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Short Communication

Cytogenetic study of the giant otter *Pteronura brasiliensis* Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae)

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Abstract

The giant otter, *Pteronura brasiliensis* Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae), was widely distributed in South America but stable populations are now only found in the Pantanal and Amazon regions and the species is classified as endangered. There is only one recognized species of giant otter, although two subspecies of doubtful value have also been cited in the literature. We present the first karyotype of four captive *P. brasiliensis* specimens, all of which posses 2n = 38 chromosomes as 14M+8SM+6ST+8A and one pair of sexual chromosomes. An heteromorphic secondary constriction, associated with the nucleolar organizer region (NOR), was seen on the long arms of chromosome pair 17. The C-banding technique revealed heterochromatin in the centromeric region of all the chromosomes and the NOR was C-banding positive. The giant otter presented the same diploid number as most mustelids, although its karyotype is quite species-specific.

Key words: Amazon, aquatic mammals, ariranha, karyotype.

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There are five Amazonian aquatic mammals, the Amazonian manatee *(Trichechus inunguis*), the pink dolphin (*Inia geoffrensis*), the tucuxi (*Sotalia fluviatilis)*, the Neotropical otter (*Lontra longicaudis*) and the giant otter (*Pteronura brasiliensis*). The World Conservation Union (IUCN) classifies the Amazonian manatee and the pink dolphin as vulnerable and the tucuxi and the neotropical otter as belonging to the "data deficient" category, while the giant otter is classified as an endangered species (IUCN, 2006).

Riverine habitats are highly vulnerable to anthropogenic activities, this being especially true in the Amazon basin, which is suffering rapid development leading to degradation and loss of essential habitats for giant otters (Borobia and Rosas, 1991; Rosas, 1994; Carter and Rosas, 1997).

The giant otter, *Pteronura brasiliensis* Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae), is the largest of all otters and is endemic to South America, with historical records of sightings from north to south-central South America. However, currently stable populations are limited to the Amazon basin and upper Pantanal in the Paraná-Paraguay river basin (Carter and Rosas, 1997). Two *P. brasiliensis* subspecies have been cited in the literature but are of doubtful value (Duplaix, 1980).

Karyotypic studies have been carried out to analyze intra- and interspecific and intra- and interpopulation variations as well as to identify cryptic species, mutagenic effects and to solve taxonomic problems (Guerra, 1988; Macgregor, 1993). Nie *et al.* (2002) used chromosomal rearrangements, a rare type of genomic change, to investigate some systematic questions relating to the genome phylogeny of the domestic cat, red panda and five mustelid species. However, while cytogenetic studies have previously been carried out on other mustelid species (Freitas *et al.*, 1975; Kurose *et al.*, 2000; Graphodatsky *et al.*, 2002) there have been no published studies on the chromosomal constitution of *P. brasiliensis*.

In this paper we describe for the first time the karyotype of *P. brasiliensis*, providing valuable data to verify the validity of the alleged subspecies in future studies using specimens from the Pantanal region.

We carried out chromosomal analyses on two male and two female giant otters from the Brazilian Amazon basin held in captivity at the Aquatic Mammals Laboratory of the National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia, INPA), Manaus, Amazonas, Brazil (Figure 1). The giant otters were sedated with 1.93 ± 0.57 mg kg^{-1} of Zoletil® (Virbac, Brazil) and

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Figure 1 - Amazonian giant otter (*Pteronura brasiliensis*) held in captivity at the Aquatic Mammals Laboratory of the Brazilian National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia, INPA), Manaus, Amazonas (AM), Brazil.

