

DNA barcoding of South American mammals

Eduardo Eizirik

Lab. Biologia Genômica e Molecular, PUCRS, Brasil
Instituto Pró-Carnívoros, Brasil
Laboratory of Genomic Diversity, NIH, EUA



Applications of DNA Barcoding in Biodiversity Conservation

1. Gathering data on components of native biodiversity
 - 1.1. Baseline data
 - 1.2. Monitoring of biodiversity in impacted areas
2. Gathering data on threats to native biotas
 - e.g. invasive species, pathogens, wildlife trafficking
3. Helping to enforce actions aimed at curbing threats to biodiversity
 - e.g. wildlife forensic analyses

Neotropical mammals

How DNA barcodes can help the study and conservation of Neotropical mammals:

1. Improved reliability of surveys
 - Academic-oriented (e.g. geographic range refinements)
 - Environmental impact assessments
2. Ecological studies
 - Habitat association
 - Diet
 - Parasite interactions
 - Monitoring of species occurrence
3. Forensics and law enforcement applications

The effort towards Large-scale DNA barcoding of Neotropical mammals

History:

Precursor:

2005 – Beginning of the Brazilian DNA barcoding network

- Large-scale inventory of Brazilian biodiversity

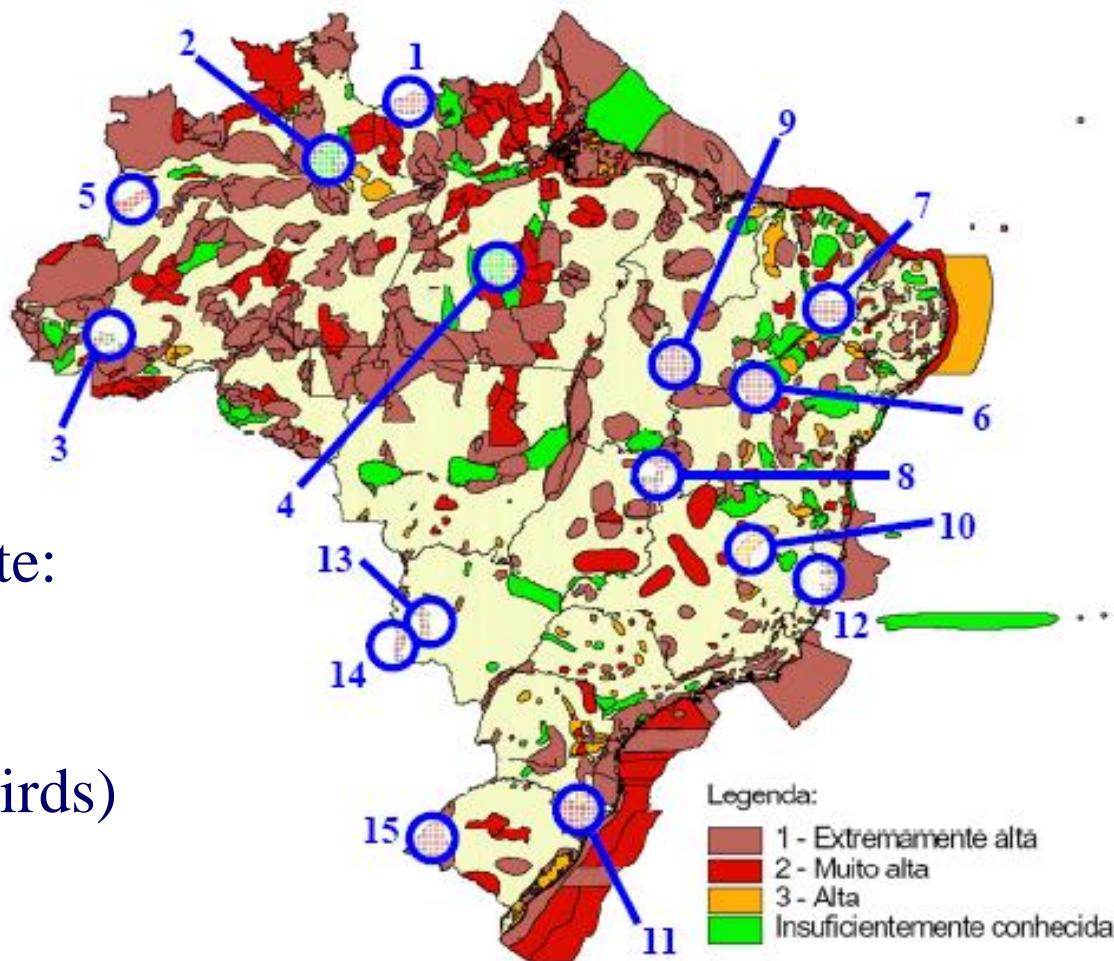
- Multi-center project designed in 2005 to boost taxonomic research in Brazil using the DNA barcode concept as a catalyst to integrate field collections, museum-based biodiversity research, genome center networks and bioinformatics advances.

Large-scale inventorying of Brazilian biodiversity (2005)

Sampling strategy

15 sites:

- 10,000 samples/site:
 - fish
 - amphibians
 - reptiles (incl. birds)
 - mammals
 - spiders
 - Leguminosae



Large-scale inventorying of Brazilian biodiversity

6 museums

- Involved in coordinating collection trips, curation of sampled specimens and their morphology-based analysis

14 Centers of Molecular Biodiversity:

- Network of genome centers and molecular biology laboratories performing high-throughput sequencing of DNA barcodes from 150,000 samples;

Bioinformatics tools: online management of data collection, analysis and integration (e.g. morphology, DNA barcodes, geography)

Training activities: courses, workshops.

The effort towards Large-scale DNA barcoding of Neotropical mammals

History:

2007 – Regional CBOL meeting in Campinas, Brazil

- Mammals emerge as a CBOL-endorsed regional priority for DNA barcoding.
- Several members of the 2005 network confirm participation, while additional partner institutions join.

The effort towards Large-scale DNA barcoding of Neotropical mammals

History:

2007 – present

Activities:

1. Talks, symposia and/or planning meetings during subsequent regional, national and international congresses:
 - 2007, 2008 – Brazilian Congress of Genetics
 - 2008 – Brazilian Congress of Zoology, Brazilian Congress of Mammalogy, Regional Genetics Meeting (RS)
 - 2009 – International Mammalogical Congress, Argentina
2. Funding proposals: CYTED (2007), PROSUL (2007, 2008).

PROSUL project proposal – 2008 (excerpt)

Goal:

To consolidate an international network whose aim is generate, analyze and apply an integrated data base of DNA barcodes for South American mammals.

Official participants: 21 research institutions from six countries (Brazil, Uruguay, Chile, Bolivia, Peru and Venezuela).

- museum-based groups working on mammal systematics
- molecular genetics laboratories

Proposed tasks:

- (i) Training of students from multiple countries in the use of molecular tools for biodiversity studies;
- (ii) Coordination meeting comprising group leaders;
- (iii) Reagent costs for the initial phase of data collection.

PROSUL 2008 participating institutions

Brazil

PUCRS - Pontifícia Universidade Católica do Rio Grande do Sul
UFMG - Universidade Federal de Minas Gerais
UFRGS - Universidade Federal do Rio Grande do Sul
UFES - Universidade Federal do Espírito Santo
FZB/RS - Fundação Zoobotânica do Rio Grande do Sul
URI – Universidade Regional Integrada
FURB – Universidade Regional de Blumenau
UFPR –Universidade Federal do Paraná
UFRJ - Museu Nacional
INCA - Instituto Nacional de Câncer
FIOCRUZ - Fundação Oswaldo Cruz
UERJ - Universidade do Estado do Rio de Janeiro
USP - Instituto de Biociências
USP/ESALQ - Escola Superior de Agricultura Luiz de Queiroz
INPA - Instituto Nacional de Pesquisas da Amazônia
UFMS - Universidade Federal de Mato Grosso do Sul
UCB-DF - Universidade Católica de Brasília
UFPA - Universidade Federal do Pará
UFAM - Universidade Federal do Amazonas

PROSUL 2008 participating institutions

Uruguay

FC - Facultad de Ciencias (Enrique Lessa)

Chile

UC - Universidad de Chile (Angel Spotorno)

Venezuela

USB - Universidad Simon Bolivar (Marisol Aguilera)

Peru

UPCH - Universidad Peruana Cayetano Heredia (Jose R. Espinosa)

Bolivia

MNHN - Museo Nacional de Historia Natural (Julieta Vargas)

