THEMATIC PROJECTS

FAPESP
SÃO PAULO RESEARCH
FOUNDATION

RELEVANT ACHIEVEMENTS IN BIOLOGICAL SCIENCES

BOTANY

ECOLOGY

ZOOLOGY

PLANT AND ANIMAL GENETICS

EXPLORATORY RESEARCH RECOGNIZED WORLDWIDE

Botany, ecology, zoology, plant and animal genetics. In these and other sub-areas of Biological Sciences, Brazilian scientists contributed with results recognized worldwide.

FAPESP, São Paulo Research Foundation, is one of the main Brazilian agencies for the promotion of research. The foundation supports the training of human resources and the consolidation and expansion of research in the state of São Paulo.

Thematic Projects are research projects that aim at world class results, usually gathering multidisciplinary teams around a major theme. Because of their exploratory nature, the projects can have a duration of up to five years.

SCIENTIFIC OPPORTUNITIES IN SÃO PAULO, BRAZIL

Brazil is one of the four main emerging nations. More than ten thousand doctorate level scientists are formed yearly and the country ranks 13th in the number of scientific papers published.

The State of São Paulo, with 40 million people and 34% of Brazil's GNP responds for 52% of the science created in Brazil. The state hosts important universities like the University of São Paulo (USP) and the State University of Campinas (Unicamp), the growing São Paulo State University (UNESP), Federal University of São Paulo (UNIFESP), Federal University of ABC (ABC is a metropolitan region in São Paulo), Federal University of São Carlos, the Aeronautics Technology Institute (ITA) and the National Space Research Institute (INPE).

Universities in the state of São Paulo have strong graduate programs: the University of São Paulo forms two thousand doctorates every year, the State University of Campinas forms eight hundred and the University of the State of São Paulo six hundred.

In addition to the three state universities, the state has 19 research institutes, three federal universities of international research level and most of Brazilian industrial R&D. The state houses more than 10 thousand fulltime faculty and 130 thousand students. São Paulo alone, produces more scientific papers than any country in Latin America, except for Brazil.

FAPESP: SUPPORT FOR RESEARCH IN SÃO PAULO

The São Paulo Research Foundation (FAPESP) promotes scientific research in the State of São Paulo, Brazil. Through a robust program of fellowships and research grants it supports fundamental and applied research.

Created in 1962, the foundation is entitled by the State Constitution to 1 per cent of the tax revenues of the state of São Paulo. FAPESP has a sizable endowment and has already supported, over these 48 years, 100,000 fellowships and 90,000 research awards.

In 2010 FAPESP will invest US$ 415 million in fellowships and research grants. The success rate for proposals in the fellowship programs ranges from 40 per cent to 63 per cent. In the grants programs the proposal success rate ranges from 40 per cent to 60 per cent, depending on the particular type of grant.

OPPORTUNITIES AND CHALLENGES

One of FAPESP's goals is the broadening and diversification of the research system in the state of São Paulo, strengthening the existing centers of excellence, by supporting their research, and stimulating the creation of new centers or research groups tackling new lines of activity. This is achieved mainly by funding Young Researchers Awards, the Biota-FAPESP Program, the FAPESP Research Program on Bioenergy (BIOEN), The FAPESP Research Program on Global Climate Change (RPGCC), the RIDC (Research, Innovation and Dissemination Centers) Program and the Thematic Projects.

All of these have in their teams, in addition to experienced scientists, young researchers as post-doctoral fellows, from Brazil and from abroad. FAPESP supports more than one thousand post-doctoral fellowships.

RESULTS OF GREAT IMPACT

When the research program for Thematic Projects was created, in 1990, FAPESP's objective was to provide a qualitative leap in Brazilian scientific research and to meet the state of São Paulo's own particular demands for development. Since then, 1,200 projects in all fields of knowledge have been selected and supported. Selection is through a stringent peer reviewing process, using multiple reviewers for each proposal.

Thematic Projects are characterized by the breadth of their research and the boldness of their objectives. They are supported for five years (as opposed to two years for a regular research grant) and are lead by teams of experienced researchers.

Thematic Projects are funded, on the average, with 450 thousand dollars, plus fellowships. The salaries for the investigators and staff are not included in this amount, since, in Brazil, they are paid by their universities. Each project is lead by 3 PI's and involves several undergraduate and graduate students.

Thematic Projects create opportunities for scientists in São Paulo to advance knowledge by creating internationally competitive science, while, simultaneously, educating a new generation of researchers.

CONTACT FAPESP

Contact FAPESP (www.oportunidades.fapesp.br/en) or a coordinator from the Thematic Project which interests you and see how to obtain a post-doctoral internship.

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THE ROLE OF DISSOLVED ORGANIC MATTER RELEASED BY PHYTOPLANKTON IN THE HYPEREUTROPHIC RESERVOIR OF BARRA BONITA (SP, BRAZIL)

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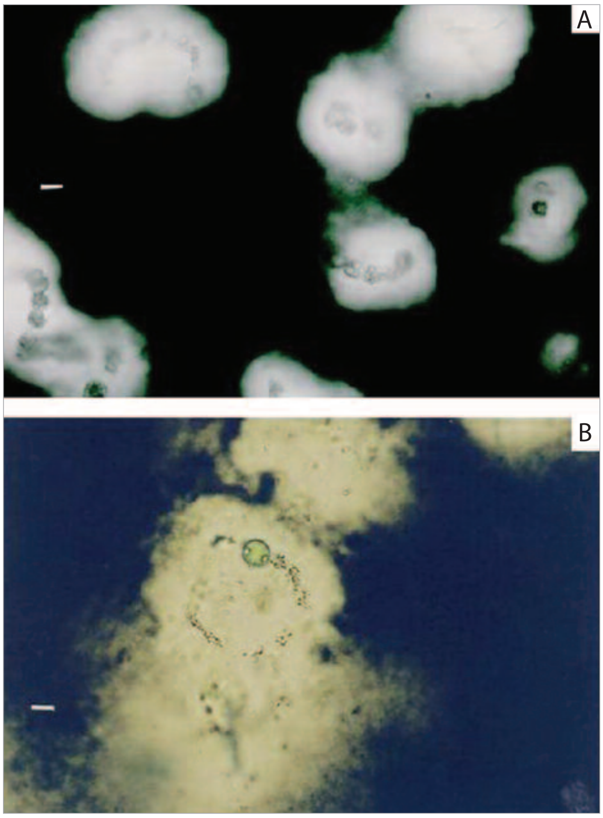
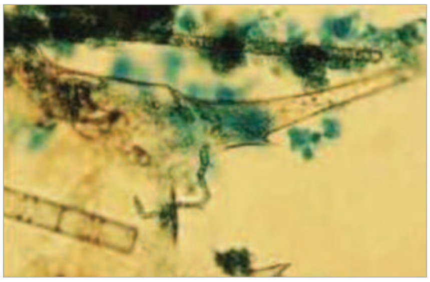


Details of biotic production of TEP by Anabaena spiroides. *(a) In the senescent growth phase, the cells burst inside the capsules, which are transformed into TEP. (b) Detail of cell remains, including a heterocyst within the former capsule. Scale bars: (a) 50 µm and (b) 10 µm. The formation of TEP potentiates the transport of intracellular toxins into the food web, via TEP filtering by the zooplankton*

There are approximately 30,000 artificial water reservoirs in the world, holding around 10,800 km^3 of water. Unfortunately, the eutrophization of the water arising from human activity leads to recurring episodes of excessive proliferation of microalgae and cyanobacteria, affecting the use of local water and placing the health of the population at risk. This is the situation at Barra Bonita Reservoir, created at the confluence of Tietê and Piracicaba Rivers, which covers an area of 384 km^2. Blooms of toxic cyanobacteria such as *Anabaena spiroides* and *Microcytis spp.*, besides eukaryotes such as the diatom *Aulacoseira spp.*, are common around the years, being recently becoming heavier. These organisms produce an enormous biomass which, after death and decomposition, cause appreciable changes, for the worse, in the water quality. Even so, much of the biomass generated by photosynthesis is released directly, by active or passive excretion, into the pool of dissolved organic matter (DOM) in the body of water, without cell death intervention. The general aim of the present project is to determine how this excreted part of the DOM interacts with physical and biological environments. The specific objectives of the project are to investigate the roles of organic compounds as substrates for heterotrophic bacteria and zooplankton. The organic compounds would act as complexing agents for heavy metals producing heavy particles aggregates and transparent exopolymer particles (TEP) out of colloidal polysaccharides, which can carry adsorbed toxins into the food chain via filter-feeding zooplankton. The project will also analyze the action of the specific bacteria/cyanobacteria associations in raising or lowering the compounds concentrations involved in allelopathic interactions. Finally, it is also under investigation, the effect of UV-B radiation on the DOM released bioavailability, by *Microcystis aeruginosa* cells.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Most of the DOM released by the phytoplankton consists of extracellular polysaccharides (EPS). Around 11,500 tons of carbon is produced in this form annually in the Barra Bonita reservoir. About 7% of the EPS are readily broken down by heterotrophic bacteria and the activity of various glycosidases can be quantified in the water column. Non-glycosidic organic compounds are also utilized by bacteria. At the surface, natural UV-B irradiation on such compounds leads to variation in their bioavailability and even selection among bacterial species. The EPS released by *Anabaena spiroides* supports the entire life-cycle of the cladoceran *Ceriodaphnia cornuta* when used as the unique substrate. In the form of mucilaginous capsules, these substances act as a protective buffer against high concentrations of toxic metals. For example, the chlorophycean *Kirchneriella aperta* is protected from copper by this way. These capsules also behave as a selective barrier, regulating the transport of molecules into the cell, as we confirmed in electron paramagnetic resonance experiments. The capsules of several phytoplankton species allow associations with species of bacteria immersed in the mucilage, which succeed one another as cell aging. After the death of a cell-forming-capsule, the empty capsule can become a TEP (quantified by a technique developed by this project), enabling the transport of trapped toxins into the food chain. In the case of the diatom *Thalassiosira duostra*, we have discovered that selective bacterial degradation can alter the hydrophobicity of the EPS by increasing their fraction of methylated monosaccharide units, such as rhamnose and fucose, making them stickier and thus promoting cell aggregation. The diatom *Aulacoseira granulata* has a sticky film of EPS coating the cells: when chain-to-chain collisions are provoked by shear rates, in the environment, the cells clump together, forming aggregates heavy enough to allow them to sink or to avoid being washed away. Thus, a part of the population is retained for seeding by easy resuspension due to the turbulence. A sensor array with nanostructured conducting polymers, for the identification and quantification of cyanotoxins in the reservoir waters, is under development.



