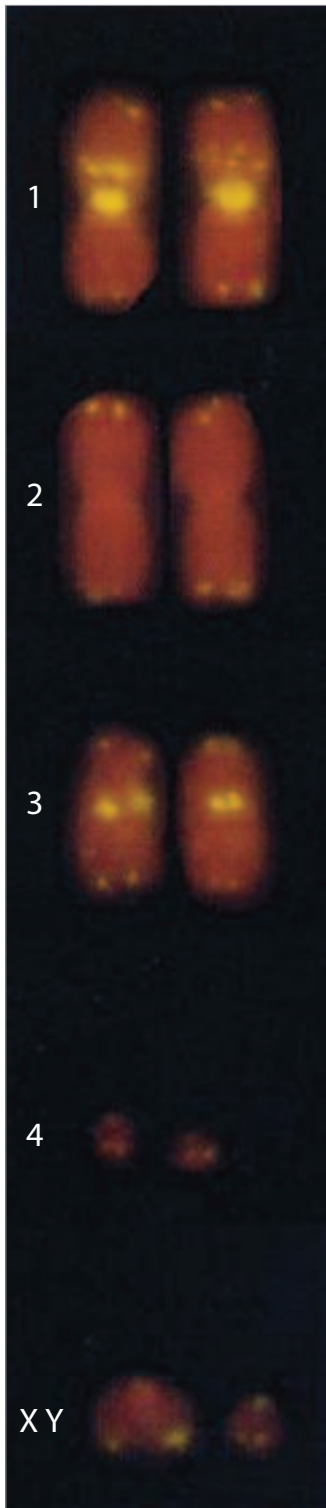


### EVOLUTIONARY STUDIES IN VERTEBRATES: I. CYTOGENETIC, MOLECULAR AND MORPHOLOGICAL ANALYSES. II. KARYOTYPICAL SURVEY OF BRAZILIAN FAUNA. III. CELL AND TISSUE COLLECTION OF MAMMALS, LIZARDS AND AMPHIBIANS

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The present project has been conducted under a multidisciplinary perspective and it aims the study of Brazilian biodiversity, including rodents, marsupials, lizards and amphibians on the basis of cytogenetic, molecular and morphological data.

Chromosomal studies have been carried out based on conventional (Giemsa and Ag-NOR-staining; C-, G-, R-bands) and molecular cytogenetics (fluorescence *in situ* hybridization or FISH) using telomeric and/or ribosomal probes, and also ZOO-FISH with probes generated from specific micro dissected chromosomes. This approach has achieved solutions for a number of cases in different groups of vertebrates. In lizards and amphibians, by instance, differential chromosome staining still represents important information to be reported and these data have contributed for characterization of populations and species, and for a better understanding of the chromosomal and evolutionary processes that groups have undergone.

Regarding morphologic issues of *Akodon*, it has been evinced which traits are the most relevant ones to be considered as informative characters for the genera. Furthermore, the chromosomal polymorphism and the range achieved for each karyomorph ( $2n=14, 15, 16$ ) of *A. cursor* throughout the Atlantic rainforest have been informative in order to have the distribution of the species mapped.

DNA sequences from mitochondrial and nuclear also represent an important tool that we are using to recover phylogeny of amphibians, reptiles and mammals. These data associated with geographic distribution, cytogenetic and morphological information of the species have helped to reconstruct the evolutionary history of each different group.

We have also been involved in fauna surveys and fauna rescues in hydroelectric power plants which occurred in different Brazilian biomes, e.g. Amazonian and Atlantic forests, Cerrado, Caatinga, etc., and the results revealed a number of unknown species within rodents, marsupials, lizards and amphibians.

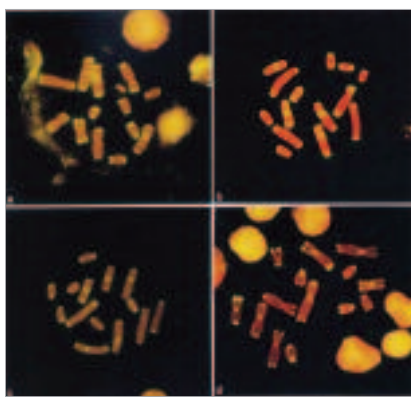
Our laboratory also stores tissues and cells obtained from fibroblast cultures in a "Collection of Cells and Tissues of mammals, reptiles and amphibians", which represents a valuable stock for rare, endangered and ordinary species collected in the Brazilian territory for about the last 20 years, as a result of our collaboration with Dr. Miguel T. Rodrigues (Zoology Department, Biosciences Institute, USP-SP) and Dr. Mario de Vivo (Zoology Museum, USP-SP), both projects supported by FAPESP.

*Karyotype of Akodon sp., male, 2n=10, after fluorescent in situ hybridization (FISH) with telomeric probes*

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In our project, cytogenetical analyses have already performed in species of 25 rodents, 13 marsupials, six bats, 32 lizards and 16 amphibians, most of them of unknown or misidentified species, which were characterized on the basis of karyotypic data.

A population level study in the rodent *Akodon cursor* has been conducted by different authors, since the description of the species in 1972 in our laboratory, with about 600 specimens sampled, in order to estimate the frequencies of the chromosomal polymorphism ( $2n=14, 15$  e  $16$ ) within this species.



Intraspecific variation of pericentromeric signals after fluorescent *in situ* hybridization (FISH) with telomeric probes in the marsupial *Micoureus demerarae* ( $2n=14$ ). a) Two interstitial signals. b) Four. c) Five. d) Six

the cytochrome b gene and chromosomes. It is underway the karyotypic description and comments on morphology of the rodent *Callistomys pictus*, an endemic and threatened species from State of Bahia, Brazil.

In the marsupial *Micoureus demerarae* ( $2n=14$ ), we detected an intraspecific variation in the distribution of interstitial telomeric sequences, after fluorescent *in situ* hybridization.

Several species of lizards have been karyotyped, and chromosomal mechanisms of sex determination of the XX:XY type and multiple ones, and also an extensive karyotypic variability in the family Gymnophthalmidae, have been detected by our group. Within a molecular perspective, a study assembling DNA sequences for 26 genera of gymnophthalmids resulted in a new classification for the family was proposed.

The amazing diversity of Brazilian amphibians with problematic issues relative to systematics, and threaten to several species in face of environmental degradation, makes this group very suitable for biological studies. We are studying this group under cytogenetical, molecular and morphological perspectives.

The multidisciplinary studies we have conducted have contributed to a better understanding of the evolutionary processes responsible for the differentiation and maintenance of vertebrate species of the enormous Neotropical biodiversity.

Presently, we are analyzing sequences from mitochondrial and nuclear regions of *Akodon* within a phylogenetic perspective. In 1998, a karyotype of an unknown species of *Akodon* with  $2n=9$  and  $10$ , the lower diploid number among rodents, was described by us. In 2006, a study about the phylogenetic relationships and karyotype evolution of this new *Akodon* and another related species was published, on the basis of sequences of

## MAIN PUBLICATIONS

Amaro-Ghilardi RC, Rodrigues MT, Yonenaga-Yassuda Y. 2007. Chromosomal studies in four species of genus *Chaunus* (Bufonidae, Anura): Localization of telomeric and ribosomal sequences after fluorescent *in situ* hybridization (FISH). *Genetica*. (DOI: 10.1007/s10709-007-9218-6).

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