South American Network for Mammalian DNA barcoding

Regional Nodes

Brazil

1. Rio Grande do Sul – PUCRS/UFRGS

2. Espírito Santo – UFES

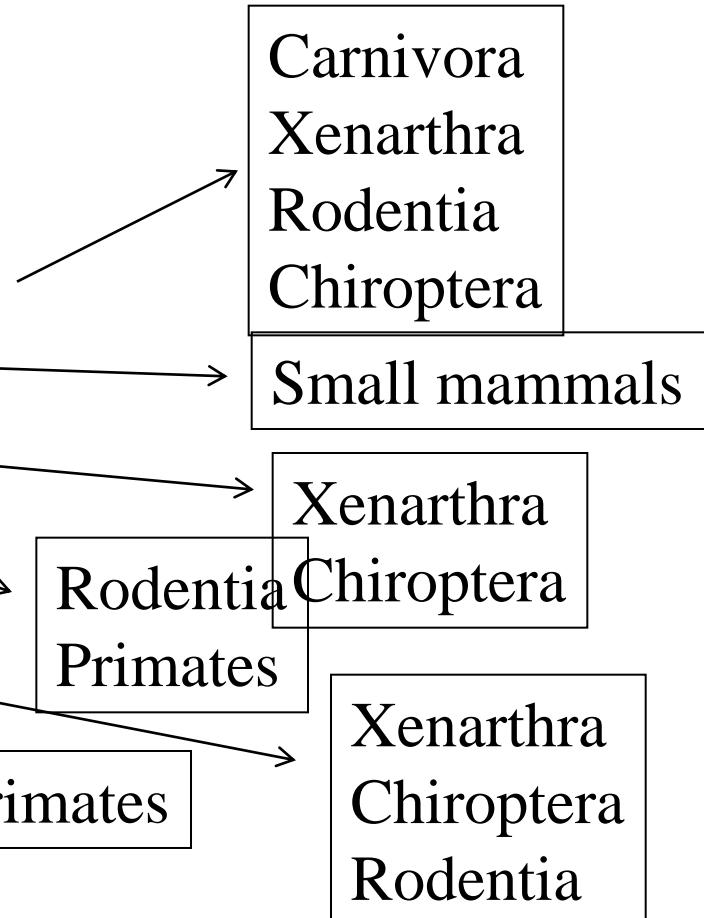
3. Minas Gerais – UFMG

4. Rio de Janeiro – INCA/UERJ

5. São Paulo - USP

6. Pará - UFPA

7. Goiás/Brasília - UFG



Uruguay

1. Universidad de la Republica

Species sampled so far:

Porto Alegre (PUCRS/UFRGS)

Xenarthra – 5 spp.

Carnivora – 23 spp.

→ Next targets: Cetacea, Primates

Rodentia – 43 spp.

Cetartiodactyla – 4 spp.

Chiroptera – 15 spp.

Total: 90 spp (~ 600 specimens)

Belo Horizonte (UFMG)

Xenarthra – 7 spp.

Chiroptera – 35 spp. (> 200 specimens)

Espírito Santo (UFES)

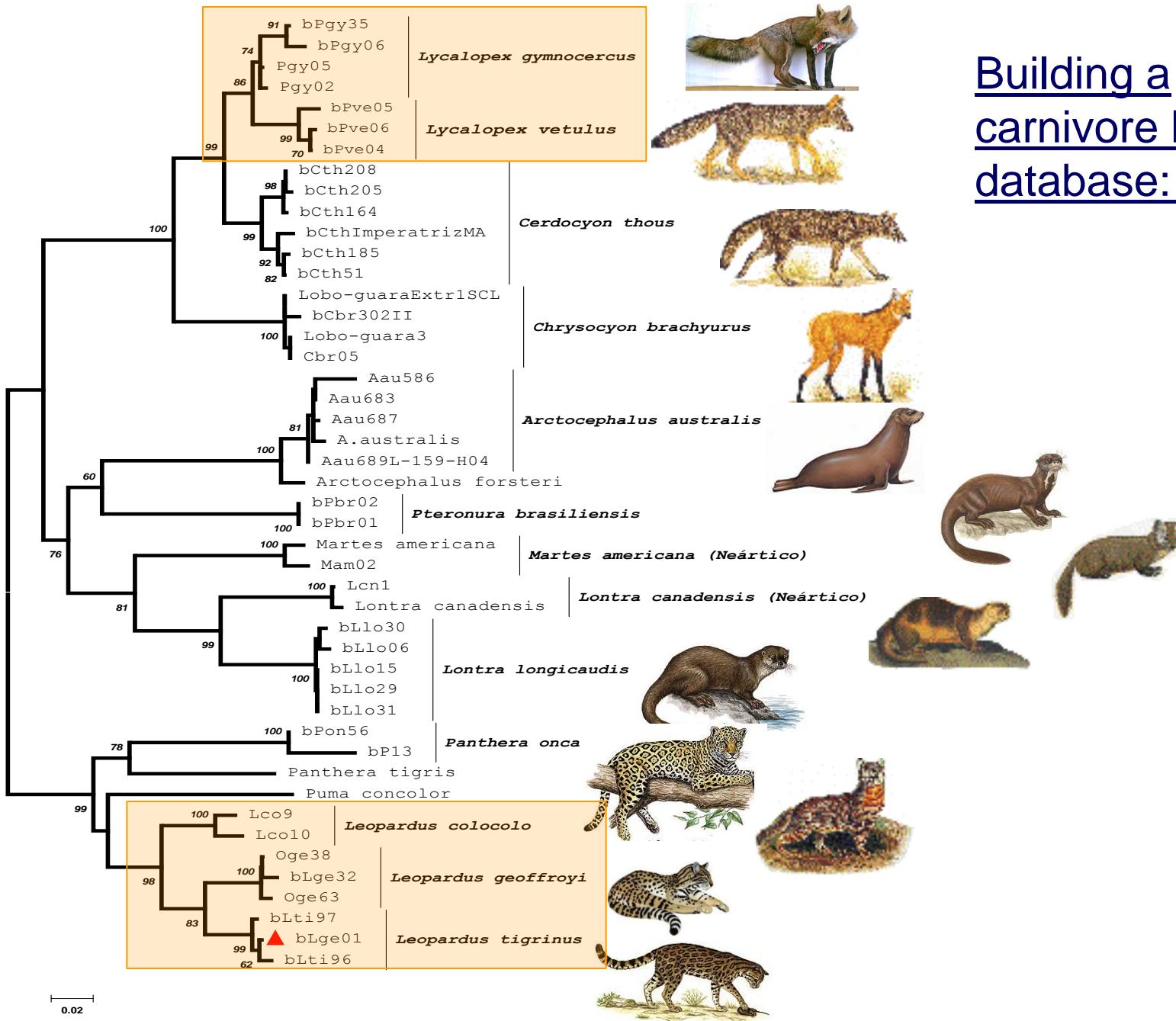
Rodentia, Didelphimorphia, Chiroptera – 85 spp. (259 specimens)

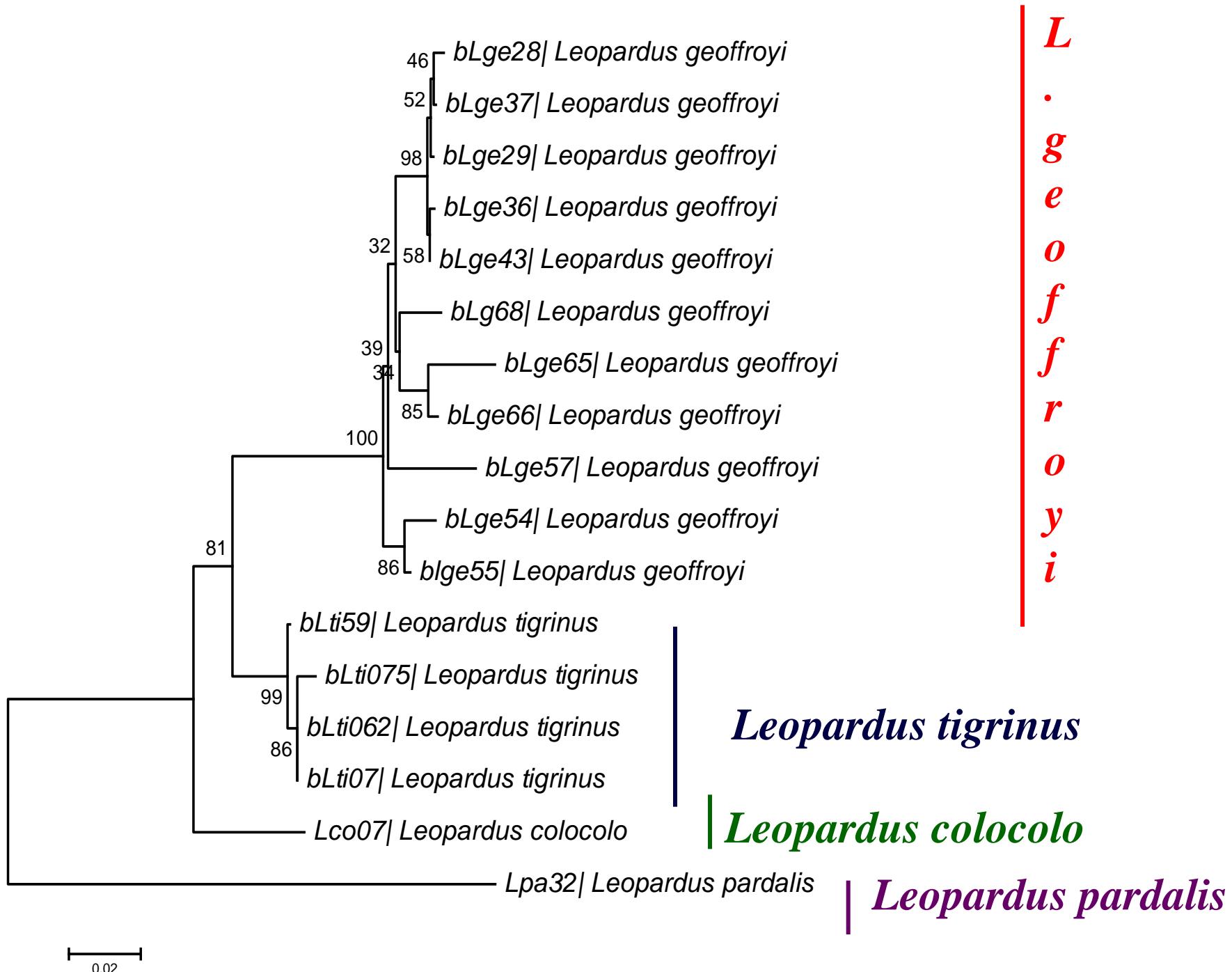
São Paulo (USP)