Natural gelatinous aggregate formed by EPS and other colloidal polysaccharides. Two filaments of the diatom Aulacoseira granulata *can be seen, as well as filaments of the cyanobacterium* Planktothrix *sp. and zooplankton fragments. The films of hydrophobic EPS covering the diatom cells promote aggregation, resulting with this species being present in more than 90% of aggregates found in the reservoir*

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CARBOHYDRATES AS MODULATORS OF TROPICAL SPECIES ECOPHYSIOLOGICAL PROCESSES AND AS ENVIRONMENTAL STRESS RESPONSES MARKERS

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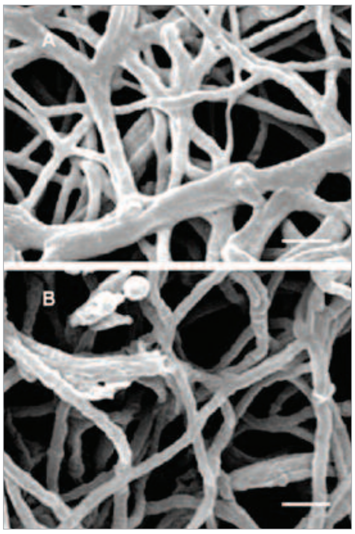
Cerrado general view and native herbaceous species. A restricted cerrado area at Mogi-Guaçu, SP, Brazil (A), Vernonia herbacea *(Vell) Rusby (B),* Viguiera discolor *Baker (C) and* Gomphrena macrocephala *St Hil.(D). Figueiredo-Ribeiro et al. 2007. In: Silva, J.T. (ed.),* Functional Ecosystems and Communities. *Global Science Books Ltd. (UK), Kenobe, Japan.* ***2****: 42-48*

The role of carbohydrates in physiological and biochemical processes in plants has been investigated primarily using crops from temperate climates as model plants. Recent studies performed by our group, with Cerrado and Atlantic Forest native tropical species have shown a high structural, metabolic, and functional diversity of carbohydrates, indicating the presence of a great variety of plant adaptive strategies to environmental conditions. Additionally, our studies has demonstrated the biotechnological potential of carbohydrates and the molecules related to their metabolism, which have novel and specific characteristics. Therefore, the main goal of this project is to study carbohydrates of native tropical species, focusing on their role as modulators of physiological processes and markers of stress conditions in plants. Three model systems were chosen to perform our studies: 1) seeds with different desiccation tolerance, from native species that accumulate different storage carbohydrates, for the analysis of the relationship between these carbohydrates and drought tolerance, maturity and defense; 2) tropical trees explants and fungal cultures cultivated *in vitro*, for the evaluation of carbohydrates as inducers and markers of specific steps in the somatic and zygotic embryogenesis and in morphological differentiation of filamentous fungi; 3) leaves and thickened underground organs of native species, storing starch and soluble sugars, for the investigation of carbohydrates roles as markers of developmental processes and environmental stress responses.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES



Scanning electron micrographs of Penicillium janczewskii *mycelium grown for 5 days on Czapek solid medium containing (A) sucrose or (B) inulin. Scale bars = 5.0μm. Pessoni et al. 2005.* Mycologia. ***97****: 304–311*

Differences in cell wall composition and concentration of soluble sugars and fatty acids between recalcitrant and orthodox tropical legume seeds suggest that these compounds are related to desiccation and freezing tolerance. Fragments of *Sesbania virgata* seed storage carbohydrates induce the production of allelochemicals in the seeds, revealing that cell wall polysaccharides, besides being used as reserve, contain in their own structure, signal molecules that may trigger defensive responses during the plantlet establishment. Seven putative orthologs of *Populus tremuloides* cellulose synthase (*CesA*) genes have been isolated and identified in *Hymenaea courbaril*. These genes are important in understanding the evolution and functional diversification of the *CesA* gene family and may be used for gene expression analysis in woody native species. Studies performed with *Vernonia herbacea*, an herbaceous plant that accumulates fructans, showed that, water deficiency, and low temperature, have caused changes in the activity of the fructan metabolizing enzymes, resulting in increased oligosaccharides level, compounds related to osmoregulation under stress conditions. A fructan exohydrolase (FEH) cDNA sequence was obtained from *V. herbacea* rhizophores, and the functional protein showed to hydrolyze predominantly inulin-type fructans. This is the first study concerning the cloning and functional analysis of a 1-FEH from the Brazilian Cerrado native species. Nutritional studies revealed that nitrogen increase plant growth and productivity in *V. herbacea*. Other environmental stressing factors, such as ozone and high CO_2, have also shown to affect plant growth and fructan metabolism in native and cultivated species. Studies performed with *Penicillium janczewskii*, a filamentous fungus that produce invertases and inulinases, has indicated that changes in the carbon source led to the production of extracellular β-fructofuranosidases with different properties and mycelia walls with altered physical and biological properties. These differences have been attributed to the presence of fructose in the medium. These results highlight the importance of the carbon source for the production of fungal β-fructofuranosidases for biotechnological purposes.

MAIN PUBLICATIONS

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USE OF SECONDARY TREATED EFFLUENTS BY BIOLOGICAL PROCESSSES (STABILIZATION POND SYSTEMS AND UASB REACTOR/ ACTIVATED SLUDGE PROCESS) IN AGRICULTURAL SOILS

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Within human activities, common agricultural irrigation represents, doubtlessly, one of the most consumptive practices of natural water resources. One alternative to solve and to minimize this high water consumption is the use of residual water (effluents) generated by biological sewage treatment systems.

The increasing agribusiness in Brazil and especially in São Paulo State, combined with the fact that sewage treatment systems generate high amounts of effluents, reveals the great potential of using treated sewage effluents for irrigation, minimizing the use of fresh water.

Crops irrigated with effluents from wastewater treatment plants (EWTP)

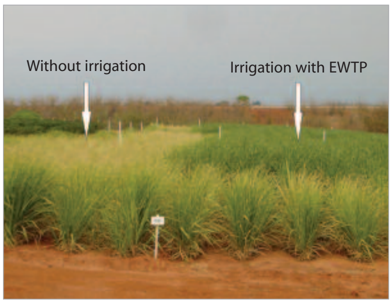
Thus, the present thematic project aims at investigating the sustainable utilization of secondary treated sewage effluents, disinfected through chlorination and ultraviolet radiation, in agriculture. For this purpose, effluents of different physico-chemical characteristics, produced by stabilization pond systems and up flow anaerobic sludge blanket (UASB) are utilized. After application to the soil, effects on the soil-plant-water system will be monitored. To understand the dynamics of the soil-plant-water system, detailed studies will be carried out: (i) to characterize soils hydrodynamics functioning, soil solution dynamics and to evaluate the soil capacity for element retention; (ii) to monitor nutrients and other elements in the treated sewage effluents and to evaluate the efficiency for nutrient supply, especially for nitrogen; (iii) to observe changes of major physical and chemical soil properties due to irrigation; (iv) to characterize organic matter originating from secondary treated effluents, evaluating its impact on the soil element dynamics and simulating the dynamics using mathematical modeling; (v) to evaluate the effect of secondary treated effluents on soil microbial biomass and its metabolic activity; (vi) to evaluate the nutritional status of plants and the benefit on dried plant material production. Moreover, the sustainability of the system depends on sanitary, technical and economical aspects. Thus, it is fundamental to evaluate: (vii) the disinfection processes of secondary treated effluents from the different treatment systems and to compare their technical and economical aspects; (viii) the formation of toxic sub-products (Trihalomethanes, THMs, and Halo-Acetic-Acids) during the chlorination process under different conditions, especially associated to ammonia concentration; (ix) the drip operability and the sprinkler irrigation system; (x) the economical viability and the environmental impact of utilizing secondary treated effluents.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

By means of the study on various crops (coffee, maize, sunflower, Tifton 85 bermudagrass and sugar cane) it was possible to evaluate the impacts of treated wastewater irrigation on the plant-soil-water system.

The results showed the feasibility of using effluents from a wastewater treatment plant (EWTP) as a nutrient and water source in agriculture. The nutrients present in EWTP, especially nitrogen, has reduced the use of N-fertilizers leading to a significant savings of fresh water, representing an important economical and environmental alternative.

Despite the presence of nutrients, only the EWTP was not sufficiently to supply the plants nutritional demands. Thus, it is necessary to implement corresponding management strategies adapted to the EWTP characteristics in order to keep the nutritional balance.



Sugar cane with and without irrigation with effluents from wastewater treatment plants (EWTP)

Considering soil quality, the main limitations found was the presence of high levels of Na in the EWPT, resulting in increased contents of dispersed clay, leading to a reduction of soil hydraulic conductivity in the experiments carried out with corn and sunflower. However, it has to be emphasized that the same effect was also observed in areas irrigated with potable water naturally rich in sodium, as it is used in Lins (SP, BR).

Although the input of N to the system by EWTP irrigation represents an attractive alternative, it can result in environmental problems due to nitrate leaching followed by contamination of groundwater. High levels of nitrate, below the root zone, were observed in soil solution in the experiments with maize, sunflower and sugar cane, however, it was not found for bermudagrass. These findings strengthen the importance of adequate crop selection and management for a successful and sustainable irrigation with EWTP in agriculture.

It can be concluded that from the agronomical and environmental point of view, EWTP may efficiently substitute conventional irrigation water, resulting in economical benefits, as well as in increased crops productivity and quality. Moreover, the use of EWTP has positive effects on soil acidity and other chemical properties, important for tropical soils.

Concerning health aspects, it was found that a dose of 10 mg L^{-1} chlorine, over a treatment time of 20 minutes, was sufficient for inactivation of total coli forms, *E. coli* and *Salmonella sp* in the EWTP. The trihalomethane concentrations were below the drinking water standards, probably due to the priority reaction of chlorine with ammoniac nitrogen.

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TROPHIC CASCADES IN A DEFAUNATED LANDSCAPE: THE ATLANTIC RAINFOREST PERSPECTIVE

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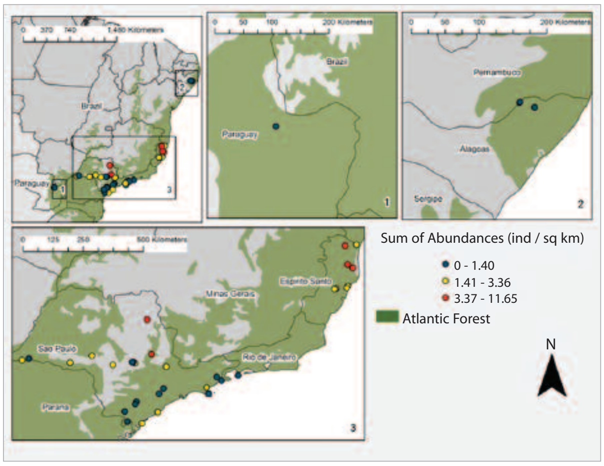


Priority areas for conservation of large mammals in the Atlantic Brazilian forest. This project sampled 12 Protected Areas in São Paulo state

Dominant and keystone species are recognized by their profound impacts on the composition, dynamics, and functioning of ecological communities. In Neotropical rain forests, large predators (such as jaguars, pumas, and harpy eagles) can control the populations of dominant herbivorous mammals (such as deers, tapirs, and peccaries), which in turn, control the populations of plants.

Deforestation, fragmentation and poaching have eliminated the top predators and now are also affecting the populations of meso- and mega-fauna over vast tracts of tropical forest. Thus, the removal of these mammals may have profound effects on the diversity and species composition of forest plants and other trophic levels.