blood samples collected from the femoral vein using an heparinized syringe which was maintained in a vertical position for 30 min after collection to allow the lymphocytes to separate. After separation, the lymphocyte layer was added to Complete Medium for Karyotyping (Cultilab, Campinas, SP) and the cultures incubated for 72 h at 37 °C. One hour before the end of incubation, 0.3 mL of colchicine at 0.0125% (w/v) was added. The cells were harvested by centrifugation at 1000 rpm for 10 min and 10 mL of hypotonic 0.075 M KCl solution at 37 °C was progressively added in 2 mL aliquots at 10 min intervals, after which the cells were fixed using four changes of Carnoy fluid (methanol/acetic acid 3:1 v/v). The methodology was modified from the protocol described by Verma and Babu (1995). Constitutive heterochromatin was detected using C-banding (Sumner, 1972) and the nucleolar organizer regions (NORs) by silver nitrate staining (Ag-NORs) as described by Howell and Black (1980). The chromosomes were identified by the arm ratio criteria (Levan *et al.*, 1964) where the metacentric (M), submetacentric (SM) and subtelocentric (ST) were considered as bi-armed chromosomes and the acrocentric (A) as one-armed chromosomes. In each group, the chromosomes were arranged in order of decreasing size.

The four giant otters examined showed a diploid number of 38 chromosomes, of which 36 were autosomes (14 metacentric + 8 submetacentric + 6 subtelocentric + 8 acrocentric) and two were sex chromosomes (Figure 2a and b). The fundamental number of autosomes (FNa) was 64. The C-banding technique identified positive centromeric heterochromatin in all the chromosomes, with chromosomes 8, 10 and 13 also showing telomeric heterochromatin (Figure 2c). Chromosome pair 17 showed a nucleolar region which was C-banding positive (Figure 2c) and corresponded to a secondary constriction in the proximal region of the long arms of this chromosome (Figure 2d).

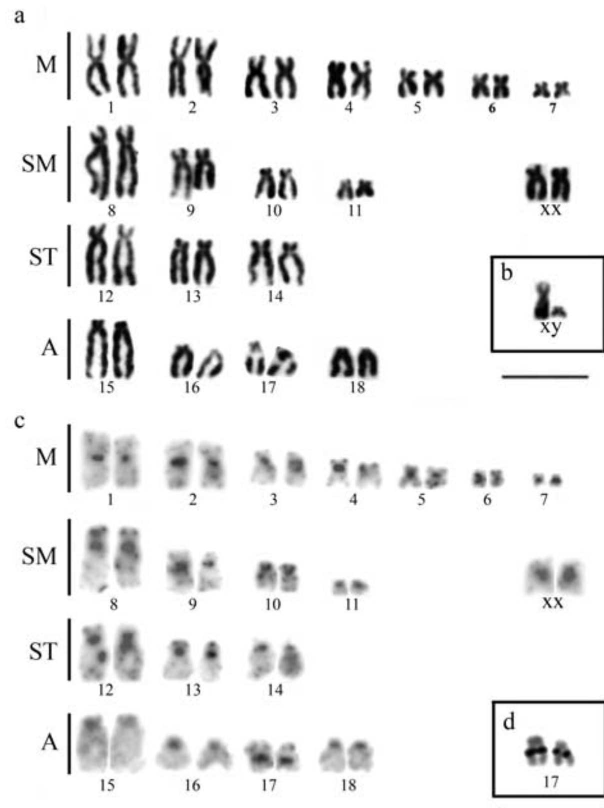

Figure 2 - Karyotype of *Pteronura brasiliensis*: (a) female and (b) sex chromosomes of male standard staining; (c) C-banding and (d) Nucleolar pair stained with silver nitrate (Bar = 10 μm).

The diploid chromosome number in Mustelidae ranges from 30 in *Mustela vison* (Graphodatsky *et al.*, 2000) to 44 in *Mustela altaica* (Graphodatsky *et al.*, 1976), *Mustela ermina orientalis* and *Mustela mustela anakuma* (Obara, 1991). However, in more than 60% of mustelids the diploid number is 2n = 38 (Table 1). Although mustelid karyotypes are conserved in regard to diploid number they present considerable differences in chromosome structure, indicating that several rearrangements occurred during the evolution of this group (Couturier and Dutrillaux, 1986). Sex chromosomes also differ in morphology (M, SM, ST, A) and size (large, medium, small), but the Y chromosome is always the smallest.