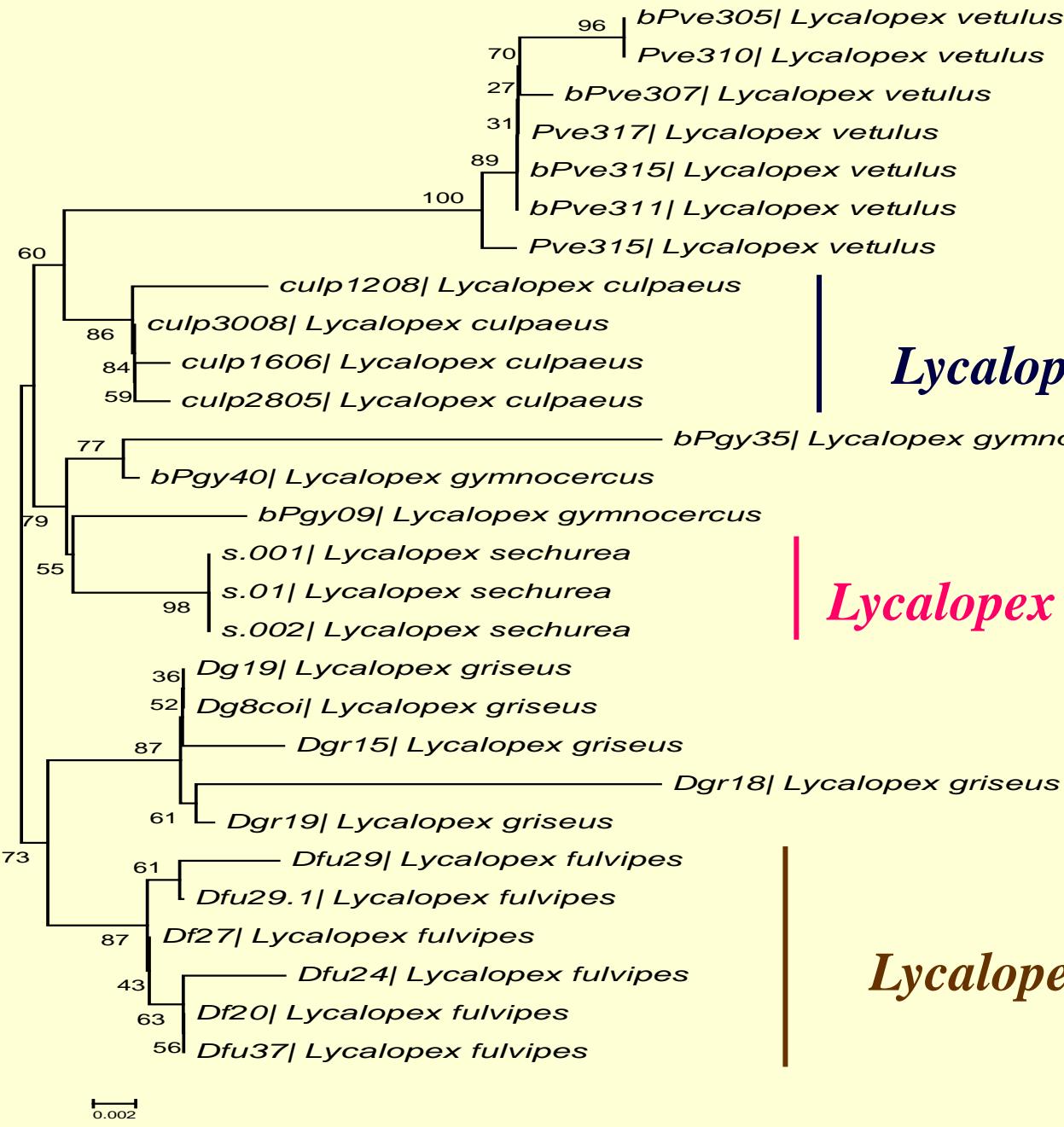
Xenarthra – 6 spp. (149 specimens) [N. Moraes-Barros]

→ Next targets: Chiroptera, Rodentia [F. Martins]

