ID Artigo: 21-07
envio: 04/12/2007
Diagramador: Rodrigo
cubomultimidia publicações
e-mail: bjb@infocentral.com.br

Identification of pumas (*Puma concolor* (Linnaeus, 1771)) through faeces: a comparison between morphological and molecular methods

Miotto, RA.[a]*, Ciocheti, G.*[b]*, Rodrigues, FP.*[a] *and Galetti Jr., PM.*[a]

[a]Departamento de Genética e Evolução, Universidade Federal de São Carlos – UFSCar, Rodovia Washington Luís, Km 235, CEP 13565-905, São Carlos, SP, Brazil

[b]Instituto de Biociências, Universidade de São Paulo – USP, São Paulo, SP, Brazil

*e-mail: remiotto@yahoo.com.br

Received May 31, 2006 – Accepted October 23, 2006 – Distributed December 1, 2007

(With 2 figures)

Pumas (*Puma concolor*) occur in low densities and possess habits that hinder observation (Ernest et al., 2000). Often, the presence of these animals may be confirmed through the identification of their faeces, avoiding the elevated costs of capture and its possible damages (Wayne and Morin, 2004). Faecal analysis allows the monitoring of populations, their movements (Kohn and Wayne, 1997; Taberlet et al., 1997; Prugh et al., 2005), and provides diet-related information (Farrel et al., 2000).

Traditionally, the identification of the species that originally deposited the faeces is based on the deposition site, size, odor, and tracks associated with faeces, although these characteristics may be subjective or inconsistent (Becker and Dalponte, 1999). Body size can vary greatly within a species, and an individual can leave scats in a broad range of sizes (Farrell et al., 2000). In addition, puma and ocelot (*Leopardus pardalis* (Linnaeus, 1758)) faeces may also present similar sizes (Miotto et al., 2007), a condition that increases the chance of these species to be misidentified.

Complementary methods for faecal identification are important tools in works based on this kind of material. We compared two methods to identify the species to which sample faeces collected in field belong: analysis of faecal DNA and analysis of guard hairs contained in the faeces. As part of a self-cleaning behavior, felines often extract their own hairs with the tongue and swallow them (personal observation). Once isolated from faeces, guard hair analysis represents an alternative to identify the species that originally deposited faecal samples.

Another possibility is by analyzing DNA content, considering that few grams of faeces contain DNA from thousands of intestinal mucosal cells from the individual that made the deposit (Albaugh et al., 1992). In the last decade, a large number of population studies have been based on faecal DNA analysis (Taberlet et al., 1997; Wasser et al., 1997; Palomares et al., 2002; Riddle et al., 2003; Deagle et al., 2005; Prugh et al., 2005).

From October 2004 to August 2005, we collected 32 faeces of supposed pumas in two protected cerrado areas in Southeastern Brazil: Vassununga State Park (approximately 21° 41' S and 47° 34' W) and Jataí Ecological Station (approximately 21° 51' S and 47° 82' W). Feline faeces are segmented, with tapered ends, and a characteristic odor, aspects that differ them from the faeces of other large carnivores (Chame, 2003). Of the 32 collected faeces, 12 were dissolved by rainwater or extremely deteriorated by the action of insects and were discarded from the analyses. We placed the samples in sterile preservative-free plastic tubes without any conservative solution and kept at –22 °C in the laboratory until DNA extraction. As a control during the DNA amplification reactions, we used blood samples from pumas (*Puma concolor* and *Puma yagouaroundi* (É. Geoffroy Saint-Hilaire, 1803)) and ocelots (*Leopardus pardalis*), species found in the studied areas (Talamoni et al. 2000). Both DNA and guard hair analyses were done independently by two distinct researchers in such a manner that one did not know the results from the other.

We extracted faecal DNA using a QIAmp DNA Stool Mini Kit (Quiagen) according to the manufacturer's recommendations and used phenol/chloroform/isoamylic alcohol (Sambrook et al., 1989) for DNA extraction of blood samples.