Our project takes advantage of a unique defaunation gradient at the São Paulo state coastal Atlantic rain forest in Brazil. In this area, there are many large and connected protected parks, which could harbor all native vertebrates. Extensive surveys through our previous BIOTA project has found clear defaunation gradient, with parks with complete mammal assemblage contrasting with "empty" parks. We are now examining aspects of the early regeneration of forest trees including seed dispersal and predation, seedling survival and trampling impact of large-bodied mammals and species richness; seedlings diversity and above-ground biomass. Based on excluded plots replicated in different areas, and vegetation types in this defaunation gradient, we will look for evidences to support or reject the "top down" effects on forest regeneration.

In addition, as elusive mammals (such as felids, tapirs and peccaries) are difficult to survey, since they are rarely seen during line transects, we will take advantage of the development of molecular biology and use non-invasive sampling (such as faecal and hair samples) and specific DNA markers to estimate the population sizes of these species. Moreover, we can also study behavioral and population characteristics of these mammals, such as dispersal and genetic structure, which can be difficult to determine using other methods.

In order to sample the populations of elusive mammals (tapirs and felids), we are developing DNA markers for individual identification. These large mammals are hardly seen in the forests, but frequently signs of their presence are found as footprints and stools. Faecal samples are valuable especially for elusive species because few grams provide DNA from thousands intestinal cells of these animals.

Stool samples of tapirs, peccaries and felines have been collected in the studied areas to obtain DNA. All sampled have being georeferenced and individual identification will be carried out using DNA markers. Thus, we can estimate the minimum population size of these animals in the studied areas. Moreover, after plotting the individuals genetically identified on maps, and the pattern of movement of these organisms will be examined.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

1. Census of large and small mammals

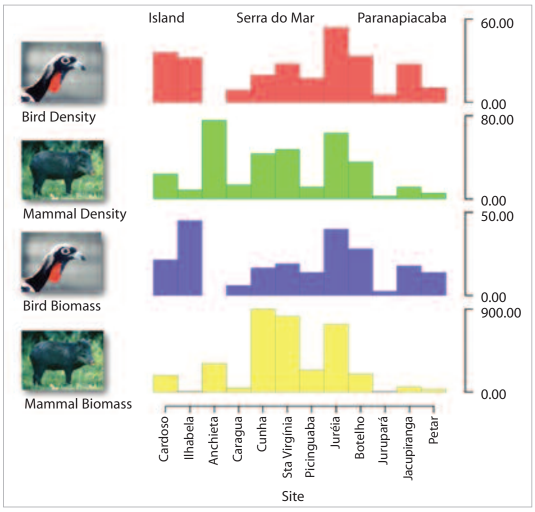
As part of the BIOTA-FAPESP biodiversity sampling program, mammal abundance was estimated for 12 protected Atlantic forest areas in São Paulo State and it was obtained from a standardized series of diurnal line-transect surveys. Each area was sampled monthly resulting in an average of 208 km of census walks per area. This reached to a cumulative total of 2,490 km surveved area. We found a great variation in bird and mammal density and biomass (Figure 1). The areas with the highest density of mammals (excluding Anchieta which has introduced mammals) was Ilhabela. However, Ilhabela has the highest population of the endangered jacutinga (*Aburria jacutinga*).

2. Developing DNA markers

We are using microsatellite markers for individual identification and to estimate the population sizes of elusive mammals. In the case of felids, we have microsatellites already described in the literature, but in the case of tapirs and peccaries, specific markers are not available, which are under development. We have already prospected and characterized 18 tapir microsatellite loci and initiated the prospection for peccaries. We have developed 18 molecular markers for tapirs. These markers have been examined and tested in some collected samples. About 30 dung sample were collected and stored in ethanol. The isolation of DNA markers of pecarids has been initiated. For felids, specific molecular markers are already described in the literature. Besides individual identification, these markers will provide the felid species identification. We have more than 40 felids faecal samples collected and we have already started to extract their DNA.

3. Top down effects on plant diversity

We have monitored the herbivory, trampling, seed predation and dispersal of two areas with distinct mammal abundances. Our preliminary results show that one area has 35-fold higher abundance of peccaries than the other one. This higher abundance has affected positively on trampling, and plant diversity. Areas with higher density of tapirs and peccaries have higher diversity of seedlings than defaunated areas, suggesting key effects of these large herbivores on plant diversity.



Density and biomass of game birds and mammals in 12 Protected Areas in São Paulo State

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ECOLOGY OF RESERVOIRS

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Figure 1. Bariri reservoir (above) and Nova Avanhandava reservoir (below)

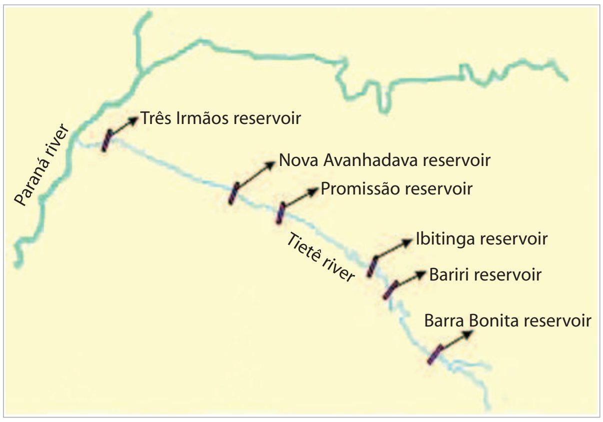

Figure 2. Reservoirs in the Tietê river (SP)

The present project is composed by five sub-projects: (1) *Historical evolution of the trophic and limnological relationships in the Itaipu reservoir: top-down and bottom-up effects in the fish production* through which we study the biotic and abiotic factors of the reservoir. This project aims to explain why the fish production of the Paraná basin reservoirs is comparatively low. (2) *Adaptation of the index of biotic integrity (IBI) to the reservoirs of Bariri, Ibitinga and Nova Avanhandava, in the Tietê river (SP)*, aiming to adapt the IBI in order to evaluate the trophic conditions of these three reservoirs by means of its fish fauna.

This subject is important to delineate optimal strategies for the use of Tietê river basin soil, which minimizes its impact on the already impoverished fish fauna; (3) *Adaptation of the index of biotic integrity (IBI) to the reservoirs of PCH´s – Batista and Jorda Flor, Turvo river (SP)*. These two PCH´s were never studied and no scientific collection of its fish fauna was accomplished to now; (4) *Bio-economical evaluation of the small-scale fisheries of Barra Bonita reservoir (SP)*, whose main objective is to estimate the net profit of the fishers at Barra Bonita reservoir, in the Tietê river. This sub-project inserts in the social and economical problems of the unemployment in the rural area, where sugarcane monoculture prevails, and then fishing constitutes the last job opportunity for many; (5) *Food webs assembly and their resistance to the introduction and exclusion of fish species: resulting properties of an individual-based model*, through which we intend to develop a theoretical computational individual-based model, for the evaluation of new food webs assembled due to invasions and exclusions of fish species. This subproject is very opportune, pioneer in our country, since invasions and exclusions of fish species is a common phenomenon in reservoirs, which are no-deliberate human experiments when considering its fish fauna. Under this strategy, we intend to understand the intricate dynamics of the original fish communities when faced to invaders.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The adaptation of the Biotic Integrity Index (IBI) for dams is a matter of particular interest, in order to evaluate the conditions of these ecosystems starting from some ecological attributes of its fish assemblies. The use of the fish is justified by the fact that they have an important position in the food chain and so they favor an integrated vision of the aquatic environment revealing its degradation.

The dams of Bariri, Ibitinga and New Avanhandava belong to the system of six dams of Tiete River (SP) reservoir cascade. The priority use of the water is electrical power generation followed by navigation and, secondarily, public and industrial provisioning, reception of domestic and industrial effluents, irrigation and fishing. A total of 24 points (six in Bariri, six in Ibitinga and 12 in New Avanhandava reservoirs) were selected for fish collection, located in three different types of habitat in each reservoir: lateral, mouth of tributaries and reservoir center. The fish fauna sampling methodology was standardized, using 10 gill nets with mesh sizes ranging from 3 to 12 cm between opposite knots and fish traps. In each point, we have collected fish and some physiochemical and environmental variables were annotated. The samples were collected in two seasons: dry (August 2007) and rainy periods (February 2008). In the data analyzes, it was also included previously collected data from Barra Bonita reservoir, the first of the cascade system. The following stages for the construction of the RFAI (Reservoir Fish Assemblage Index) a derivative of IBI were: (1) definition and selection of the metrics; (2) the reference conditions determination; (3) the punctuation attribution for each metric and RFAI calculations and (4) the results validation. The IAPR seasonal and space (dams and its habitats) variability of IAPR was evaluated by ANOVA, and the importance of the physiochemical and environmental variables were evaluated through an ANCOVA.

The results obtained in this study show a decreasing gradient of the values of IAPR along the reservoir cascade in Tiete River. In particular, the best values are calculated for Barra Bonita reservoir, the first and most polluted of the cascade, and the smallest values were calculated for Nova Avanhandava, the last of the studied sequence. So, an improvement of the water quality (increasing IAPR), due to biological processes of self-purification and sedimentation expected, as hypothesized, seems to be wrong. In this context, the result of the IAPR is not different from previous studies, but it allows improving the interpretation of this phenomenon. In particular, the poverty condition emphasized by IAPR of Nova Avanhandava may be a direct result of human impact upon the fluvial system. Three aspects stand out: (i) the alteration of the River Continuum Concept due to transformation of the Tiete river in a system reservoir cascade; (ii) the dominance of carnivore fishes increased due to the introduction of species as *Plagioscion squamosissimus* and *Cichla spp*; and (iii) the reservoir age.

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OPENMODELLER: A NEW COMPUTING ENVIRONMENT FOR MODELING SPECIES DISTRIBUTION

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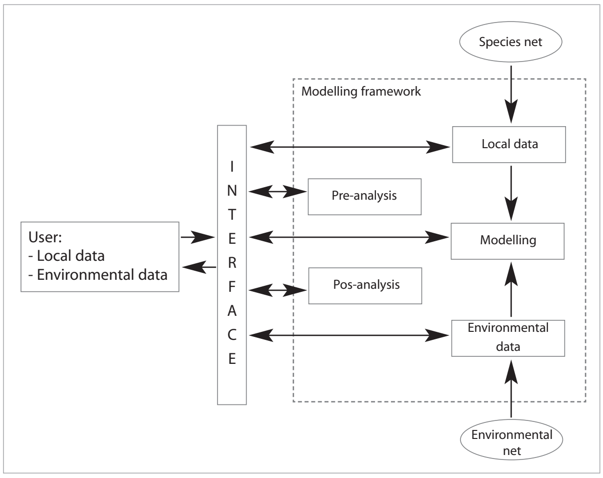

Figure 1. Original architecture of the openModeller *framework*

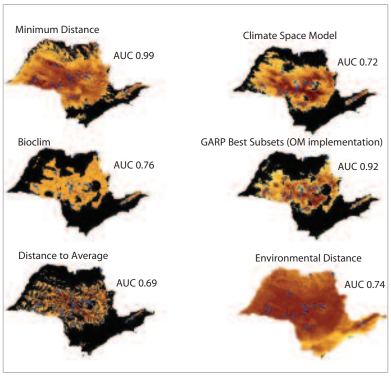

Figure 2. Results of six modeling experiments using the openModeller framework with the same points for Ouratea spectabilis*, the same raster layers with the respective AUCs, and comparing six different algorithms*

Conciliating social and economical development with environmental conservation is one of the biggest challenges today. There is a growing demand for quick answers to solve problems related to the occurrence and distribution of biological species, including threatened species, invasive species, and disease vectors. The formulation of strategies for sustainable development depends on these answers, which can only be obtained by accessing various types of data from different sources and by using advanced tools to analyze, visualize results, and build different scenarios.

