

BIOTA-FAPESP PROGRAM



THE STATE OF SÃO PAULO  
RESEARCH FOUNDATION

# BRAZILIAN BIODIVERSITY RESEARCH: A PROMISING FUTURE



# KNOWLEDGE TO SUPPORT CONSERVATION AND SUSTAINABLE EXPLOITATION OF BIODIVERSITY



From Amazonia to the Cerrado (the Brazilian savanna), from the Atlantic Rainforest to Caatinga, from the Pantanal to the Southern grasslands, Brazil displays one of the richest diversities of natural environments and of plant and animal species in the world.

FAPESP, the State of São Paulo Research Foundation, one of the main Brazilian agencies for promoting scientific research, plays an outstanding role in the effort to map and understand the biological diversity essential for the survival of human species.

The Biota-FAPESP program, started in 1999, brings together hundreds of scientists in a Virtual Institute for Biodiversity. Their aim is to map and analyze the origins, diversity and distribution of the flora and fauna in the State of São Paulo. The institute generates scientific knowledge that has a role in supporting the formulation of public policy on the environmental conservation or restoration of forest remnants and to assess the possibilities for the sustainable exploitation of plants or animals with economic potential.

## 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 15th in the number of scientific papers published.

The State of São Paulo, with 40 million people and 34% of Brazil's GNP responds for 53% of the science created in Brazil. The state hosts the University of São Paulo (USP) and the State University of Campinas (Unicamp), both classified among the 200 best in the world by the Times Higher Education Supplement (THES), the growing University of The State of São Paulo (UNESP), 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 State of 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 46 years, 89,000 fellowships and 80,000 research awards.

In 2008 FAPESP will invest US\$ 388 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 Thematic Projects, Young Researchers Awards, the RIDC (Research, Innovation and Dissemination Centers) Program and the Biota-FAPESP Program.

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.

Contact FAPESP ([www.opportunidades.fapesp.br](http://www.opportunidades.fapesp.br)) or the coordinator from the Biota project which interests you and see how to obtain a post-doctoral internship.





## BIOTA-FAPESP PROGRAM: MAPPING BIODIVERSITY IN THE STATE OF SÃO PAULO, BRAZIL

Since 1999 the Virtual Institute of Biodiversity (<http://www.biota.org.br>) has been studying biodiversity in the state of São Paulo, Brazil. The institute's mission is to catalogue and characterize the biodiversity in the State of São Paulo, and define the mechanisms for its conservation and sustainable use.

Scientists from the main public universities, some private universities, research institutes and NGOs participate in the program. The program involves approximately 400 scientists holding PhDs, plus 500 graduate students. In addition there are 80 collaborators from other Brazilian states and approximately 50 from abroad.

In nine years, the Biota-FAPESP Program has supported 80 major research projects, trained 150 science Masters and 90 PhD students, produced and stored information on approximately 12,000 species and managed to link and make available data from 35 major biological collections.

This effort is summarized in 548 articles published in scientific journals. In addition to this, the researchers have published 16 books and two atlases.

In 2001, the program launched an open-access electronic peer-reviewed journal, Biota Neotropica (<http://www.biotaneotropica.org.br>), to report original research on biodiversity in the Neotropical region. The journal is becoming an international reference in its area and it is indexed by the Zoological Record, CAB International, Directory of Open Access Journals and the Scientific Electronic Library Online/SciELO.

In 2002 the program began a new venture called BIOprospecTA (<http://www.bioprosecta.org.br>), in order to search for new compounds of economic interest – extracts and pure compounds from plants, microorganisms, marine organisms and other natural sources – which has already submitted patents for 3 new drugs.





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## ATLANTIC OMBROPHYLUS DENSE FOREST: FLORISTIC COMPOSITION, STRUCTURE AND FUNCTIONING WITHIN THE “SERRA DO MAR” STATE PARK