We amplified a 146 base pairs (bp) fragment of the cytochrome b gene from the mitochondrial genome via polymerase chain reaction (PCR) using primers designed by Farrel et al. (2000). We compared the obtained sequences with GenBank reference sequences from pumas and other felids that are also found in the sampled regions (Miotto et al., 2007). Of the 20 analyzed samples, 12 amplified the mitochondrial DNA fragment - ten from pumas and two from ocelots (Figure 1).

For the guard hair analysis, we determined the cuticular pattern by means of impression of the hair surfaces and compared it to a reference collection (Quadros, 2002). Since the medular pattern (large medula with fimbriated edges) is similar among all species belonging to the Felidae group (Quadros, 2002), we carried out only the cuticular pattern analysis from the hair shaft. This guard hair microstructure analysis found the same results obtained by DNA analysis. We submitted the remaining eight samples that did not amplify the mitochondrial DNA only to the microstructure analysis of the guard hair, and all were identified as belonging to pumas.

The cuticular patterns observed in Felidae are petal, diamond petal, or regular waved (Quadros, 2002). For pumas, we found the transversally waved pattern, i.e., scales with a waved contour and disposed transversally

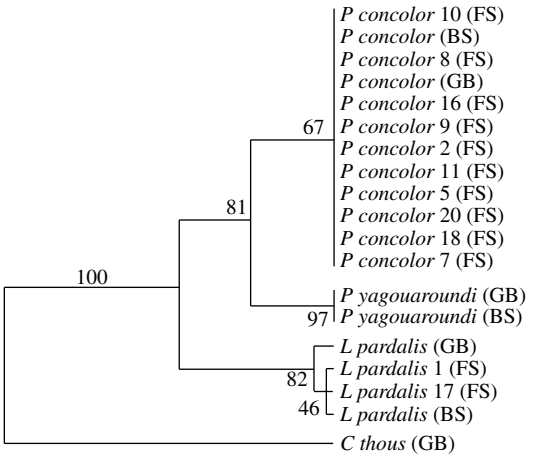

Figure 1. Cytochrome b neighbor-joining tree of the reference sequences and sequences from the analyzed faecal samples. *Cerdocyon thous* is an outgroup; *Puma yagouaroundi* and *Leopardus pardalis* are other felids present in the study area. The bootstrap values, based on 1,000 randomizations, are shown on the branches. The FS and BS nomenclature refers to faecal and blood samples, respectively; GB refers to the other sequences obtained at GenBank (access numbers DQ469940 - DQ469953, DQ790891).

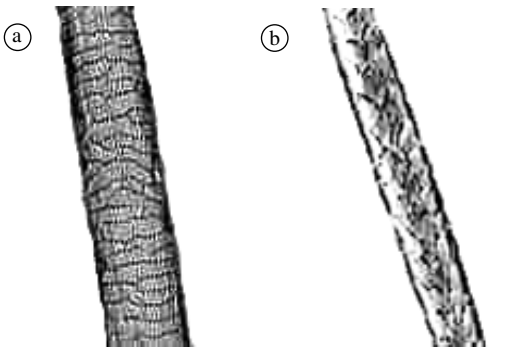

Figure 2. Photomicrography of the cuticular patterns from hair shaft of (a) *Puma concolor* and (b) *Leopardus pardalis* obtained from faeces collected in field (200x increase).

to the hair shaft (Figure 2a). For ocelots, we found hairs with the intermediary petal cuticular pattern (Quadros, 2002), with imbricated and wider scales (Figure 2b).

The confirmation of the presence of two ocelot faeces among those supposedly belonging to pumas indicated that only field experience is not enough to tell them apart, pointing out the need of complementary methods that may aid their identification.

The guard hair microstructure study proved to be a useful tool in the identification of faecal samples. The guard hairs are long hairs that stick out in the fur with a mechanoreceptor function, or even the hairs whose individual coloring produces the general color pattern of the pelage (Quadros, 2002). Thus, we emphasize that the simple observation of characteristics such as hair color and size could generate incorrect results, due to a large fur variation according to the geographical distribution and season of the year (personal observation).

In contrast, the genetic identification of field-collected faeces becomes practical when there are doubts regarding identification of the guard hairs, when they are scarce, or when there is not a good reference collection. It is mostly efficient when individual identification is aimed (Miotto et al., 2007).

