SEMA/GTZ, Curitiba.

Pardini R (1998) Feeding ecology of the neotropical river otter, *Lontra longicaudis*, in an Atlantic Forest Stream, southeastern Brazil. *Journal of Zoology*, **245**, 385–391.

Pardini R (1999) Use of shelters by the neotropical river otter (*Lontra longicaudis*) in an Atlantic Forest stream, southeastern Brazil. *Journal of Mammalogy*, **80**, 600-610.

Parera A (1996) Las "nutrias verdadeiras" de la Argentina. *Boletin Tecnico de la Fundacion Vida Silvestre Argentina*, **21**, 1-38.

Passamani M, Camargo SL (1995) Diet of the river otter *Lutra longicaudis* in Furnas reservoir, south-eastern Brazil. *IUCN Otter Specialist Group Bulletin*, **12**, 32-33.

Pérez-Haro M, Viñas J, Mañas F, Batet A, Ruiz-Olmo J, Pla C (2005) Genetic variability in the complete mitochondrial control region of the Eurasian Otter (*Lutra lutra*) in the Iberian Peninsula. *Biological Journal of the Linnean Society*, **86**, 397-403.

Pertoldi C, Breyne P, Cabria MT, Halfmaerten D, Jansman HAH, Van Den Berge K, Madsen AB, Loeschcke V (2006) Genetic structure of the European polecat (*Mustela putorius*) and its implication for conservation strategies. *Journal of Zoology*, **270**, 102-115.

Pope LC, Domingo-Roura X, Erven K, Burke T (2005) Isolation by distance and gene flow in the Eurasian badger (*Meles meles*) at both a local and broad scale. *Molecular Ecology*, **15**, 371–386.

Quadros J, Monteiro-Filho ELA (2000) Fruit occurrence in the diet of the neotropical otter, *Lontra longicaudis*, in southern Brazilian Atlantic forest and its implications for seed dispersal. *Mastozoología Neotropical*, **7**, 33-36.

Quadros J, Monteiro-Filho ELA (2001) Diet of the Neotropical otter, *Lontra longicaudis*, in Atlantic Forest area, Santa Catarina State, southern Brazil. *Studies on Neotropical Fauna and Environment*, **36**, 15-21.

Randi E, Davoli F, Pierpaoli M, Pertoldi C, Bo Madsen A, Loeschcke V (2003) Genetic structure in otter (*Lutra lutra*) populations in Europe: implications for conservation. *Animal Conservation*, **6**, 93-100.

Reuther C, Dolch D, Green R, Jahrl J, Jefferies D, Krekemeyer A, Kucerova M, Madsen AB, Romanowski J, Roche K, Ruiz-Olmo J, Teubner J, Trindade A (2000) Surveying and monitoring distribution and population trends of the Eurasian otter (*Lutra lutra*). *Habitat*, **12**, 143pp.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution*, **1**, 1-10.

São Paulo (Secretaria de Estado do Meio Ambiente) (1998) *Fauna ameaçada no Estado de São Paulo*. SMA/CED, São Paulo.

Sato J, Hosada T, Mieczyslaw W, Tsuchiya K, Yamamoto Y, Suzuki H (2003) Phylogenetic relationships and divergence times among mustelids (Mammalia: Carnivora) based on

nucleotide sequences of the nuclear interphotoreceptor retinoid binding protein and mitochondrial cytochrome *b* genes. *Zoologial Science*, **20**, 243-264.

Schlötterer C (1998) Microssatelites. In: *Molecular genetic analysis of populations: a pratical approach*, 2nd edn. (ed. Hoelzel AR), pp. 237-261. Oxford University Press, New York.

Schlötterer C (2004) The evolution of molecular markers – just a matter of fashion? *Nature Reviews Genetics*, **5**, 63-69.

Serfass TL, Brooks RP, Novak JM, Johns PE, Rhodes Jr OE (1998) Genetic variation among populations of river otters in North America: considerations for reintroduction projects. *Journal of Mammalogy*, **79**, 736-746.

Silva F (1994) *Mamíferos silvestres do Rio Grande do Sul*. Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre.

Sivasundar A, Bermingham E, Ortí G (2001) Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South America rivers. *Molecular Ecology*, **10**, 407-417.

Soldateli M, Blacher C (1996) Considerações preliminares sobre o número e distribuição espaço/temporal de sinais de *Lutra longicaudis* (Olfers, 1818) (Carnivora, Mustelidae) nas lagoas da Conceição e do Peri, Ilha de Santa Catarina, SC Brasil. *Biotemas*, **9**, 38–64.

Spinola RM, Vaughan C (1995) Abundancia relativa y actividad de marcaje de la nutria Neotropical (*Lutra longicaudis*) en Costa Rica. *Vida Silvestre Neotropical*, **4**, 38-45.

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revelead for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology*, **12**, 2357-2369.

Swimley TJ, Serfass TL, Brooks RP, Tzilkowski WM (1998) Predicting river otter latrine sites in Pennsylvania. *Wildlife Society Bulletin*, **26**, 836-845.

Tchaicka L, Eizirik E, Oliveira TG, Cândido Jr JF, Freitas TRO (2007) Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). *Molecular Ecology*, **16**, 819–838.

Terborgh J (1990) The role of felid predators in neotropical forests. *Vida Silvestre Neotropical, **2**, 3-5.

Terborgh J (1992) Maintenance of diversity in tropical forest. *Biotropica*, **24**, 283-292.

Utreras V, Rodríguez M, Araya I (2002) Preliminary study on the diet of the Neotropical otter *Lutra longicaudis* in the Tiputini river, Yasuni National Park, Ecuadorian Amazonia. In: *Proceedings of the VII International Otter Colloquium*.

Waits L, Taberlet P, Swenson JE, Sandegren F, Franzen R (2000) Nuclear DNA microsatellite analysis of genetic diversity e gene flow in the Scandinavian brown bear (*Ursus arctus*). *Molecular Ecology*, **9**, 421-431.

Waldemarin HF (1997) *Estudo da ecologia de lontras (Lontra longicaudis) no Parque Nacional da Lagoa do Peixe: Manejo e Conservação*. Monografia de Graduação. Fundação Universidade do Rio Grande, Rio Grande.

Waldemarin HF, Colares EP (2000) Utilization of resting sites and dens by the neotropical river otter (*Lontra longicaudis*) in the south of Rio Grande do Sul State, Southern Brazil. *IUCN Otter Specialist Group Bulletin*, **17**, 14-19.

Walker CW, Vilà C, Landa A, Lindén M, Ellegren H (2001) Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 53-63.

Wisely SM, Buskirk SW, McDonald DB, Ostrander EA (2002) Genetic diversity e fitness in black-footed ferrets before e during a bottleneck. *Journal of Heredity*, **93**, 231-237.

Wright SJ, Gompper ME, DeLeon B (1994) Are large predators keystone species in neotropical forest? The evidence from Barro Colorado Island. *Oikos*, **71**, 279-294.

Van Zyll de Jong CG (1987) A phylogenetic study of the Lutrinae (Carnivora; Mustelidae) using morphological data. *Canadian Journal of Zoology*, **65**, 2536-2544.