The Use of Molecular Tools in Biodiversity Studies

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Molecular Markers in Biodiversity Studies

1. Inventories of biodiversity
   - Systematics, species delimitation
   - Species identification
     - DNA barcoding

2. Geographic distributions, community composition, habitat association, diet, pathogen/parasite/symbiont interactions

3. Demographic and genetic monitoring of species and populations

4. Phylogeography, population structure and demographic history
   - Definition of units for conservation

5. Behavioral ecology (social structure, dispersal patterns)

6. Forensics

7. Adaptation and natural selection (including at the genomic level)
Ongoing projects: carnivore phylogeny, phylogeography, population genetics and molecular ecology
Different Markers for Different Tasks …

1. Deep phylogenetics
2. Shallow phylogenetics and phylogeography
3. Species identification
4. Population genetics
5. Parentage/kinship analyses
6. Individual identification
7. Sexing
8. Pathogen identification
9. Analysis of genes involved in immune response
10. Analysis of genes involved in potentially adaptive phenotypes
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Evolutionary History of Neotropical Carnivores

Meta-analysis of molecular phylogenies and divergence dates derived from nuclear gene supermatrices.

- Contrasting patterns of diversification among carnivoran families.

Opportunity to investigate recent speciation processes and test species-delimitation criteria for carnivores.

Eizirik (in press)
Eizirik (in press)
Distributions of *L. tigrinus*, *L. geoffroyi* e *L. colocolo*
Sampling for genetic analyses (mtDNA e STRs)

mtDNA Control Region

Brazilian populations of *L. tigrinus* may have mtDNA lineages from three distinct species!
Bayesian analysis of microsatellite genotypes for *L. geoffroyi* and *L. tigrinus*
Expanded sampling

- $\Delta$ **Leopardus tigrinus**
- • **Leopardus geoffroyi**
- • **Leopardus colocolo**
mtDNA haplotype network

L. tigrinus clade

L. geoffroyi clade

L. guigna

L. pardalis

L. wiedii

L. colocolo clade

L. tigrinus (C, NE Brazil)
Haplotype network (intron 2, PLP1 gene, X chromosome)
Haplotypic network (intron 2, *PLP1* gene, X chromosome)

*Haplotype network (intron 2, PLP1 gene, X chromosome)*

- **L. tigrinus** (S, SE Brazil)
- **L. guigna**
- **L. pardalis** + **L. wiedii**

Diagram showing haplotype network with labels for each haplotype and their geographic distribution.
Haplotype network (intron 2, ZFY and SMCY3 genes, Y chromosome)

$L. tigrinus$
(S, SE Brazil)

$L. Geoffroyi$

$L. colocolo$

$L. wiedii$
Genus *Lycalopex*

- *L. culpaeus*
- *L. griseus*
- *L. gymnocercus*
- *L. sechurae*
- *L. vetulus*
- *L. fulvipes*

Tchaicka *et al.* (in review)
Genus *Lycalopex*

**Phylogeny:**
mtDNA control region (588 bp)

Neighbor-Joining

0.005 substitutions/site

Tchaicka *et al.* (in review)
Genus *Lycalopex*

ML phylogeny for 3 concatenated mtDNA segments (2.5 kb)

- **L. culpaeus**
- **L. fulvipes**
- **L. griseus**
- **L. gymnocercus**
- **L. sechurae**
- **L. vetulus**

0.005 substitutions/site

M. Favarini *et al.* (in prep)
L. vetulus and L. gymnocercus in Brazil
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Development of molecular tools for carnivore species identification from faecal DNA

Felis catus numt (~7.9 Kb)

Panthera numt (~12.5 Kb)

Carnivora mtDNA (~16.7 Kb)

5’  ND5  ND6  CYTB  CR  12S  16S  ND1  ND2  COI  COII  ATP8  ATP6  3’

MINICOI (187 bp)

COI barcode region (658 bp)

ATP6 (126 bp)

ATP8

A (160 bp)
B (385 bp)
C (307 bp)
D (110 bp)

E (421 bp)
F (500-1000 bp)

CYTB
Development of molecular tools for carnivore species identification from faecal DNA

- bPon56 (faecal samples of Panthera onca in alcohol)
- bPon55 (faecal samples of Panthera onca in DET)
- bPon306 (faecal samples of Panthera onca)
- bPon307 (faecal samples of Panthera onca)
- bPon56 (faecal samples of Panthera onca - 98 mg)
- bPon32 (blood samples of Panthera onca)
- bPon56 (faecal samples of Panthera onca in DET)
- P3-16 (fur of Panthera onca)
- bPon305 (faecal samples of Panthera onca)
- P3-2 (hair of Panthera onca)
- bPon56 (faecal samples of Panthera onca - 48 mg)
- bPon24 (blood samples of Panthera onca)
- P31-1 (hair of Panthera onca)

- F2-636 (faecal samples of large cat)
- Puma concolor (blood)
- L.V. (faecal samples of large cat)
- F57-1C (faecal samples of large cat)
- F2-635 (faecal samples of large cat)

Haag et al. 2009
Identification of carnivores using short DNA segments

Goal: global standardized system for carnivore identification.