Non-invasive analyses represent a new possibility to populational study and monitoring of large carnivores with elusive habits. Our results indicate that the analyses of the DNA and guard hair found in the faeces are efficient and, when used together, may provide higher accuracy in identification of faeces collected in the field.

Acknowledgments – The authors thank CENAP/IBAMA and the Associação Mata Ciliar for supplying the blood samples and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

References

ALBAUGH, GP., IYENGAR, V. and LOHANI, A., 1992. Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. Int. J. Cancer, vol. 52, p. 347-350.

BECKER, M. and DALPONTE, JC., 1999. *Rastros de mamíferos silvestres brasileiros.* Brasília, DF, Editora UnB. 180p.

CHAME, M., 2003. Terrestrial mammal feces: a morphometric summary and description. *Mem. I. Oswaldo Cruz*, vol. 98, p. 71-94.

DEAGLE, BE., TOLLIT, DL., JARMAN, SN., HINDEL, MA., TRITES, AW. and GALES, NJ., 2005. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Mol. Ecol.*, vol. 14, p. 1831-1842.

ERNEST, HB., PENEDO, MCT., MAY, BP., SYVANEN, MS. and BOYCE, WM., 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Mol. Ecol.*, vol. 9, p. 433-441.

FARREL, LE., ROMAN, J. and SUNQUIST, ME., 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Mol. Ecol.*, vol. 9, p. 1583-1590.

KOHN, MH. and WAYNE, RK., 1997. Facts from feces revisited. *Trends Ecol. Evol.*, vol. 12, p. 223-227.

MIOTTO, RA., CIOCHETI, G., RODRIGUES, FP. and GALETTI JR, PM., 2007. Determination of the minimum population size of pumas (*Puma concolor*) through faecal DNA analysis in two protected cerrado areas in the Brazilian southeast. *Biotropica*, vol. 39, p. 647-654.

PALOMARES, F., GODOI, JA., PIRIZ, A., O'BRIEN, SJ. and JOHNSON, WE., 2002. Fecal genetic analysis to determinate the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. *Mol. Ecol.*, vol. 11, p. 2171-2182.

PRUGH, LR., RITLAND, CE., ARTHUR, MA. and KREBS, CJ., 2005. Monitoring coyote population dynamics by genotyping faeces. *Mol. Ecol.*, vol. 14, p. 1585-1596.

QUADROS, J., 2002. *Identificação microscópica de pêlos de mamíferos brasileiros e sua aplicação no estudo da dieta de carnívoros.* 127 p. (Tese de Doutorado) – Universidade Federal do Paraná, Curitiba, PR.

RIDDLE AE., PILGRIM, KL., MILLS, LS., MCKELVEY, KS. and RUGGIERO, LF., 2003. Identification of mustelids using mitochondrial DNA and non-invasive sampling. *Conserv. Genet.*, vol. 4, p. 241-243.

SAMBROOK, J., FRITSCH, EF. and MANIATIS, T., 1989. *Molecular Cloning: A Laboratory Manual*. Cold Springs Harbor, NY, Cold Springs Harbor Laboratory Press. 1859p.

TABERLET, P., CAMARRA, JJ., GRIFFIN, S., HANOTTE, O., WAITS, LP., DUBOI-PAGANON, C., BURKE, T. and BOUVET, J., 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.*, vol. 6, p. 869-876.

TALAMONI, SA., MOTTA JUNIOR, JC. and DIAS, MM., 2000. Fauna de mamíferos da Estação Ecológica de Jataí e da Estação Experimental de Luiz Antônio. In SANTOS, JE. and PIRES, JSR. (eds.). *Estudos integrados em ecossistemas, Estação Ecológica de Jataí.* vol. 1. São Carlos, Editora Rima, p. 317-329.

WASSER, SK., HOUSTON, GM., CADD, GG. and FAIN, R., 1997. Techniques for application of fecal DNA methods to field studies of Ursids. *Mol. Ecol.*, vol. 6, p. 1091-1097.

WAYNE, RK. and MORIN, PA., 2004. Conservation genetics in the new molecular age. *Front. Ecol. Environ.*, vol. 2, p. 89-97.