Building a carnivore barcode database: COI







Lycalopex vetulus

Lycalopex culpaeus

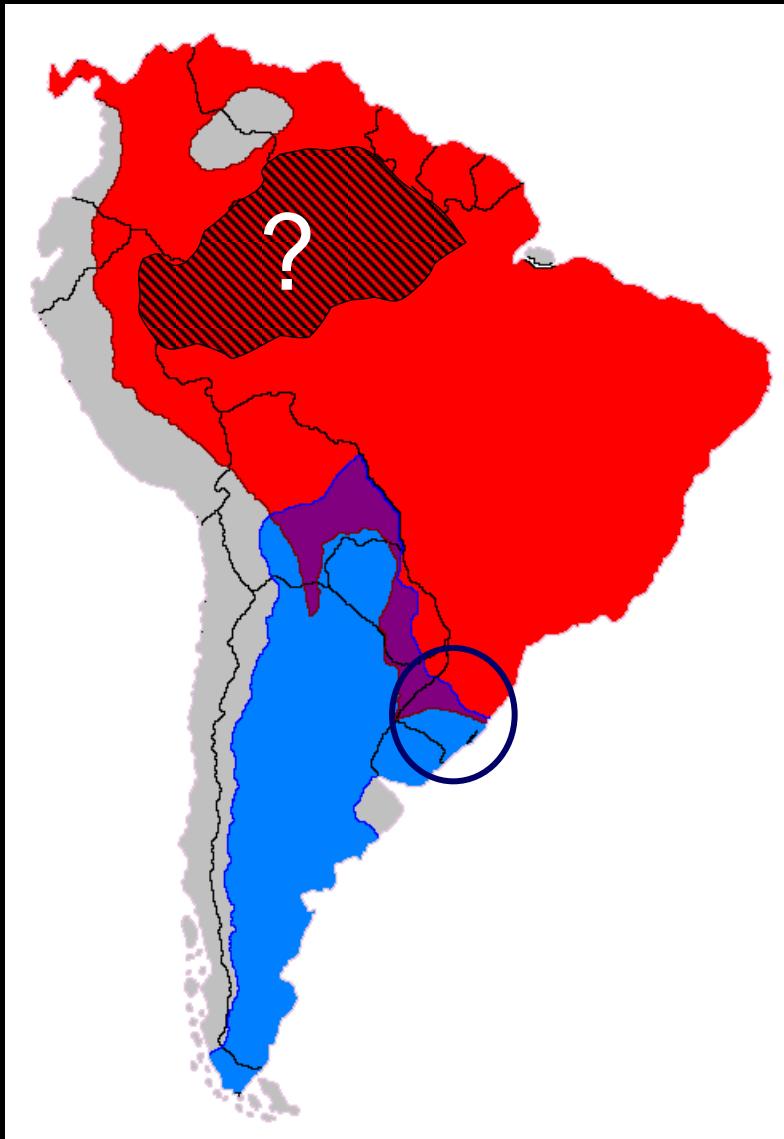
*Lycalopex
gymnocercus*

Lycalopex sechurae

Lycalopex griseus

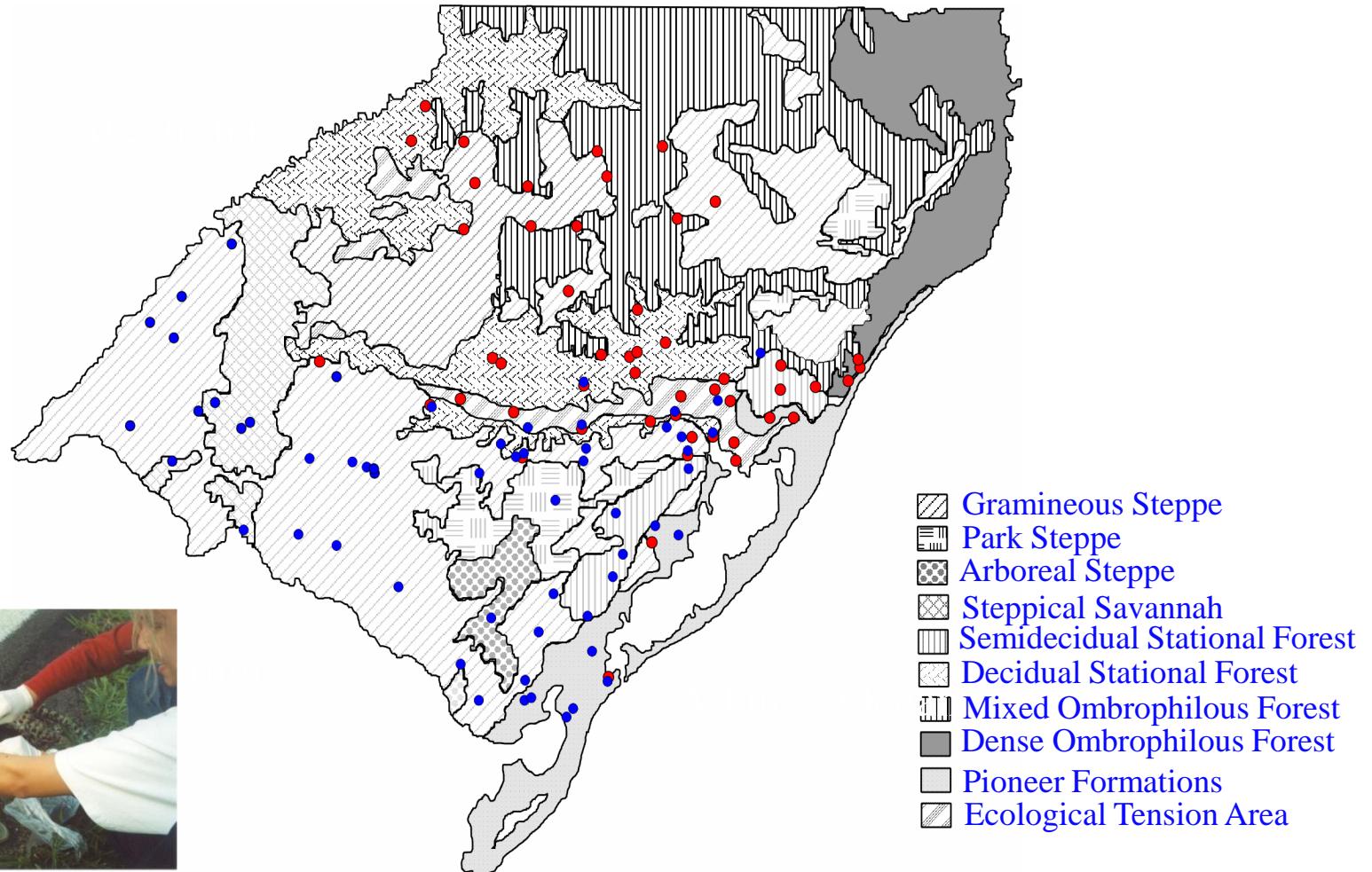
Lycalopex fulvipes

Evolutionary ecology of *L. tigrinus* and *L. geoffroyi*

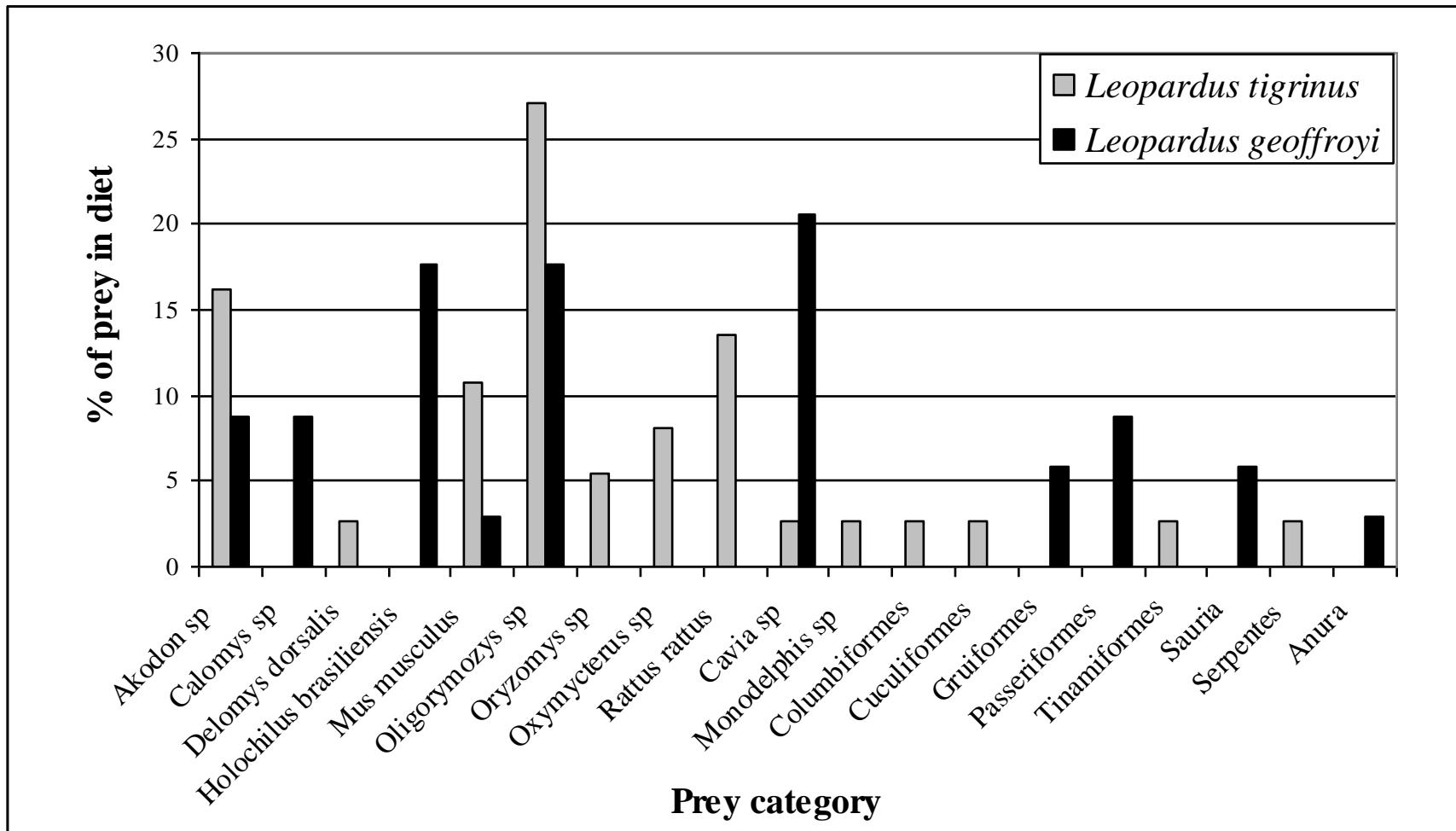




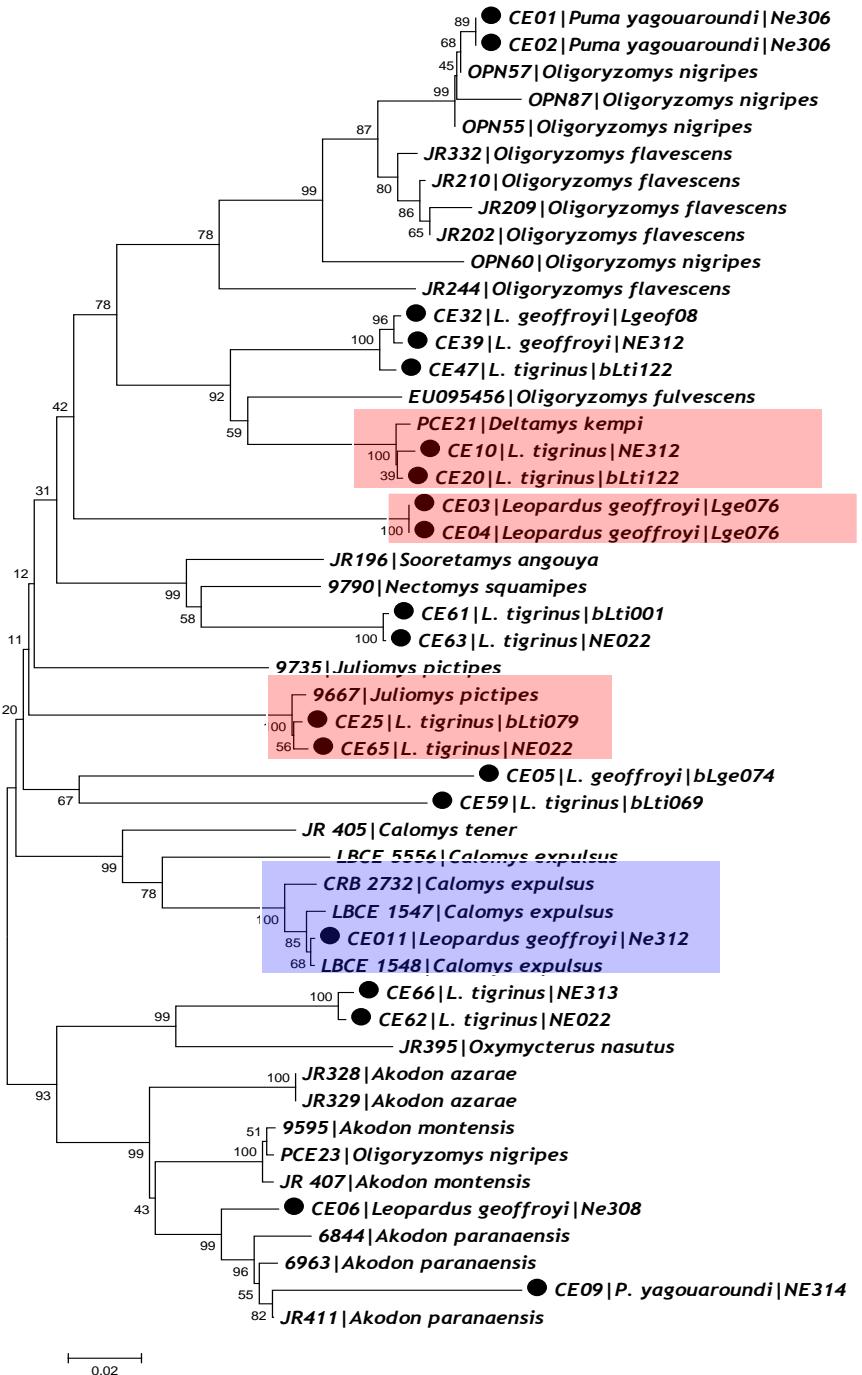
Habitat use of *L. tigrinus* and *L. geoffroyi* in RS state