This project aims to develop a new computing environment to model the potential distribution of species. It basically consists of a multiplatform framework specialized in performing modeling tasks, compatible with a service-oriented architecture. High-performance computing techniques are used, and the whole functionality is available to researchers through different interfaces. Study cases are being carried out to test and improve different modules of the framework and to compare the different algorithms that are being added.

Since the modeling task depends on different types of data, the scope of this project also includes activities related to species occurrence data (presence and absence) and environmental data stored in different formats and located in different places (local or remote).

The institutions involved in the project are: Reference Center of Environmental Information (CRIA), Polytechnic School of the University of São Paulo (USP) and National Institute for Space Research (INPE).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Species occurrence data. The speciesLink network is being maintained, improved and expanded as part of this project. Several tools have been created to help collections to improve the quality of their data. The modeling framework allows, not only direct access to speciesLink online database, but also to the Global Biodiversity Information Facility (GBIF) data.

Environmental data. Approximately 4,000 environmental layers have been cataloged. All layers are available in modeling servers and will soon be accessible through a new web service based on TerraLib, implementing a special protocol adapted to handle large volumes of raster data (Web Coverage Service – WCS).

Computing infrastructure. Modeling servers are accessible through a new protocol (OM-WS) that was developed through this project. One of the modeling servers available is a computing Cluster with 11 nodes (Core 2 Quad), which allows users to efficiently carry out complex experiments involving several species, several algorithms and a large number of layers.

Modeling framework. During the last 3 years, 12 new versions of the modeling framework have been released as part of this project. The framework has 8 modeling algorithms. It also offers pre-analysis tools to help the selection of the most relevant environmental layers, the post analysis tools, for models validation, and a command-line interface that can be used to perform the main functionalities of the framework. *Figure 1* shows the original architecture proposed.

Interfaces. The access to modeling functionalities is also available through advanced graphical user interfaces. A desktop interface – "OpenModeller Desktop" – is available for all 3 platforms (GNU/Linux, Mac OS X, and Windows) and allows the use of remote modeling servers. Recent versions of TerraView, a geographic information system developed by National Institute for Space Research (INPE), offers a new modeling plug in that was also developed through this project. The GBIF data portal has also developed a demonstration interface making use of one of the CRIA modeling servers. Lastly, a new web interface is currently under development and will be released before the end of the project in March, 2009.

Study cases. Study cases were carried out for São Paulo State, the Brazilian Savannah (Cerrado), and the Amazon region. New study cases are being carried out for other regions of interest to test technical aspects of the framework, such as the comparison of different algorithms and to understand the modeling process. Figure 2 shows some modeling results for the potential distribution of a tree species that occurs in São Paulo State.

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RECONSTRUCTION OF THE EVOLUTIONARY HISTORY AND PHYLOGENETIC STUDIES OF NEOTROPICAL BIRDS BASED ON MOLECULAR MARKERS

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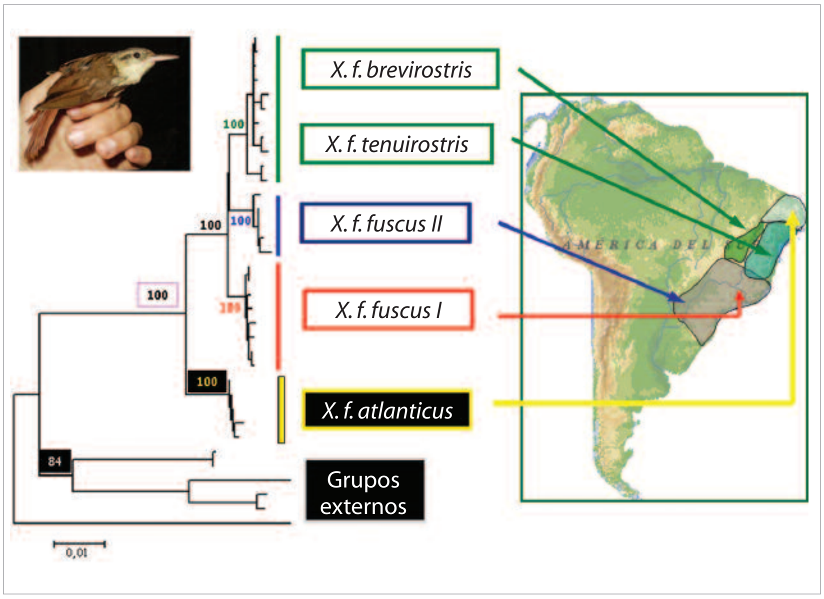
The Neotropics is a mega-diverse region. The origin of such richness may be related to the geologic and climatic changes and its conservation depends on combined efforts. Birds comprise one of the most diverse groups of neotropical animals. Their endemic patterns and the association between the systematic and biogeographic relationships are appropriate for historical biogeography studies. Their occurrence in all biomes is also suitable to study the origin of diversity in the Neotropics. In the present research project, molecular techniques have been applied in phylogenetic and phylogeographic studies of birds. We have been studying parrots, cracids, and toucans and will start the study of hawks and eagles. We will also start the study of Atlantic Forest birds to understand the biogeography and phylogeography. The dates of divergence between lineages will be estimated. Taxonomy uncertainties may be resolved, and the level of isolation between populations will be determined. These data will contribute to a better knowledge of the origin, distribution, and maintenance of Neotropics biodiversity.

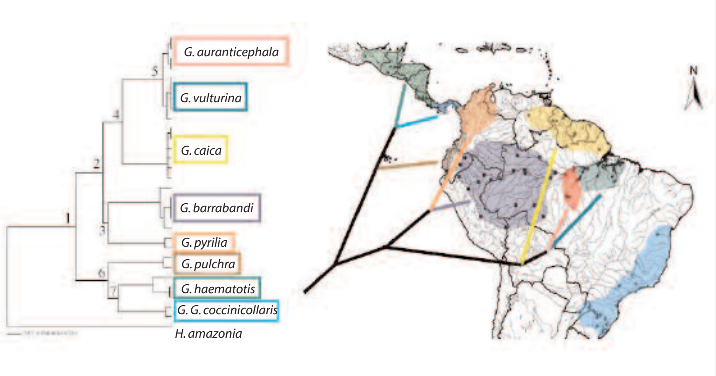


Relationships and geographic distributions of generic lineages of Xiphorhynchus fuscus

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The present project has been gathering data on the historic biogeography of birds and their biomes based on their phylogenies and population structure studies. The reconstructions of the evolutionary history of birds (including estimates of divergence dates of lineages) are used to infer possible factors that can be related to these divergences. These analyses suggest that past climate and geological changes in South America has contributed to speciation events and intra-specific diversification. The results have indicate a complex biogeographical history in the Neotropical region in contrast with less complex patterns in the Neartic and Paleartic regions. Also, our data indicate that taxonomic revisions are needed in some groups. Finally, we have found rates previously undescribed. Among those there are populations that need conservation actions. We have counted on many collaborators to develop these subprojects.

Another main area of the present project is to provide genetic data for the management of endangered avian species. Given the conservation complexity, we have been working with various collaborators. We have recommended actions for captive programs and for diminishing the illegal wildlife traffic.



Molecular phylogeny of the genus Gypopsitta and corresponding areas of distribuition of its species

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DECIPHERING THE MOLECULAR MECHANISMS INVOLVED IN INTRACELLULAR PROTEIN TRAFFICKING IN PLANT CELLS

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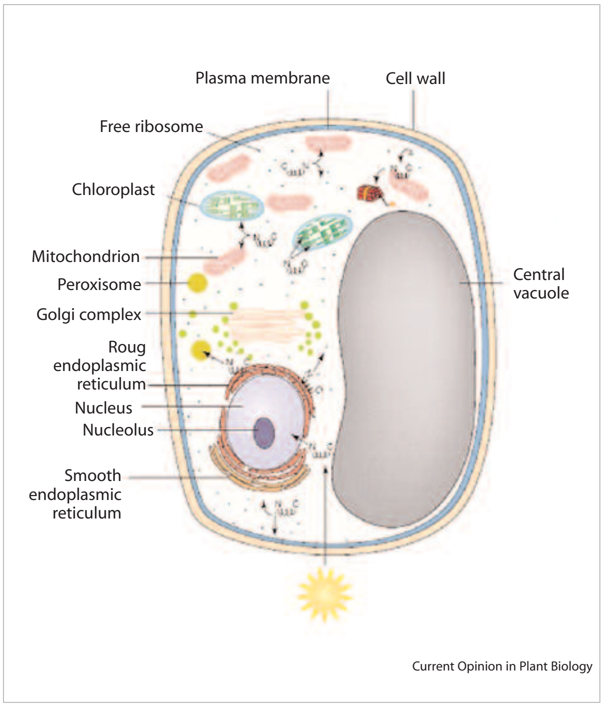
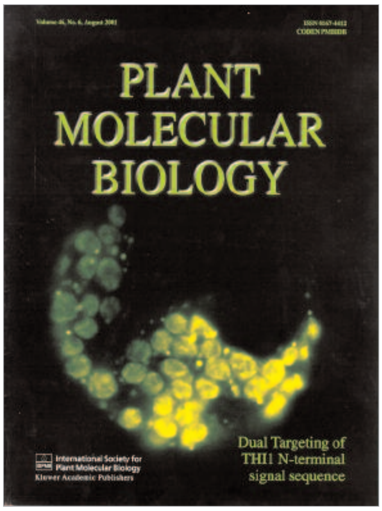


Current Opinion in Plant Biology

One of our main objectives is to understand the molecular mechanisms responsible for the protein localization inside plant cells, especially the proteins directed to mitochondria and chloroplasts. In this proposal, it is intended to advance in the characterization of the regulatory mechanisms involved in the intracellular localization of different proteins, also integrating an evolutionary approach. Four sub-projects are presented. The first one aims to identify proteins interacting with THI1 protein of *Arabidopsis thaliana* on thiamin biosynthesis, by using the yeast double-hybrid technique. Moreover, it is intended to identify proteins responsible for the control of the double targeting of THI1, since it was shown by our group that this protein is directed simultaneously to mitochondria and chloroplasts by a post-transcriptional mechanism. The second sub-project aims to study the thiamin biosynthesis in plants. Preliminary studies have suggested that biosynthesis of thiamin in plants can involve more than one subcellular compartment, opposing previous works that suggests the occurrence of the pathway only in the plastids. In addition to in-silico analysis, experiments with yeast functional complementation of plant genes involved on thiamin biosynthesis will be carried out. Furthermore, the determination of the subcellular localization of all proteins involved in the process will be provided by gene fusions with the green fluorescent protein (GFP). The third subproject aims to understand the gene regulation mechanism, know as riboswitches. The presence of structures in mRNA capable to interact directly with molecules intervening with its own translation has driven the attention of several groups worldwide. In the case of the *Thic* genes (involved in thiamin biosynthesis in *Arabidopsis thaliana)*, results have shown that its mRNA presents an aptamer-like structure that allows its binding to either thiamin pirophosfate (TPP) or thiamin, regulating its expression. It is intended to verify experimentally if the occurrence of this structure intervenes with the expression of the *Thic* gene, as well as, to the subcellular localization of the *Thic* protein. Moreover, a study will be carried in silico with all genes whose products are involved in thiamin biosynthesis, in order to identify the occurrence of aptamer-like structures and, in positive case, to verify its function experimentally. The fourth subproject involves the characterization of the mechanism responsible for the localization of one thylakoid membrane metaloprotesease, the FtsH-p1. Members of this family have been described as belonging to the Tat system (twin-arginine translocation system) for insertion in the thylakoid membrane. In a previous work of our group, it was shown that a member of this family does not present a classical RR motif responsible for its insertion in the membrane. Therefore, it is intended to characterize the mechanism responsible for the localization of protein, which suggests a distinct mechanism of those already described in the literature.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our group has shown that a protein named THI1, from *Arabidopsis thaliana,* is encoded by a single gene and is subsequently translocated to mitochondria and chloroplasts by an alternative translation initiation mechanism based on two in-frame AUG start codons. When translation initiates in the first AUG, the protein is translocated to plastids, whereas translocation from the second AUG, the protein is delivered into mitochondria. Previous examples of dual-targeting to mitochondria and chloroplasts involved ambiguous targeting sequences recognized by both sets of import machinery.