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Structure and floristic composition will be determined in the following Atlantic Forest types: Low Land Ombrophylus Dense Forest (5 to 50 m above sea level), Sub Montana Ombrophylus Dense Forest (50 to 500 m above sea level), and Montana Ombrophylus Dense Forest (500 to 1.200 m above sea level). All trees with a DBH  $\geq$  4,8 cm that fall inside 14 1ha permanent plots, divided into a grid of 10 x 10 meter parcels, will be considered. The 14 permanent plots will be established along the altitudinal gradient, 4 independent plots in each forest type plus 1 plot of Restinga Forest (sand dunes type of forest) and 1 extra plot of Low Altitude Ombrophylus Dense Forest. In the case of botanical families with relevant ecological roles, such as the Leguminosae in the nitrogen cycle, or Bromeliaceae, Melastomataceae, Rubiaceae, Solanaceae, Moraceae and Piperaceae responsible for the maintenance of key populations of pollinators and dispersors, a comprehensive floristic survey will include herbaceous, lianas and epiphytes. Data analysis will be conducted using the FITOPAC program. Where appropriate, more detailed analyses will be conducted using multivariate methods such as Canonical Correlation, Correspondence Analysis, PCA and PCO.

The database on composition and structure of the forest will allow a choice of species for more detailed studies on reproduction biology, seed anatomy and reserves, germination, photosynthesis and water use efficiency, nitrogen assimilation, transport and metabolism, plant populations structure and dynamics, techniques, genetic structure of plant populations using molecular markers, determination of forest age by DBH classes and using  $^{14}\text{C}$ , determination of annual average growth

*Micrometeorological  
Eddy Covariance flux  
tower installed at  
Núcleo Santa  
Virgínia/PESM, São  
Luis do Paraitinga/SP*



rates of key species, and phenology. Multivariate analyses will be used to check for functional groups, or groups of species that share a common behavior and ecology. The comparison of different groups along the altitudinal gradient will allow investigation of the effect of altitude in the functioning of these groups. Simultaneously, the inputs of nitrogen through precipitation, biological fixation, and soil mineralization and nitrification will be determined, along with key parameters of N losses through denitrification and export by streams, allowing a preliminary nitrogen mass balance along the altitudinal gradient. Water and carbon balance of the forest will be estimated along with the seasonal variation of this balance through use of micrometeorological towers and Eddy-covariance technique. The photosynthesis/respiration balance of the ecosystem will be used to determine the role of the forest as a sink or source of carbon to the atmosphere.

Our final goal is to integrate the results of all activities listed above, scaling-up from individual trees, to families, to functional groups, and finally to phytophysiognomies, allowing us to understand forest structure and functioning.

The outcomes of this project will allow, for the first time, a full comparison between the Atlantic ODF and the Amazon ODF, and will enhance our capability in understanding how this biome will respond to future climatic changes.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

As established in the initial chronogram, all 14 parcels of 1ha have been set and their topography delineated through 1 meter contour lines. We have one plot in Restinga Forest, 5 plots in Low Land ODF, 4 plots in Sub Montana ODF and 4 plots in Montana ODF four parcels. In terms of structure, we can already show that the Atlantic Ombrophylus Dense Forest differs significantly from the Amazon Ombrophylus Dense Forest, since it has a lower canopy height (18 to 20 meters) and a different relation between height and diameter. Overall, we also found significant differences among the four phytophysiognomies along the altitudinal gradient. In the Restinga Forest, we found 90 species per hectare, this number increases to 130 species in the Low Land ODF (50 – 100 m of altitude) and in the Montana ODF (500 a 1000 m). In the Sub Montana ODF (100-500 m of altitude), we found so far the highest diversity, with more than 200 species per hectare.

The first results of floristics and phytossociology allowed us to choose species or group of species suitable for representing each phytophysiognomie for auto-ecology and population ecology studies. In the cariotypes of Fabaceae species, it was observed dominance of metacentric chromosomes, and a lower number of submetacentric chromosomes. Chromosome numbers varied from  $2n = 14$  to 24. Based on floristic and phytossociology studies, we choose species or group of species, like the palms for studies of population structure and distribution.

Regarding ecosystem functioning, we are developing studies about carbon and nitrogen cycles, including stock of this elements in the soil, in living biomass, recycling via litter fall, and potential losses via flux of gases. The first results showed that there are significant differences in the gas flux of carbon and nitrogen along the altitudinal gradient, suggesting that ecosystem functioning differs along this gradient. The main component of the living aboveground biomass are the trees, being responsible for more than 90% of the total; pteridophytes are responsible for less than 1% and palms from 1 to 5% depending on the altitude.