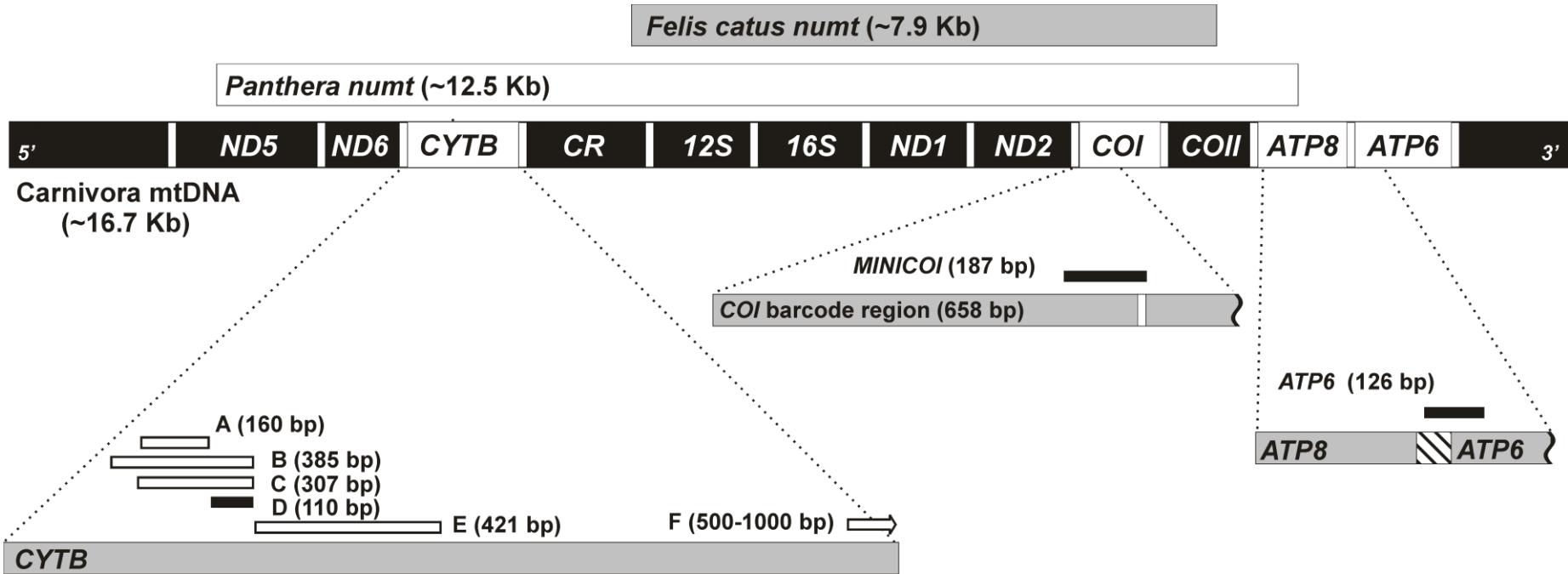
Dietary analysis of *L. tigrinus* e *L. geoffroyi* using stomach contents of roadkilled animals



Dietary analysis of *L. tigrinus* e *L. geoffroyi* using stomach contents of roadkilled animals: DNA barcoding of prey items

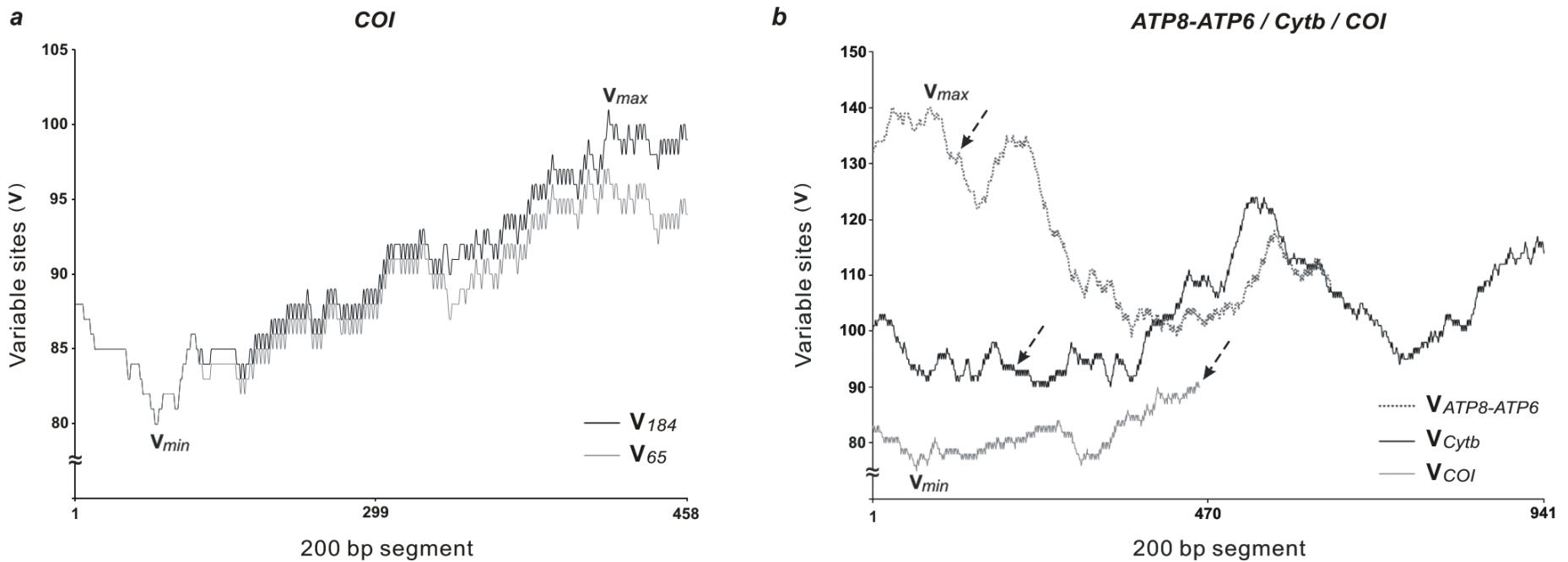


Development of molecular tools for carnivore species identification from faecal DNA