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TRANSPOSABLE ELEMENTS ARE ACTIVE CONTRIBUTORS TO GENOME STRUCTURE VARIATION, GENE REGULATION AND FUNCTION IN BACTERIA AND PLANTS

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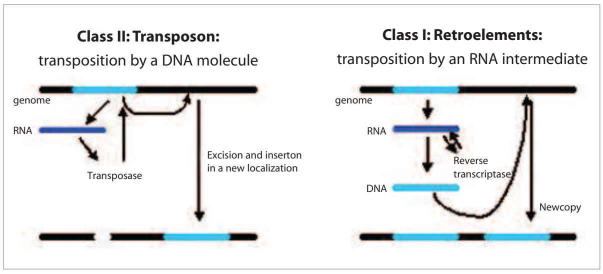


Figure 1. Both mayor groups of transposable elements (TE) and their corresponding transposition mechanisms

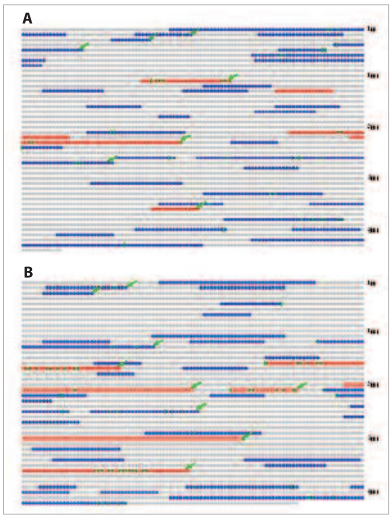
Transposable elements (TE) expression profile study using different sugarcane tissues (CL, callus; AM, apical meristem; LR, leaf roll, FL, flower). Sugarcane TE containing clones were spotted onto membranes and hybrized with radioactively labeled mRNA extracted from specified tissues supporting active expression under regular growth conditions. The original image is published in Araujo & Rossi et al. 2005. Plant J.

Transposable elements (TE) are being considered important components of genome maintenance and diversification. Transposition involves a gene function, encoded by the element, and DNA repair functions, encoded by the host. This dual configuration is essential for their activity control. Elements move by two different mechanisms: direct transposition, as observed in Class II elements, or by reverse transcribing an mRNA molecule, as in Class I elements (*Figure 1*). Our research is focused on the structure and function of genomes, specifically studying the impact of TE in Eukaryotes and Bacteria. The approach utilizes molecular genetics and bioinformatics tools. Recently, we have started exploring the association of metabolites content, locus/gene diversification among closely related plant species and the association of TE specific insertions.

Retrotransposon *Retrolyc1* was originally isolated from *Lycopersicon peruvianum* and was found to be differentially spread on the whole genus. The project now focuses on the control of the element gene expression. Among the Solanaceae, an exploratory study will be initiated to identify retrotransposons in wild and cultivated *Solanum* that harbors differential ploidy. Polymorphisms associated to retrotransposon activity is evaluated by insertional profiling and these are correlated to the domestication process.
The perception of the impact of transposable elements and phages, in the evolution of bacterial genomes, is increasing with the information input made by the genome sequencing projects. Integrase catalytic function is essential to introduce any invading DNA and they are associated to horizontal gene transfer among different bacterial species. We will compare the integrase content and associated genes in four *Xylella fastidiosa* strains. Genetic contribution of the associated integrase regions will be analyzed *in silico*, and stored in a database to be developed along the present project. Contributions are expected in the area of genome evolution and diversification among closely related organisms.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

As a consequence of the SUCEST sequencing project, a total of 21 transposable element families were identified in the sugarcane transcriptome. We will concentrate on the evaluation of the activity, polymorphism and expression patterns of four of these families which are *Mu*-like and *hAT*-like transposons and two retrotransposons families, *Tnt*1 and *Hopscotch*. Sugarcane is an interspecific hybrid genome and comparative analyses will be undertaken so as to compare the progenitor's contribution on the transposable element constitution of this hybrid genome. Differential proliferating activity of transposable elements may be a result of remodeling closely related genomes. Expression was further investigated through a combined study utilizing electronic northerns, macroarray, transient and stable sugarcane transformation. Callus was identified as the tissue with the highest number of TEs being expressed, revealing that tissue culture drastically induced the expression of different elements. Transformation experiments have demonstrated that *Hopscotch* retrotransposon is transcriptionally active.



Genomic islands identified in two Xanthomonas *genomes. Green arrows locate transposable elements at the border of such genomic islands. Shared and specific islands are depicted in blue and red, respectively. (extracted from Lima* et al.*, 2005)*

In bacterial phytopathogen genomes, we have looked at the contribution of IS elements to gene diversity pool and genome structure among closely related bacterial strains of different *Xanthomonas*, *Xylella* and *Leifsonia* species, mainly by means of bioinformatics analyses. Sugarcane and tomato species are plant models suitable for studying the impact of transposable elements in eukaryotic genomes. The pool of TEs in sugarcane has been determined initially on the EST database and subsequently with full length sequence and phylogenetic analyses. In both plant systems (sugarcane and tomato) we have studied TE activity by determining their expression pattern and genomic copy number among domesticated and wild cultivars. These studies involve the generation of genetically modified plants using GUS/GFP: promoter fusions, as well as genome imprinting of TE insertions using hybridization profiles.

In order to evaluate the impact of TEs at a higher taxonomic level, we have enlarged our studies to Poacae (sugarcane) and Solanaceae (tomato). Manetti *et al* (2007) describes the diversification of a particular Ty1/copia LTR-retrotransposon in three Solanaceae genera.

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

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

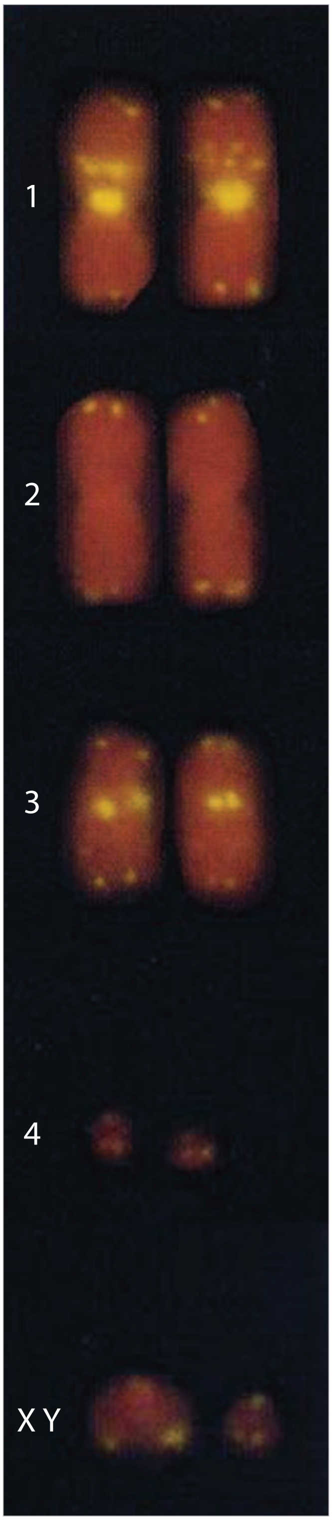
Chromosomal studies have been carried out based on conventional (Giemsa and Ag-NOR-staining; C-, G-, R-bands) and molecular cytogenetics (fluorescence *in situ* hybridization or FISH) using telomeric and/or ribosomal probes, and also ZOO-FISH with probes generated from specific micro dissected chromosomes. 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 the characterization of populations and species, and for a better understanding of the chromosomal and evolutionary processes that groups have undergone.

Regarding *Akodon* morphological issues, 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 mitochondre and nucleous also represent an important tool that we are using to recover phylogeny of amphibians, reptiles and mammals. These data associated with species geographic distribution, cytogenetic and morphological information 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.

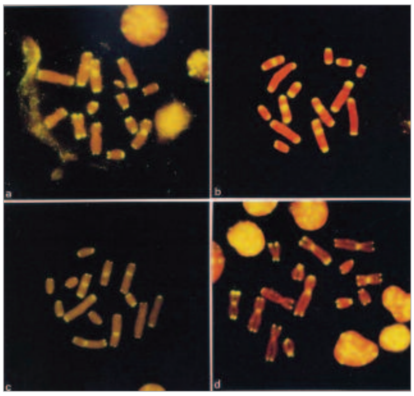


Karyotype of Akodon sp., *male, 2n=10, after fluorescent* in situ *hybridization (FISH) with telomeric probes*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In our project, cytogenetical analyses have already performed in species of 25 rodents, 13 marsupials, 6 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 and d) Six*

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.

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 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 have 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.

The amazing diversity of Brazilian amphibians with poor 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.