The highest total aboveground biomass was found in the Sub Montana ODF (235,42 Mg.ha<sup>-1</sup>) followed by the Montana ODF (223,87 Mg.ha<sup>-1</sup>). Low land ODF, both disturbed (T.B. Explorada) and not disturbed, had a lower biomass. The lowest biomass was found at the Restinga forest.

An integrated analysis of the results obtained so far, allowed us, for instance, to use species niche distribution tools, like the Genetic Algorithm for Rule-set Prediction/ GARP, coupled with global warming models, like the Hadley Center model. The preliminary modeling efforts have shown that an increase of  $\leq 2^{\circ}\text{C}$  within the next 50 years, an optimistic scenario, would result in the reduction of 30% of the area today used by 30 species of trees of the Atlantic Forest. In a more realistic scenario, with an increase of  $3^{\circ}\text{C}$ , the reduction of the potential occurrence area of these species will be in the order of 70%.

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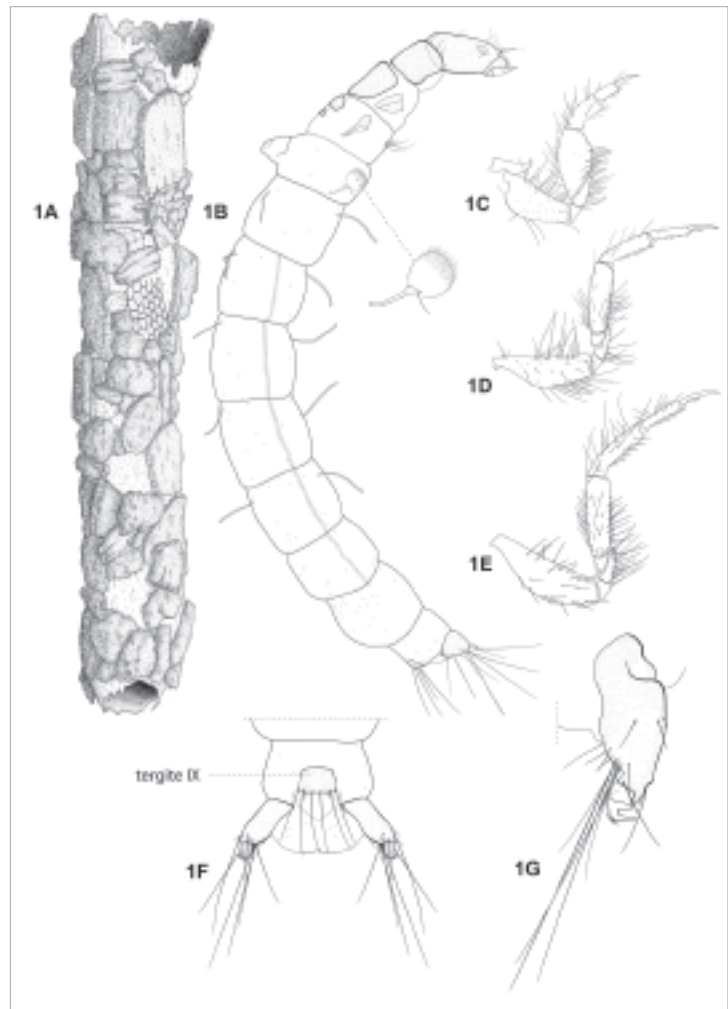
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## SURVEY AND BIOLOGY SÃO PAULO STATE OF BENTHIC FRESHWATER INSECTS AND OLIGOCHAETES

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The present project aims at carrying on faunistic survey of two groups of benthic freshwater macroinvertebrates, insects and oligochaetes, with emphasis on lotic systems. The previous project included also crustaceans and molluscs. For both, a survey was made in the main hydrographic basins of the State. The area sampled for insects was smaller due to the much poorer knowledge of their taxonomy. The emphasis was on protected areas in Serra do Mar and Serra da Mantiqueira in order to have a better knowledge of local faunas and ecological aspects included. The survey of oligochaetes also was restricted, as the group was included into the project in the beginning of the third year. For the present project, the area to be sampled is enlarged, with three new areas in the State, the State Parks of Vassununga, Fumas do Bom Jesus and Caetetus. Two areas from the first project, The State Parks of Intervales and Campos do Jordão, are maintained because there are still ongoing research projects there. Samplings in the neighborhood of the principal researchers' institutions (Araraquara, São Carlos and Ribeirão Preto) will also be continued. At the end of the second year, the inclusion of new areas will be considered. The project comprises three subprojects, to be carried on in an integrated manner, optimizing resources and field activities: Freshwater Diptera (Chironomidae, Simuliidae), Ephemeroptera, Plecoptera, Trichoptera