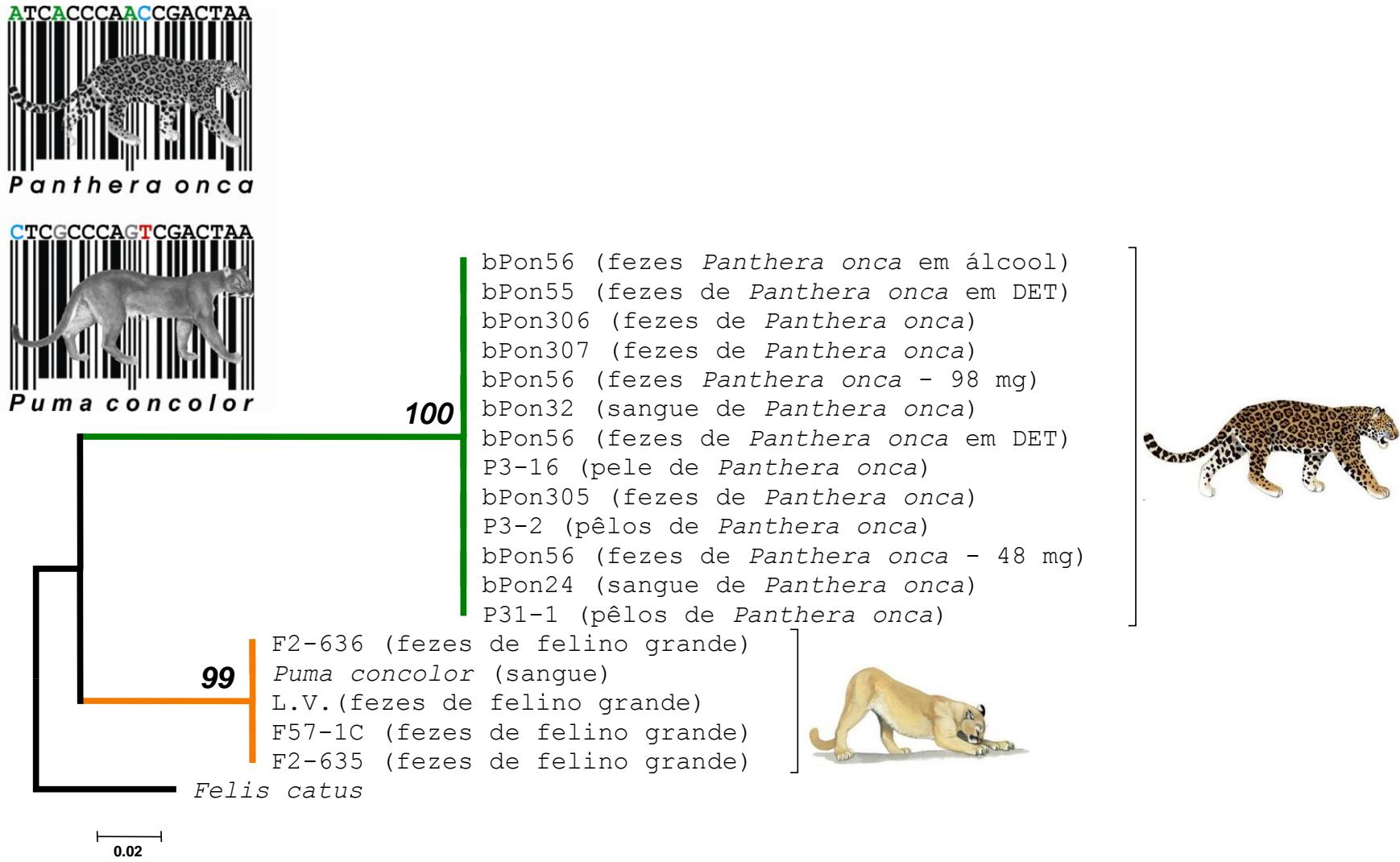
“Molecular scatology meets DNA barcoding”

Assessment of variability in mtDNA segments

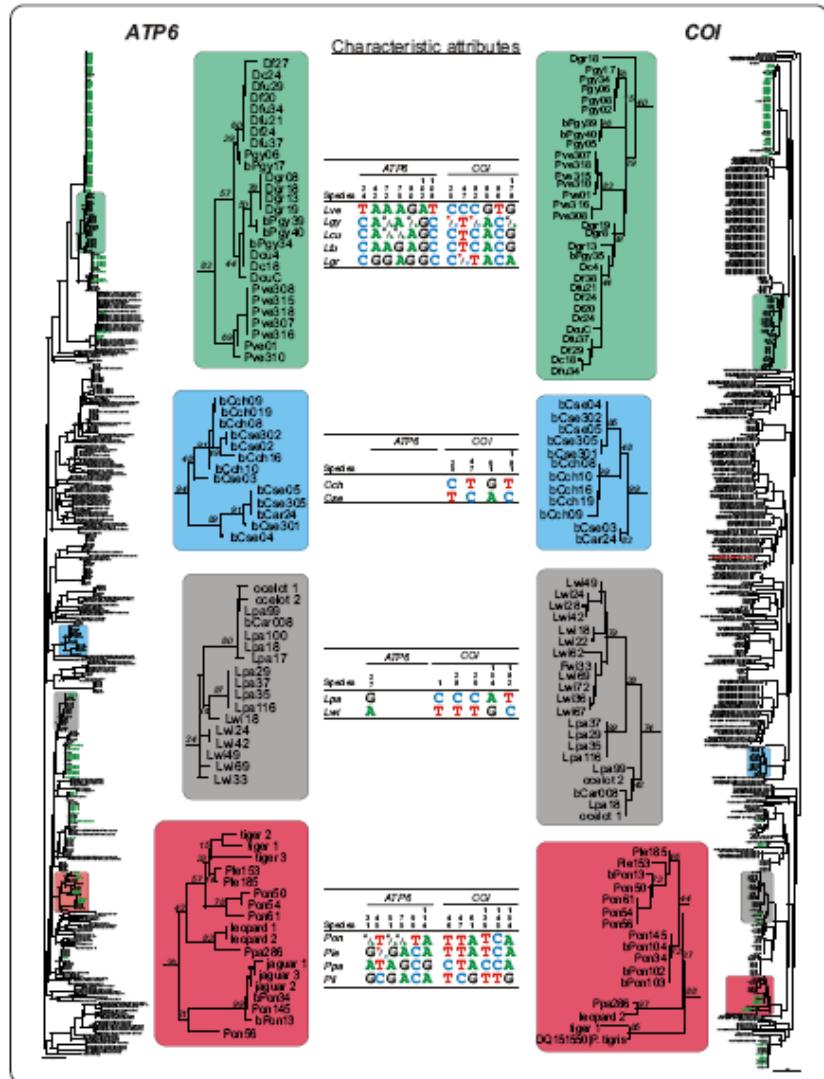


→ Generation of large data bases of carnivore sequences

Development of molecular tools for carnivore species identification from faecal DNA



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 in South America

Brazil (RS, ES, MS, MT, TO, MG, DF), Argentina.