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Apis mellifera FUNCTIONAL GENOME – SEARCH FOR NEW GENES AND INTEGRATED NETWORK IN THE CONTEXT OF DEVELOPMENT, REPRODUCTION AND CASTE DIFFERENTIATION

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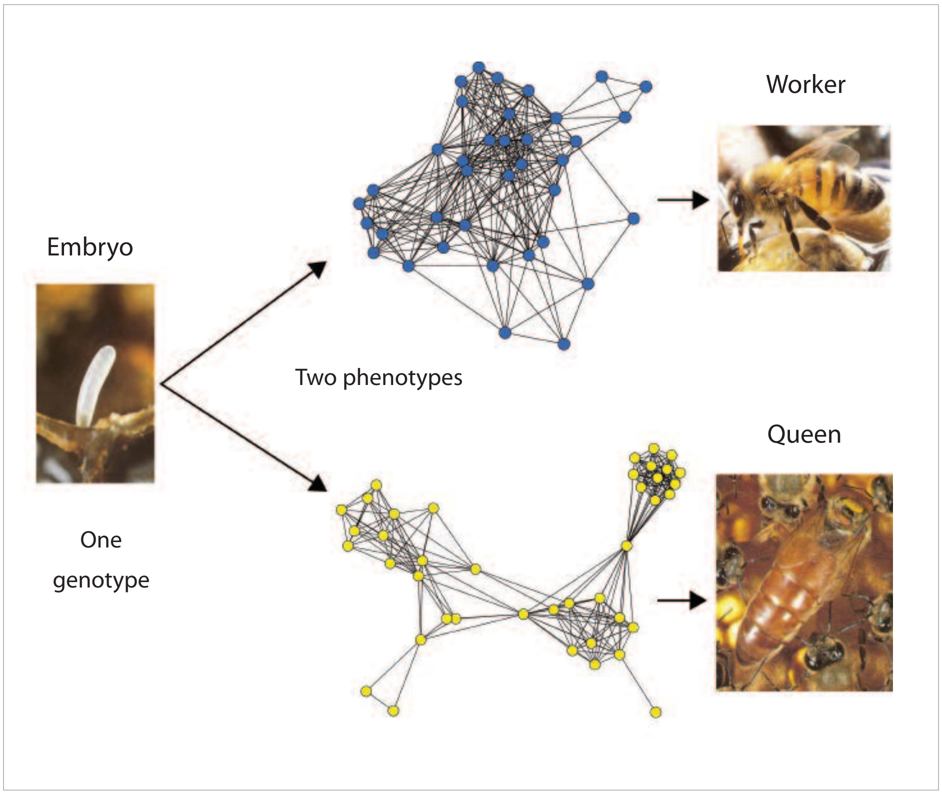
With an active contribution and participation in the annotation of the recently sequenced genome of the honey bee (5021 ORESTES, deposited in the GenBank), our group now has consolidated knowledge and expertise in all aspects required for large-scale analyses of gene expression in honey bees. Following our long term questions of interest on mechanisms underlying caste and sex determination and those involved in reproduction and labor division, already formulated in the pre-genomic era of honey bee research, we are now proposing a large scale analysis of gene expression in different phases of the honey bee life cycle. We will focus on the following questions: the identification of genes involved a) in caste development; b) in programmed cell death of the larval ovary; c) in integument differentiation; d) in the activation of the adult ovary of queens and workers; e) in the functional cycle of the hypopharyngeal glands and f) in early embryonic sex determination. In addition, we will screen our libraries for putative micro-RNAs to unveil the participation of this novel group of post-transcriptional regulators in the above contexts. The project adopts a dual strategy: a) the identification of candidate genes in the honey bee genome based on prior knowledge of gene function in other organisms, especially Gene Ontology attributes registered in flybase; b) the search for novel genes by microarrays, subtractive hybridization strategies, and the generation of an embryonic cDNA library. The differential expression of all these genes will be tested and candidates of specific interest will be investigated by quantitative RT-PCR and silencing by RNAi. The joint information on gene expression in these contexts will be subjected to network analysis in order to detect functional linkage and organization in gene expression networks.



Networks depicting gene interaction based on the occurrence of motifs in the Upstream Control Regions of Differentially Expressed Genes between A. mellifera *castes*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Investigating molecular underpinnings of honey bee caste development, differential reproduction and division of labor were perceived as core elements of our proposal. Our group had participated and coordinated the annotation of genes related to caste development and reproduction in the genome sequence project of *Apis mellifera*. The companion paper produced by our group comprised a bioinformatics analyses of genes specifically expressed in caste development. A subsequent expansion on this analysis, on differential gene expression in queen, versus worker development was based on new experimental data from micro array analyses and used a network approach to connect genes via their revealed putative upstream control region elements. These results paved the way for novel approaches to the study of developmental processes and their regulation in honey bees, for example the study of genes underlying the hormonally controlled expression of caste-specific morphological characters, such as the corbicula on the workers hind leg and the massive autophagic cell death in the ovaries of worker larvae leading to the highly divergent ovary phenotypes observed in adult honey bee queens and workers. Further analyses along these lines are now being performed which will contribute to our understanding of queen/worker development by expression analyses of candidate genes or large scale differential gene expression screens. Such expression analyses, however, are the first step towards understanding gene function, and in this direction, our group has contributed to the field of honey bee functional genomics through the establishment of a successful RNAi approach, silencing gene function of the yolk protein vitellogenin. We focused on this gene, which is abundantly expressed in the female sex, both in queens and workers. There are novel functions postulated to vitellogenin, as a major regulator of longevity, which clearly is a major difference between queens and workers. In addition, vitellogenin was postulated to interact with juvenile hormone in a negative feedback circuitry to regulate the transition of a worker bee from within-hive tasks to foraging. Such aspects of functional genomics on major life history determinants were and are successfully being explored by our group in collaboration with Dr. Gro Amdam (Arizona State University) and Dr. Dolors Piulachs (Institut de Biologia Molecular de Barcelona, CID, CSIC).

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INSTITUTE TO STUDIES OF THE PARASITIC HYMENOPTERA AT THE BRAZILIAN SOUTHEASTERN REGION – HYMPAR

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Figure 1. New Metopius sp *(Hymenoptera, Ichneumonidae, Metopiinae). General view of an adult male (digital imaging)*

Currently, in the Brazilian Southeastern region, only some areas of native forest and savannah in São Paulo State has been sampled in a systematic way through inventories of the fauna of *Hymenoptera parasitoids*. Little is known of the taxonomy and biology of the communities of Hymenoptera parasitoids associated with agroecosystems and in areas of forest fragments. Although so abundant, little is known of bionomy of many species of parasitoids Hymenoptera, including their hosts. The study of tritrophic interactions involving plants, phytophagous and parasitoids (or hyperparasitoids) or predators should provide subsidies to the understanding of the groups phylogeny and evolution. The possibility to know the identity of *Hymenoptera parasitoids*, enemies of certain agricultural pests allow the use of techniques of insects mass rearing that will put the Brazilian agriculture in the context of biological control of agricultural pests. The financial resources will enable the expansion of our collections by possibility of sampling in new areas of natural ecosystems and in agroecosystem.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project, still in data collection phase, has the objective to promote the establishment of solid foundations for the hymenopterous parasitoids biodiversity knowledge. We have installed 95 sampling points georeferenced in about 30 counties in the Brazilian Southeastern, in 30 native areas in the States of São Paulo, Rio de Janeiro, Minas Gerais and Espírito Santo with monthly collections using Malaise traps, since May 2009. Braconidae, Ichneumonidae and Chalcidoidea families have been sorted and identified. Some genera were determined so far. Four of them have not been registered in Brazil yet, namely: *Diglyphomorpha*, *Hemiptarsenus*, *Platyplectrus* (Eulophidae), *Erixestus* (Pteromalidae), *Andesipolis*, *Paphanus*, *Syntretus*, *Topaldios*, (Braconidae). About 12 000 specimens of Ichneumonoidea were mounted on entomological pins, properly preserved in dehumidified room. More than 120 Malaise samples are awaiting for sorting. Around 420 specimens of Hymenoptera were identified in Minas Gerais and São Paulo States coffee area plantations using water pan traps in 19 samples. In different Brazilian regions, collections of live and mummified aphids on different host plants were made. Larvae of Lepidoptera defoliators, from which have emerged adult specimens of *Lepidoptera*, *Hymenoptera* and *Diptera parasitoids* were collected. Species of Lepidoptera Geometridae, Noctuidae and Arctiidae were identified. Behavioral studies, including parasitism data on some species of spiders were also made. We have also been studying biology, ecology and parasitoids of Chrysomelidae (Coleoptera). This involves the study of tritrophic interactions between beetles, their plants hosts and their parasitoids, including studies of the life cycle, behavior, population dynamics and species habits. Many new species have been described in national and international journals. Several training courses in technical studies of the *Hymenoptera parasitoids* in field and laboratory, and their use for biological control and conservation of natural ecosystems, were also conducted. Transfer of *Hymenoptera parasitoids* biology basic knowledge was carried out to the community through educational exhibitions, assembly and use of educational videos.

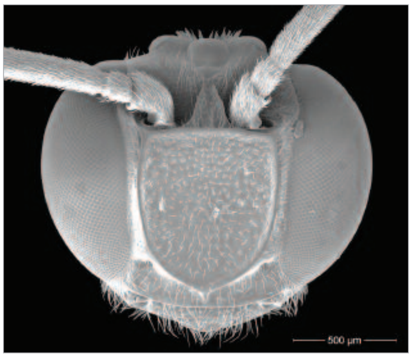

Figura 2. Metopius sp nova (Hymenoptera, Ichneumonidae, Metopiinae). Head in frontal view (scannning electron microscope image)

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BIODIVERSITY, EVOLUTION, ENDEMISM AND CONSERVATION OF THE MEDUSOZOA FROM THE SOUTHWESTERN ATLANTIC

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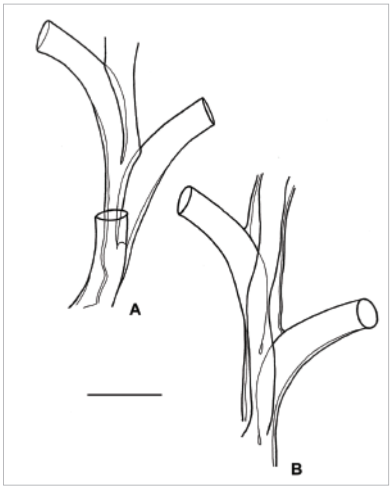
Portuguese man-of-war (photo A. E. Migotto)

Medusozoa is a diverse and widely distributed group, with complex life histories. Species of medusozoans inhabit different seas and oceans around the world. In a regional scale, some species are widespread along the Southwestern Atlantic Ocean (Brazilian and Argentinean shores), with populations distributed along a heterogeneous and structured environment. There are few biogeographical and phylogeographical studies for the group, either under descriptive or interpretative approaches, and the relation between the distribution of the species and most of the biological and environmental factors are still largely unknown. This lack of studies precludes a better understanding of its evolution and proposal for conservational actions. The present project aims to study the diversity of medusozoans in the Western South Atlantic, from Cabo Frio (RJ, Brazil) to Tierra del Fuego (Argentina), correlating distribution and life cycles. The study has two complementary approaches: a) to determine the pattern of present distribution of species and the recognition of areas of endemism and b) to provide explanations for the patterns of distribution observed and to understand the factors affecting species distribution and evolution, particularly related to the biology and the life history of the groups under study. More specific goals are: 1) to survey and evaluate the biodiversity of Medusozoa in the area under study, 2) to identify the areas of endemism, based on parsimony analyses of endemicity, in order to understand the general patterns of macro-distribution, 3) to promote phylogeographic studies that, together with life cycle and biology studies, aims to understand the general and specific patterns of micro-distribution. The project will carry out benthic and planktonic surveys along the area under study, and will also consider the available previous collections undertaken between Cabo Frio and the southern Brazilian coast. The coast of Uruguay and Argentina will also be surveyed with the collaboration of Argentinean researchers.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We carried out taxonomical studies focusing planktonic and benthic stages of Medusozoa. The surveys, still ongoing, resulted in the description of new species and several new records for the region. The plankton study resulted in a fauna of 20 species for which some distributions are associated to specific water mass. The parsimony analyses of endemicity resulted in two different areas, the first located between 50 and 200 m deep with predominance of the South Atlantic Coastal Water; the second located eastern to the 200 m bathymetric line, along the occurrence of Tropical Water, as the main water mass. In the morphometrical multivariate analyses carried out with populations of several species of medusozoans, viz. *Thyroscyphus ramosus*, *Physalia physalis*, *Pinauay ralphi*, *Chrysaora lactea*, and *Olindias sambaquiensis*, we found no geographical patterns or population structure. Ecological studies on the specificity of animal and plant substrata, acting as a constraint for the distribution of the benthic species of medusozoans, were also carried out and very few species seem to be specifically related to a given substrate. In the phylogeographical study, we gathered data on the ecology and natural history of *Olindias sambaquiensis* and *Liriope tetraphylla*, and carried out phylogeographic analyses using CO1 and 16S DNA markers. Our results have shown similar phylogeographical patterns and genetic structures for both species. The Brazilian populations are basal and have a higher nucleotidic diversity than the apical Argentinean populations. The Rio De La Plata River is not an effective barrier, and introgression possibly occurs for both species and might be related to the circulation of the water masses.