Application in ecological, biogeographic and forensic studies.

Ongoing tests in multiple field sites
Brazil (RS, ES, MS, MT, TO, MG, DF), Argentina, Namibia.

P.B. Chaves et al., in prep.
What do wild cats eat?

Leopardus tigrinus

Leopardus geoffroyi

L. colocolo
Dietary analysis of *L. tigrinus* and *L. geoffroyi* using stomach contents of roadkilled animals

Trigo et al. (in review)
Dietary analysis of *L. tigrinus* and *L. geoffroyi* using stomach contents of roadkilled animals: DNA barcoding of prey items
Akodon azarae 06VV
CE006 | L. tigrinus | bLti143
Akodon azarae 07VV

Oxymycterus sp. LC186
Oxymycterus sp. YL30
Oxymycterus sp. MBML2062
Oxymycterus sp. YL513
CE062 | L. tigrinus | bLti137
CE066 | L. tigrinus | NE313
Oxymycterus nasutus Oxy001
Oxymycterus nasutus 20VV
Oxymycterus nasutus Oxy065
Oxymycterus nasutus Oxy057
Oxymycterus nasutus 28VV
Oxymycterus nasutus 21VV
Oxymycterus nasutus 23VV

Cavia magna RS005
CE010 | L. tigrinus | bLti149 | 1
Cavia magna CM003

Rattus norvegicus AJ428514
Rattus norvegicus DQ673916
Rattus norvegicus DQ673917
CE059 | L. tigrinus | bLti069
Rattus rattus EF186584
Rattus rattus gi225622213
Rattus rattus EF186585

Sooretamys angouya LC12
CE016 | L. tigrinus | bLti068
CE017 | L. tigrinus | bLti068
CE056 | L. tigrinus | bLti160
CE061 | L. tigrinus | bLti001
Sooretamys angouya 9790

H. V. Figueiró et al. (in prep.)
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Maned wolf conservation biology

Integrating ecology, genetics, health sciences and education programs
Molecular ecology of the Neotropical otter (*Lontra longicaudis*)

Cristine Trinca
Molecular ecology of the Neotropical otter (*Lontra longicaudis*)

Cristine Trinca

10 sampling campaigns (2 years)
Individual identification / sexing

Nuclear molecular markers (microsatellites/SRY)

26 individuals
Forqueta Stream – 9 individuals
Forqueta Stream – 9 individuals

3.13 km
Forqueta Stream – 9 individuals

- Indiv. 2
- Indiv. 7

5,21 km
Forqueta Stream – 9 individuals

- Indiv. 2
- Indiv. 7
- Indiv. 3

0.7 km
Forqueta Stream – 9 individuals

- Indiv. 2
- Indiv. 7
- Indiv. 3
- Indiv. 9

3.2 km
Forqueta Stream – 9 individuals
Forqueta Stream – 9 individuals
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Conservation genetics of fragmented jaguar populations


Sampling

- UE Porto Primavera (SP/MS) (1-6) → n = 23
- P. E. do Morro do Diabo (SP) (7) → n = 8
- P.E. das Várzeas Rio Ivinhema (MS) (8-9) → n = 10
- Corredor verde (Brasil/Arg/Paraguai) (11-21) → n = 18
Population structure: Identification of migrants and admixed individuals

M. Diabo
Ivinhema
Porto Primavera

Strong evidence of substantial genetic differentiation among sites.
Molecular evidence for recent gene flow among these them.

Conclusions

- Surprising result given the geographic proximity of the field sites (ca. 69 km to 500 km) and the dispersal ability of this species

- Recent isolation (30-40 yrs, ca. 6-8 generations)

- Small effective size and restricted gene flow among areas

- Comparative data from other species

**Strong impact of human-induced genetic drift in remnant jaguar populations**
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Using scat DNA to predict a jaguar’s coat color

MC1R

Jaguar

MC1R - Δ15

Eizirik et al. 2003.
Genotyping of the jaguar *MC1R* deletion

Complete association (p<0.001)

Eizirik et al. 2003.
Molecular tracking of jaguar melanism using faecal DNA

Taiana Haag · Anelisie S. Santos · Fernanda P. Valdez · Dênis A. Sana · Leandro Silveira · Laury Cullen Jr. · Carlos De Angelo · Ronaldo G. Morato · Peter G. Crawshaw Jr. · Francisco M. Salzano · Eduardo Eizirik
Mapping jaguar melanism

L. Gonçalves

- Integration of different data types.

- Test of association with landscape variables.

Testing of adaptive hypotheses.
**Acknowledgements**

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**Support:** CNPq, CAPES, FAPERGS, NIH, NSF, FNMA, CESP, WCS, WWF, CVRD, PETROBRAS, PUCRS