Identification of carnivores using short DNA segments



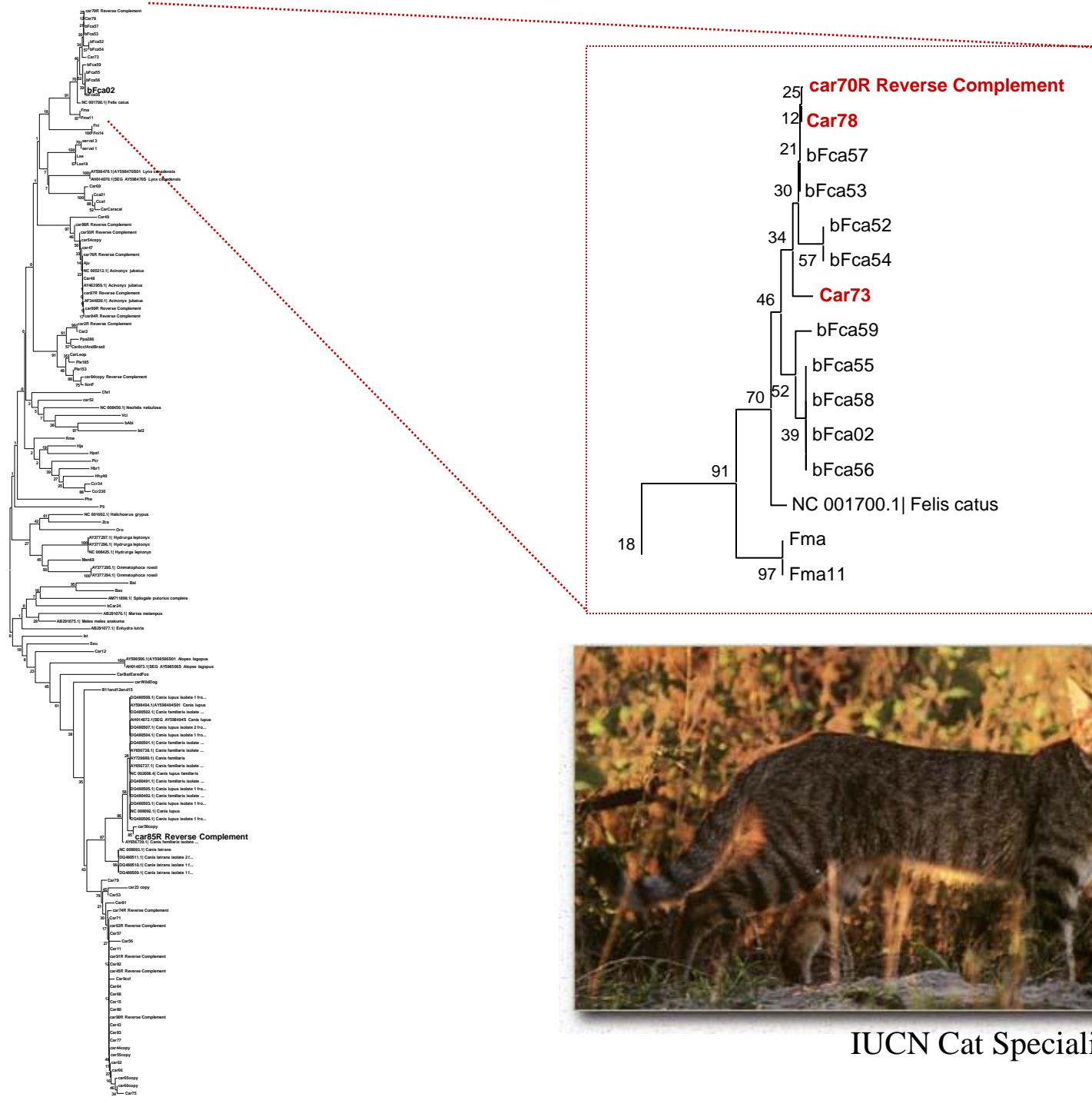
Ongoing surveys and ecological studies



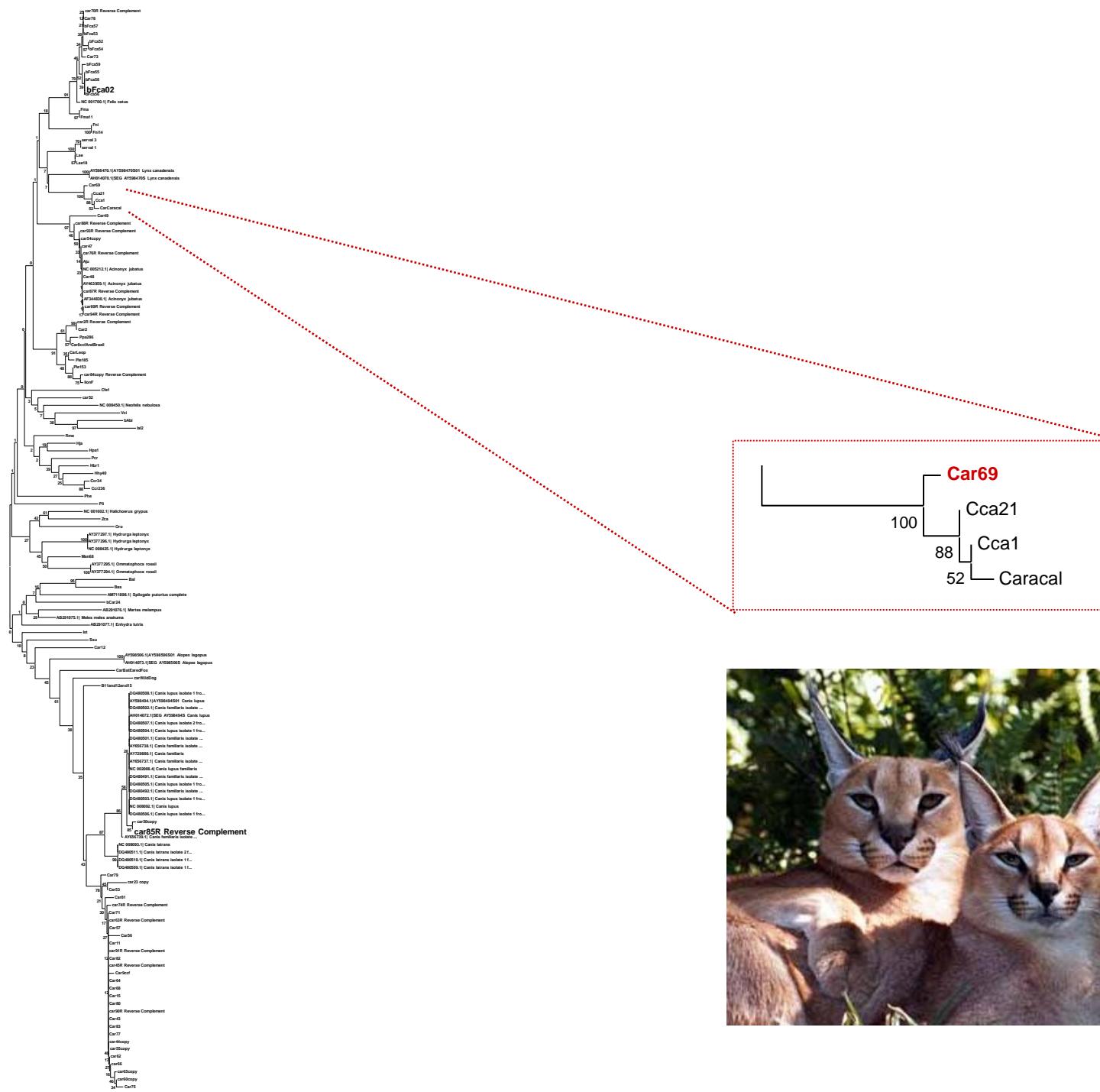
Identification of carnivores using short DNA segments

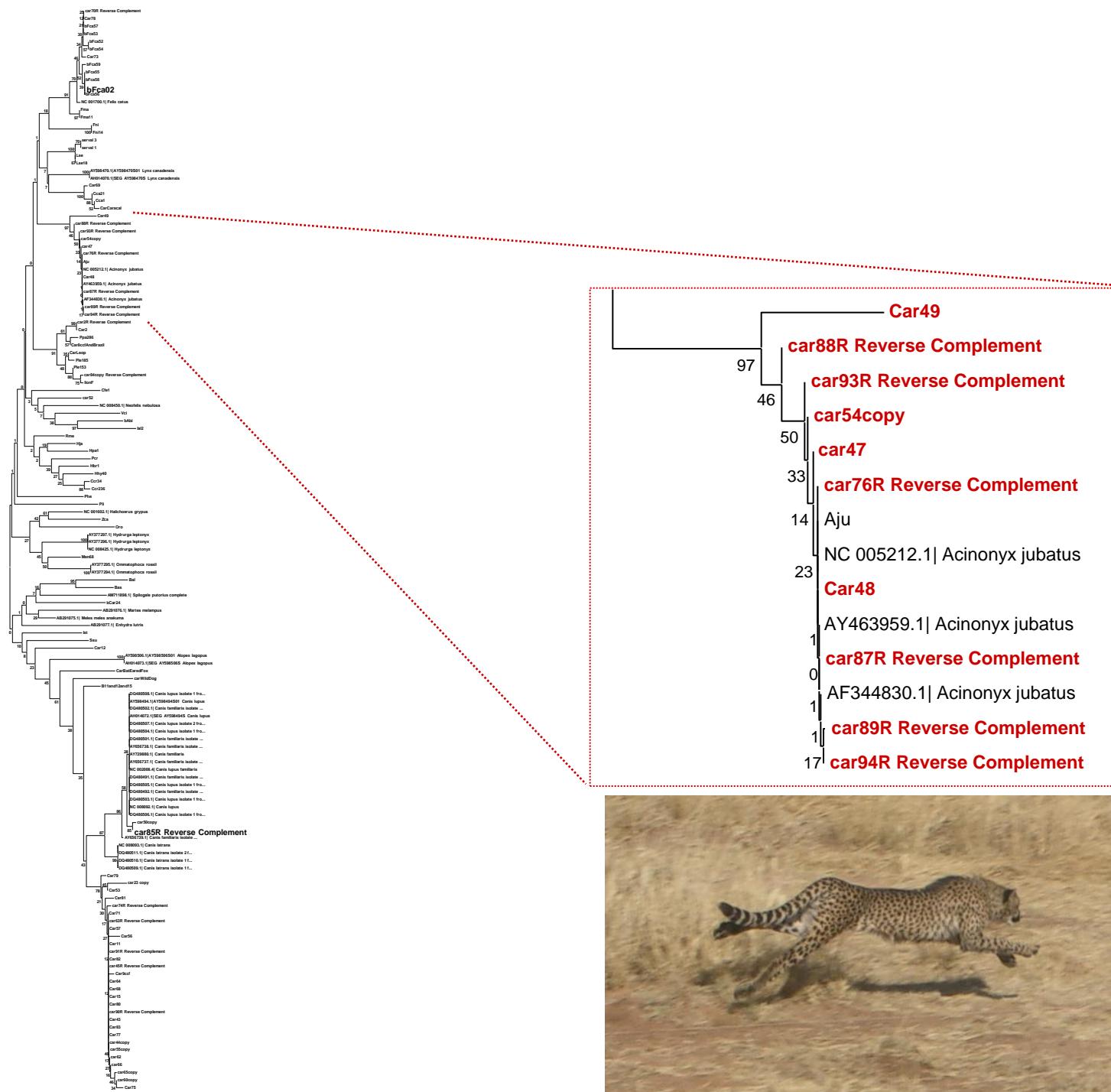
Testing and optimization of protocols for African carnivores:
Partnership with CCF, Namibia.