Acryptolaria sp.
(illustrated by A.L. Peña Cantero)

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SUBTERRANEAN AQUATIC FAUNA: BIODIVERSITY, BIOLOGY, ECOLOGY AND CONSERVATION

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Pimelodella kronei (photo A. Camargo)

R. enfurnada (photo A. Camargo)

Due to their distinctive characteristics, subterranean ecosystems are of a major scientific interest. Regarding conservation, their intrinsic fragility and vulnerability are a matter of concern. Subterranean communities include species living exclusively in hypogean habitats (troglobites). As a result of the evolution through isolation under a peculiar selective regime, characterized by permanent darkness and food limitation, troglobites generally present autapomorphies such as reduction of eyes and melanic pigmentation (classical troglomorphies), and other morphological, physiological and behavioral specializations.

In Brazil, systematic studies on the cave fauna began in the early 1980´s. Since then, there was a rapid progress in the knowledge of hypogean ecosystems and particular rate, but there are still important gaps. A most serious problem is the taxonomic impediment and the paucity of biological and ecological data for several groups.

Brazil distinguishes worldwide by its remarkably rich and diversified subterranean ichthyofauna, with a great potential for research on aquatic Biospeleology. Having as basis faunistic inventories in different Brazilian karst areas, this project encompasses several aspects of the biology and ecology of subterranean fishes and other aquatic troglobites, some new and some in a higher level of detail and methodological sophistication. For fieldwork aiming to faunistic surveying, natural history and population ecology of selected fish species, several karst areas in the States of Bahia, Minas Gerais, Goiás, Mato Grosso, Mato Grosso do Sul and São Paulo, besides non-karst areas in Amazônia, were chosen. Specimens brought alive to the laboratory are used for behavioral (including chronobiological) and physiological studies, and preserved specimens for taxonomic (morphological and molecular systematics), anatomical (brain) and biological (diet, reproduction).

The detection of "hotspots" of subterranean biodiversity and detailed studies on specific rate are important steps for effective conservation actions, especially needed in the case of the fragile subterranean ecosystems and their troglobitic species.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

After three years of surveying, the confirmed number of troglomorphic fishes raised from 18 to 24, including the first record of troglobitic species for the family Callichthyidae and the trichomycterid Glaphyropoma and Copionon. As well, the number of troglophilic populations was significantly increased. Ichthyofaunistic surveys in surface water bodies, carried out for comparative purposes, helped to establish the troglobitic *versus* troglophilic status of subterranean populations, also contributing to the knowledge of the ichthyofauna in poorly known areas.

In addition to the description of new troglobitic species, morphological, cytogenetic and molecular taxonomic studies have been carried out for rate such as the Amazonian Phreatobius catfishes, the new Rhamdiopsis catfish from Chapada Diamantina, and the catfishes of the genera Pimelodella and Rhamdia. Data on distribution, allied to these taxonomic studies have been the basis for the evolution and subterranean populations differentiation hypothese.

Santana cave (photo A.L. Guil)

Techniques of individual identification using permanent tags were used in long-term population studies, encompassing several annual cycles, thus allowing for the detection of annual patterns. The duration of this project has being fundamental for this. Studies on the heptapterids *Rhamdia enfurnada* (Bahia), *Pimelodella spelaea* (Goiás) and *Pimelodella kronei* (São Paulo) are in progress. Visual censuses were applied to fishes with sizes insuffiecient for identification and/or with low population densities. Previously studied species could be monitored. The data obtained are the basis for efficient conservation actions.

Studies on the locomotor rhythmicity were carried out for eight species, which showed different degrees of reduction in time control mechanisms, apparently correlated with their degree of troglomorphism. Other behaviors focused include the reaction to light, hiding habits and agonistic interactions. As observed for morphological characters, behavioral specializations have a mosaic distribution in the studied species, revealing interesting and complex evolutionary patterns.

Physiological studies revealed the complexity of pigmentation reduction mechanisms in troglobitic fishes, which includes changes in the response to hormones (detailed study in progress) and loss of melanin production, caused by different mechanisms.

Collections of terrestrial invertebrates also resulted in the discovery of new and interesting troglobitic species. Some of these species have already been described.

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SYSTEMATICS OF *ANOPHELES* (*NYSSORHYNCHUS*) (DIPTERA: CULICIDAE)

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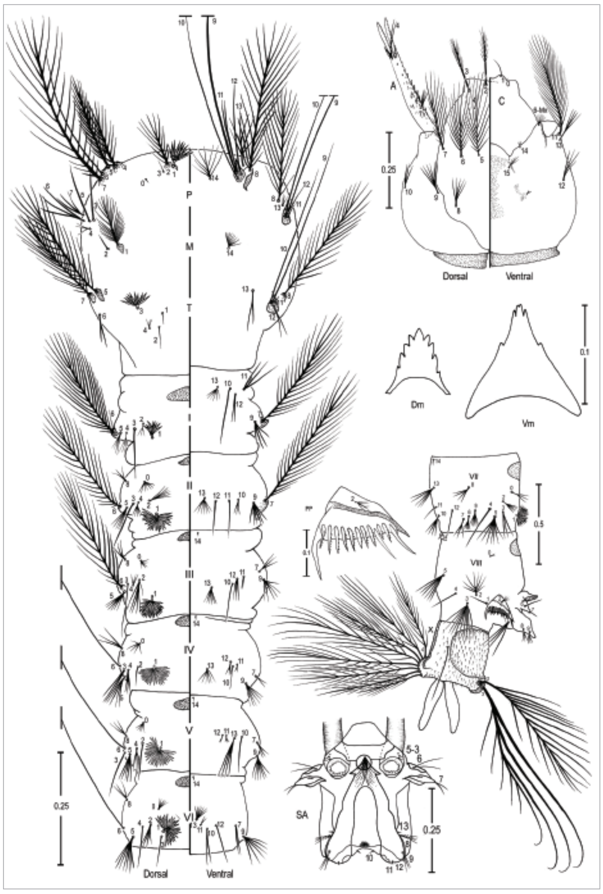

Fourth-instar larva of Anopheles oswaldoi. *A: antenna; C: cranium; Dm: dorsomentum; M: mesothorax; P: prothorax; PP: pecten plate; SA: spiracular apparatus; T: metathorax; Vm: ventromentum; I–VIII: abdominal segments; X, anal lobe. Scales in mm. Motoki et al. 2007*

The proposal aims to continue systematic studies on mosquitoes (Diptera: Culicidae), focusing on the genus *Anopheles*. The main subjects will be the Neotropical subgenera, especially the subgenus *Nyssorhynchus* and *Anopheles darlingi*. Although being the most important Neotropical vector of human *Plasmodium*, the involvement of *An. darlingi* in the dynamics of transmission of malaria in Central America and southern Brazil seems less important than in the Amazon region. In addition, its vector importance is not well established in some localities of southern Brazil. We propose to investigate the possibility that the populations of the Amazon region and southeastern Brazil, eastern of Mata Atlantica, have distinct gene pools.

The main objectives are: 1) genetic characterization of populations of *An. darlingi* of northern and southeastern Brazil; 2) estimation of the phylogenetic position of *An. darlingi* within the subgenus *Nyssorhynchus*; 3) contribution to the identification subgenus *Nyssorhynchus* species, since some species are involved in the transmission of human *Plasmodium*. Additionally, some members of the subgenus *Nyssorhynchus* are able to adapt to human environments. Within this context, we intend to carry out systematic studies on *An. darlingi* and species of the *Nyssorhynchus*, employing both morphological and molecular tools. Fragments of the mitocondrial genes cytochrome c oxidase subunit I (*Cox1*), the ATP synthase F0 subunit 6 (*Atp6*) and the NADH dehydrogenase subunit 6 (*Nd6*), the nuclear elongation factor 1-alpha (*Ef1*-alpha), the nuclear *white* gene, and the second internal transcribed spacer region (ITS2) of the ribosomal DNA will be employed to evaluate genetic structure of *An. darlingi* populations, to estimate its phylogenetic placement within *Nyssorhynchus*, and to examine the existence of species complexes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Results of genetic analyses of Brazilian *Anopheles darlingi Cox1* mitochondrial gene (from the states of Amapá, Pará, São Paulo, Acre, Paraná, Rio de Janeiro, Espírito Santo, Minas Gerais and Mato Grosso), showed that individuals from the localities situated eastern of Serra do Mar, in the Mata Atlântica, share haplotypes that are distinct from those from the Amazon region and Central America. Individuals from Rio de Janeiro are more closely related to individuals from Amapá, rather than those from São Paulo and Minas Gerais. Considering the high genetic distance between individuals from Espírito Santo and Rio de Janeiro and those from other Brazilian localities, we have hypothesized that the population from eastern Mata Atlântica may have either a substantially different behavior or distinct vector competence and capacity to transmit human *Plasmodium*.

Photographs depicting morphological characteristics of the male genitalia of Anopheles benarrochi *s.l.. Form 1, A: ventral claspette; B: detail of ventral claspette (AC15-109); Form 2, C: ventral claspette; D: detail of ventral claspette (AC18-115); E: dissected ventral claspette; F: detail of dissected ventral claspette. Sallum et al. 2008*

Nyssorhynchus is one of the most studied subgenus of Neotropical *Anopheles*. However, there are several questions regarding the taxonomy of this group. The combination of morphological and gene sequence data obtained from several species of the genus *Anopheles,* either suggest or corroborate the presence of several species complexes and undescribed rate within *Nyssorhynchus*. Moreover, it is important to consider the possibility that junior synonyms of species may be valid.

Anopheles oswaldoi s. l. is a sibling species complex in South America. In the project, we redescribed and performed the molecular characterization of *An. oswaldoi*, using samples from the type locality, and also designated the lectotype.

Anopheles strodei includes five species in the synonymy, *An. ramosi*, *An. arthuri*, *An. artigasi* and *An. albertoi*, described in Brazil, and *An. lloydi*, in Panamá. Morphological and molecular evidences from the *Cox1* and *ITS2*, corroborate the fact that there are at least four species under the name *An. strodei* in Brazil and, thus, its junior synonym may be a valid species.

Regarding *An. lutzii*, morphological and molecular evidences of the *ITS2* rDNA support the presence of at least three species that can be misidentified as *An. lutzii* when using characteristics of the adult female.