According to Graphodatsky *et al.* (2002), the differentiation mustelid karyotypes from the common ancestor probably occurred by means of several chromosomal rearrangements (*e.g.* centric fusions and fissions, addition of heterochromatin, pericentric inversions) which caused the large karyotype variability observed in the Mustelidae family. Comparative analysis of the chromosomal segments in mustelids and out-group species revealed 18 putative conserved autosomal segments, which are probably ancestral chromosomes or chromosome arms (Graphodatsky *et al.*, 2002).

Table 1 - List of the chromosome number of 22 mustelid species.

Species	Karyotype					Reference
		Autosomes		Sex chromosomes		
	Diploid number (2n)	Number of autosomic arms (FNa)	Karyotype formula	X	Y	
Amblonyx cinerea	38	64	–	sm	a	Couturier and Dutrillaux, 1986
Ictonyx striatus	38	–	–	–	–	Graphodatsky *et al.*, 2002
Lontra longicaudis	38	62	8m+18sm+10a	sm	sm	Freitas *et al.*, 1975
Martes foina	38	68	6m+28sm-st+4a	–	–	Yigit *et al.*, 1998
M. melampus melampus	38	68	10m+6sm+16st+4a	m	a	Obara, 1991
M. melampustsuensis	38	68	10m+6sm+16st+4a	m	a	Tsuchiya, 1979
M. zibellina brachyura	38	66	10m+6sm+14st+6a	m	a	Iwasa and Hosoda, 2002
M. flavigula	40	68	–	m	st	Nowak, 1991
Meles meles anakuma	44	62	20m-sm-st+22a	m	st	Obara, 1991
Melogale moschata	38	–	–	–	–	Nowak, 1991
Mustela vison	30	–	–	–	–	Graphodatsky *et al.*, 2000
M. eversmanni	38	–	–	–	–	Graphodatsky *et al.*, 1976
M. itatsi	38	64	14m +14sm-st + 8a	sm	a	Kurose *et al.*, 2000
M. lutreola	38	58	–	sm	m	Graphodatsky *et al.*, 1976
M. nivalis namiyei	38	66	18m+12sm-st+6a	m	sm	Obara, 1991
M. sibirica	38	58	14m + 8sm-st + 14a	sm	–	Kurose *et al.*, 2000
M. putorius	40	64	–	sm	m	Graphodatsky *et al.*, 1976
M. nivalis nivalis	42	74	10m+24sm-st+6a	m	sm	Obara, 1991
M. altaica	44	–	–	–	–	Graphodatsky *et al.*, 1976
M. erminea orientalis	44	64	6m +16sm-st+20a	m	st	Obara, 1991
Pteronura brasiliensis	38	64	14m+8sm+6st+8a	sm	sm	Present paper
Vormela peregusna	38	68	10m+22sm+4a	sm	–	Ozkurt *et al.*, 1999

a = acrocentric, m = metacentric; sm = submetacentric; st = subtelocentric. A dash (–) indicates that data is unavailable.

Our results show that the *P. brasiliensis* karyotype is very similar to the karyotype of the Japanese weasel, *Mustela itatsi* (Kurose *et al.*, 2000), since both species present the same karyotypic formulae, a secondary constriction on the long arm of chromosome pair 17 and a submetacentric X chromosome. However, it is clear that the *P. brasiliensis* and *M. itatsi* karyotypes are species-specific since, among other factors, the Y chromosome is submetacentric in *P. brasiliensis* and acrocentric in *M. itatsi*.

The mustelids are also very diverse in regard to the C-banding pattern, with some presenting several heterochromatic short arms while others only show heterochromatic centromeric regions (Freitas *et al.*, 1975; Kurose *et al.*, 2000). We found that *P. brasiliensis* showed constitutive heterochromatin blocks at the centromeric region of all chromosomes and on the telomeric region of some of chromosomes. This pattern is totally different from that of the neotropical otter *Lontra longicaudis* which belongs to the same subfamily (Lutrinae) (Freitas *et al.*, 1975).

The karyotype and chromosome constitution of the giant otter described in this paper could provide useful information to clarify the situation regarding the existence of the *P. brasiliensis* subspecies mentioned in the literature (Duplaix, 1980), if similar chromosomal studies are carried out with giant otters from the Pantanal region.

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