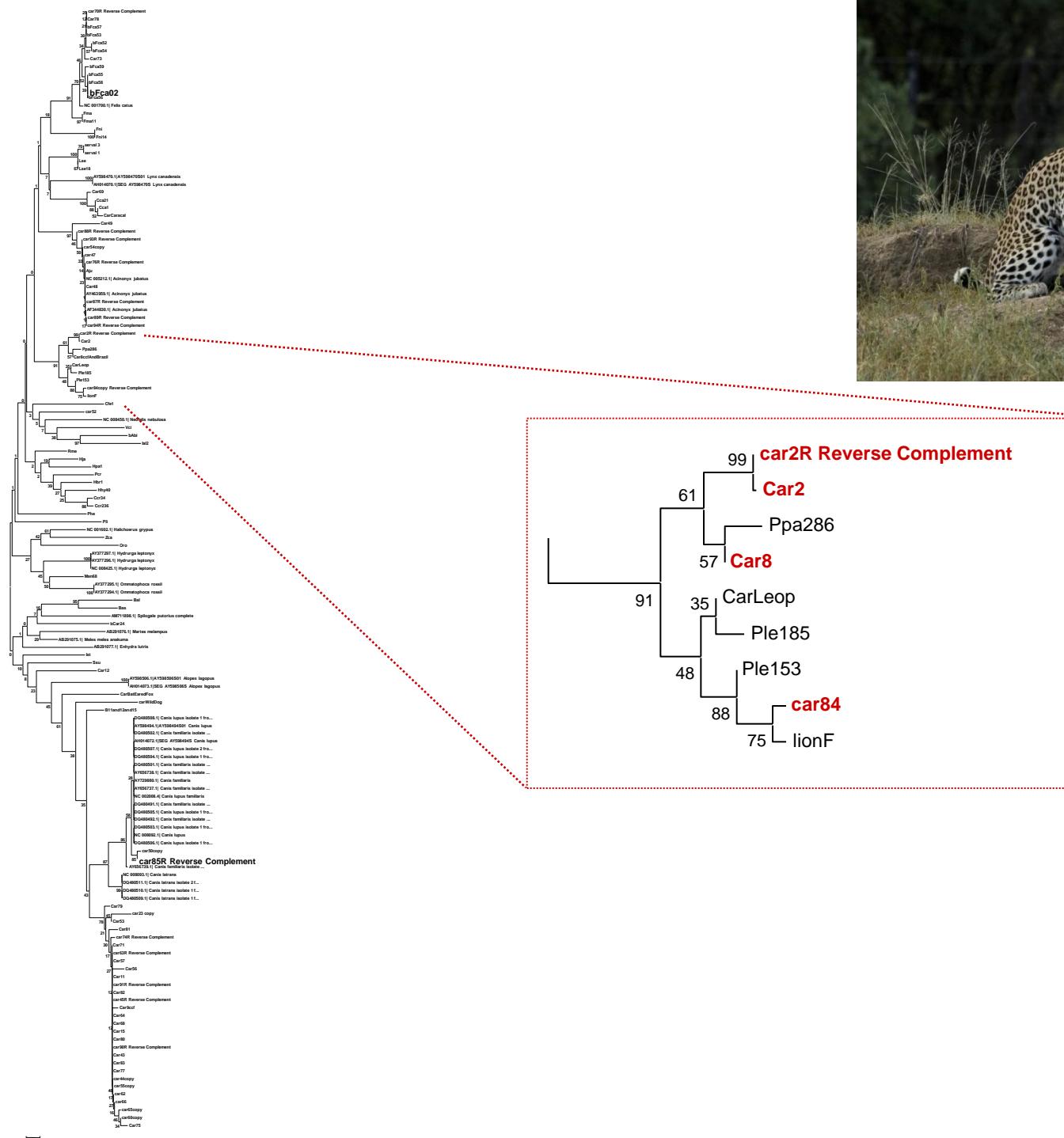


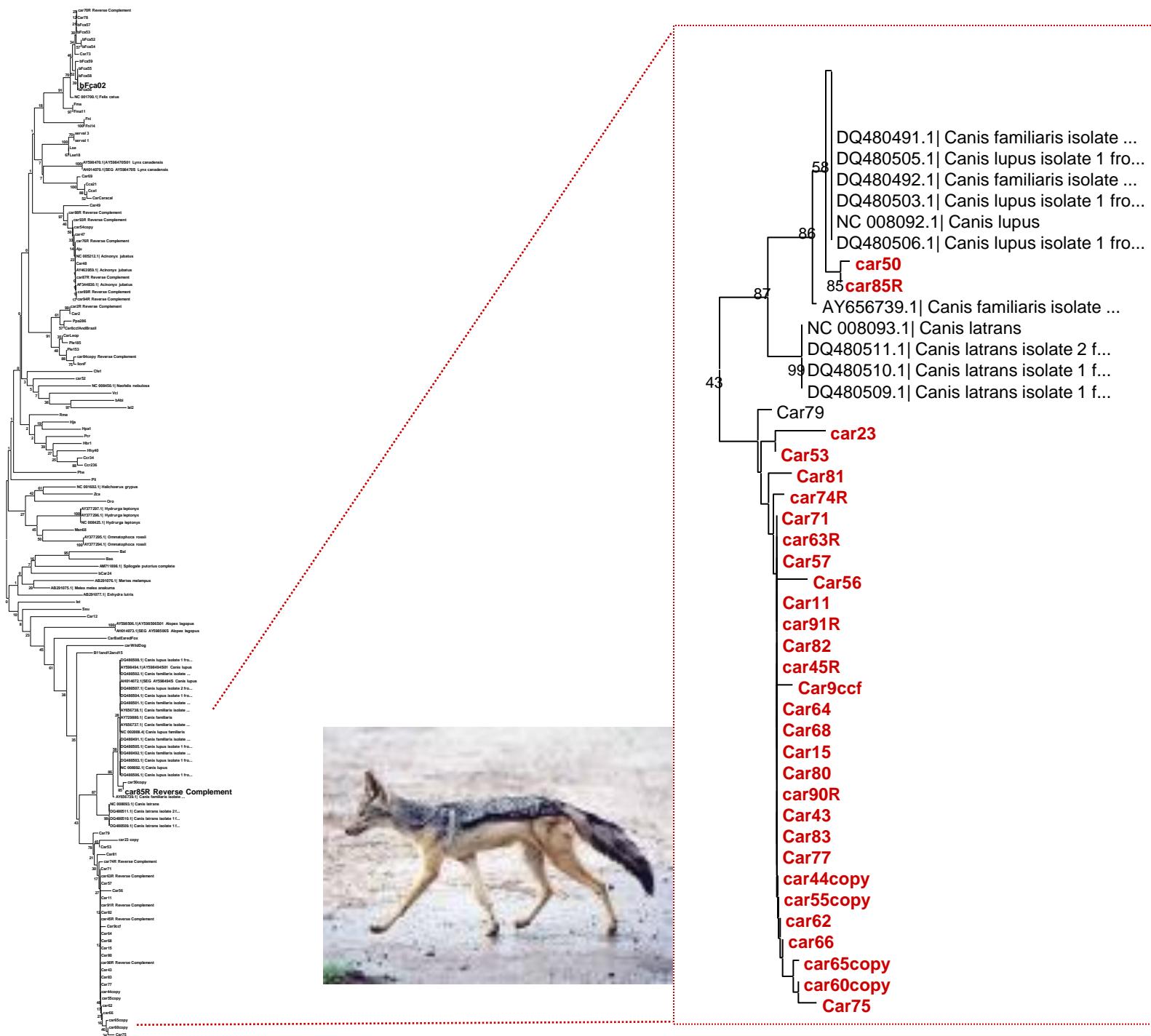


IUCN Cat Specialist Group









Current challenges for large-scale DNA barcoding of Neotropical mammals:

1. Samples with no vouchers (especially medium-sized and large species).
2. Museum vouchers with no tissue samples.
3. Unsampled species.
4. Poor geographic coverage for most species.
5. Taxonomically complex taxa (need for thorough revision).
6. Lack of manpower, lack of large-scale funding.

ACKNOWLEDGEMENTS:

Students

Henrique Figueiró
Marina Favarini
Fernanda Valdez
Ezequiel Fabiano
Flávia Tirelli
Cristine Trinca
Camila Jaeger
Paulo B. Chaves
Vanessa G. Graeff
Gabriel Macedo

Carnivore scat ID projects

Fernanda Michalski (Pró-Carnívoros)
Laurie Marker (CCF)
Anne Schmidt-Küntzel (CCF)

ACKNOWLEDGEMENTS:

Y. Leite, L. P. Costa, V. Fagundes, A. Ditchfield (UFES)
F. R. Santos (UFMG)
L. Müller, P. C. Estrela, T. R. O. Freitas (UFRGS)
J. S. Morgante, N. Moraes-Barros, F. M. Martins (USP)
L. R. Oliveira (UNISINOS/GEMARS)
V. Valiati (UNISINOS)
S. L. Bonatto (PUCRS)
A.R. Percequillo (ESALQ/USP)
C. R. Bonvicino, H. Seuánez (INCA)
J.A. Oliveira (Museu Nacional)
Salvatore Siciliano (FIOCRUZ)
R. Collevatti (UFG)
H. Schneider, M.I. Sampaio (UFPA)
M. A. Jardim (FZB-RS)
F. B. Peters, L. F. Machado (ULBRA)
J. Koenemann (PUCRS – Uruguaiana)
M. Bordignon (UFMS)
A.C. M. de Oliveira (UFPA)
L. Geise (UERJ)
J.I.R. Porto (INPA), I. Farias (UFAM), L. Tchaika (UEMA)
F. Braga (Museu Capão da Imbuia - PR)