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ZOOLOGY

THEMATIC PROJECTS

SYSTEMATIC AND EVOLUTION OF NEOTROPICAL HERPETOFAUNA

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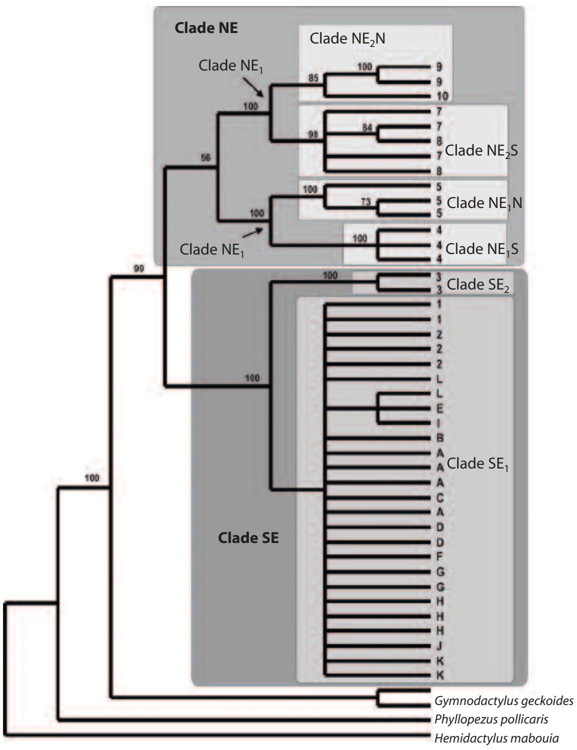
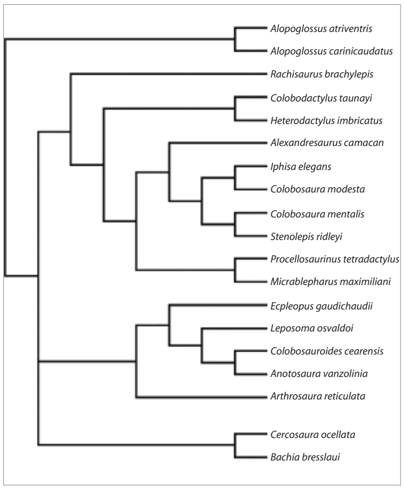


Molecular phylogeny of the Gymnodactylus darwinii *complex for a region of mtDNA cytochrome b recovered by maximum parsimony (L=699; CI=0.72; RI=0.88). Numbers at nodes are bootstrap values (>50%)/posterior probability (Bayesian searches). Numbers and letters at terminals correspond to sampling localities (Pellegrino et al., 2005)*

The present proposal aims to continue and expand the ongoing multidisciplinary research on the systematic and evolution of the herpetological fauna from Neotropical areas, as well as the study of historical biogeography of Neotropical reptile, amphibian and small mammal faunas. We intend to: (1) expand the karyological data so far gathered for Brazilian lizards, amphibians and rodents, in order to better understand their chromosomal evolution and detect useful characters for phylogenetic analyses; (2) complete the ongoing studies on taxonomy, karyotypes and DNA sequencing on the herpetological fauna of São Francisco dunes, obtaining intra and interspecific rates of divergences for endemic ratio; (3) study the heterocrony of the genus Calyptommatus and the ontogeny of the related Gymnophthalmini genera in order to drive future studies on the developmental biology of the group; (4) proceed with the ongoing collection of karyotypes and DNA sequences data on lizards of genus *Leposoma* to better understand its diversity and phylogeny; investigate the origin of parthenogenesis in *L. percarinatum* comparing the process with that of occurring in *Gymnophthalmus underwoodii*; (5) increase the taxonomic and character sampling to the study of the phylogenetic relationships within the Gymnophthalmidae, in order to obtain a robust hypothesis based on multiple data sets; (6) increase the sampling for lizards of genera Gymnodactylys, Coleodactylus, and Mabuya to allow phylogenetic and historical biogeography studies using morphological, karyological and molecular data;
(7) conduct preliminary comparative studies on selected ratio of herps and small rodents with discontinuous distribution on Amazon and Atlantic forests and on rainforest remnants in the northeastern Caatingas, in order to obtain approximate times of divergence for different groups in these forested areas.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have described the adult skeleton and developmental series for five lizard genera of tribe Gymnophthalmini (*Calyptommatus, Notobachia, Procellosaurinus, Psyllophthalmus, Scriptosaura,* and *Vanzosaura*). A series on studies on the genetical mechanisms involved in limb degeneration, and ecological and physiological studies on adaptation to fossoriality in some of the lineages of Gymnophthalmidae are being conducted.



Phylogenetic tree for gymnopthalmid genera estimated under Bayesian method of the combined morphology and molecular data set and posterior probabilities (above nodes) for clades recovered at the 50% majority rule consensus topology (Rodrigues et al. 2007)

A preliminary phylogeny of Gymnophthalmidae was recently published. We are now working on a new molecular based hypothesis for the Gymnophthalminae with 40 terminals (27 genera and 38 species), using as external groups, exemplars of the sub-families Cerocasaurinae, Rhachisaurinae and Alopoglossinae.

Our studies on the phylogeny of other Neotropical lizard's genus *Cnemidophorus, Enyalius, Leposoma, Coleodactylus,* and *Gymnodactylus* have also been fruitful. Molecular data on the small forest litter lizards genus *Coleodactylus* has shown a high and unexpected diversity, as well as very high species divergence rates, oldest clades dating from Cretaceous. The morphological data shows that there are cryptic species to be described, but the morphology seems to be highly conservative in the large Amazonian group, where molecular data shows high levels of differentiation. The molecular studies on the forest lizards genus *Gymnodactylus* and *Enyalius* has also revealed the existence of undescribed new species and general patterns of differentiation for the Atlantic forest populations. We are now exploring along the role of the Doce River, as a barrier to speciation using selected populations of frogs and lizards obtained from North and South of Doce River.

The results on anuran chromosomal characterization have been published for genera *Chaunus*, *Leptodactylus*, and *Proceratophrys*. The later has been subject of molecular studies considering mitochondrial and nuclear genes revealing different degrees of divergence among populations of southeastern Brazil. We are working also on the construction of new hypothesis of relationships for specific anuran groups using morphological data.

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PHYLOGENETIC RELATIONSHIPS IN THE CHARACIDAE (OSTARIOPHYSI: CHARACIFORMES)

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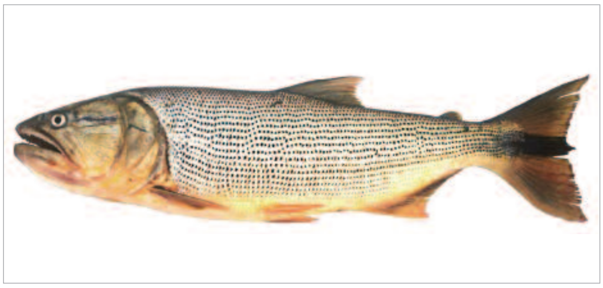


Examples of small (above, Moenkhausia sanctafilomenae*), medium (*Brycon hilari*) and large (bellow,* Salminus brasiliensis*) size Characidae species (original photographs)*

The neotropical freshwater fish fauna is the richest and most diversified in the World, consisting of 71 families, several hundred genera, and approximately 6,000 species. The family Characidae, with 12 subfamilies, 167 genera and about 980 recognized species, contain approximately 21% of the fishes species now recognized in the neotropical freshwater ichthyofauna. Characids are commonly known in Brazil as dourado fishes, tetras, dogtooth characins, elongate hatchetfishes, characins, pacus, tambaquis, redbelied pacus and piranhas among other names and range in size from small (up to 15 cm length), medium sized to large species (20 to 100 cm length). The confused and unresolved phylogenetic relationships among the characid ratio presents a major impediment to the advancement in the understanding and the conservation of the neotropical freshwater fish fauna. Our goal is to undertake an analysis of the phylogenetic interrelationships of the Characidae by the use of cladistic methodologies, applied to anatomical (mostly skeletal) and genetic/molecular characters applied to an group of at least 145 generic terminals.

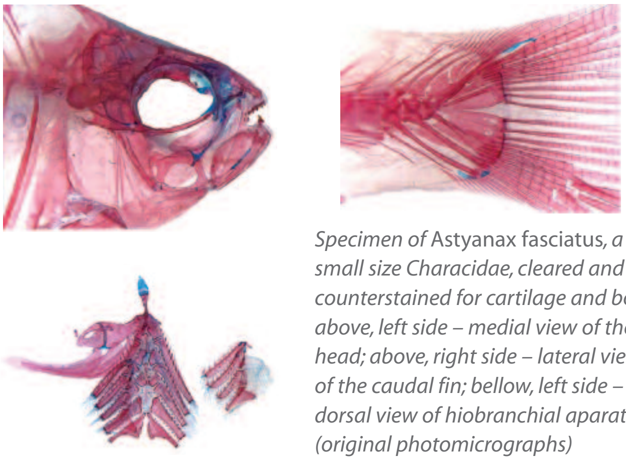
These analyses, when completed, would result in: 1) a phylogenetic hypothesis based on a broader ratio sample than was previously attempted, with a particular focus on the about 90 *incertae sedis* genera; 2) a test of the hypothesized monophylus of all of the 12 recognized characid subfamilies; 3) the examination of the phylogenetic position and limits of the Acestrorhynchidae, Cynodontidae, and in particular the Gasteropelecidae, a group that has been typically considered to be *incertae sedis* in the Characiformes; 4) the formulation of a more robust hypothesis about the limits of the Characidae based on the large number of terminal ratio in the analysis; and 5) provide a framework for the study of phylogenetic relationships within the genera presently included as *incertae sedis* in the Characidae.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

At the present date, we have obtained, for the anatomical part of the analysis, representative specimens of 102, in addition to representative specimens of five additional terminals not originally included in our in-group. All the approximately 200 representative specimens had their taxonomic identifications carefully checked and were submitted to a method for clearing and counterstaing for cartilage and bone, utilizing alcian blue for cartilage and alizarin red S for bone, together with proteolytic enzyme digestion for tissue clarification. The specimens were dissected and the resulting homologous skeletal complexes and/or structures were photomicrographed.

For the genetic/molecular part of the analysis, we have obtained partial sequences of the 16S RNA gene of at least 50 generic terminals of our in-group.

As partial results, we have produced a phylogenetic analysis of the genera *Brycon* and *Henochilus*, a complete species-level phylogeny of the gasteropelecid freshwater hatchet fishes, plus some important preliminary hypothesis about the true composition and phylogenetic position of the characid subfamilies Agoniatinae and Clupeacharacinae. Also, as byproducts and complements of the project main investigative objectives, we are preparing a myological atlas of *Brycon orbignyanus*, a generalized characid, plus species-level phylogenetic analyses of the characid genera *Agoniates*, *Bario*, *Deuterodon*, *Ctenobrycon*, *Microschemombrycon*, *Moenkhausia* and *Tetragonopterus*.



Specimen of Astyanax fasciatus*, a small size Characidae, cleared and counterstained for cartilage and bone: above, left side – medial view of the head; above, right side – lateral view of the caudal fin; bellow, left side – dorsal view of hiobranchial aparatus (original photomicrographs)*

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