*Notalina morsei* Holzenthal. Larva: A) larval case; B) body, legs removed; lateral sclerite of abdominal segment I enlarged; C) fore leg; D) mid leg; E) hind leg; F) Abdominal segments IX and X, showing tergum IX; G) abdominal proleg and anal claw, lateral

(EPT), and Oligochaeta. Other aquatic insects will also be collected. A significant part of the material will be deposited in the Museum of Zoology of the University of São Paulo.



## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Research areas covered include taxonomy, biology and ecology, extending to environmental impacts and biomonitoring.

The necessity of bringing together the creation of scientific knowledge and its effective use in decision making and environmental management is one of the great challenges of the project. In this context, an agreement has been made between participants of the project and CETESB, the State's Sanitation Organization, aiming to develop a firm taxonomic basis needed to develop metrics to be used in the quality standards of its laboratories.

Checklists and identification keys, important as tools for the evaluation of the environmental status of streams and rivers are being made and included in the site.



*Gripopteryx*: *Gripopteryx* sp.,  
nymph (*Gripopterygidae*: *Plecoptera*).  
Photo L.S. Lecci.

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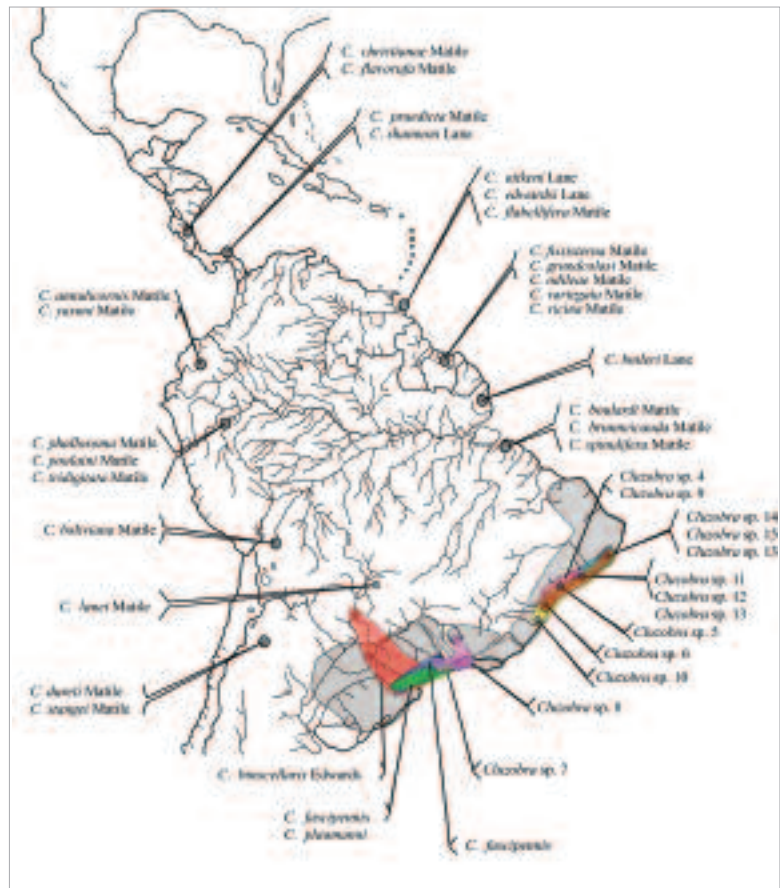
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## GEOGRAPHIC LIMITS AND CAUSAL AGENTS OF DIPTERA ENDEMISM IN THE ATLANTIC FOREST

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Biological diversity is heterogeneously distributed in space, so biodiversity knowledge cannot properly be reduced to a list of names. Even though attention given to biodiversity has grown in the last decades, quite few is still known about the geographic patterns of distribution of species and the historical causes of these patterns. It is still not possible to map the distribution of Brazilian species of most groups of animals and plants – not even in the most well studied areas – and it is neither possible to determine the sequence of events in the geological history of the continent that resulted in these patterns of distribution. This deficiency has implications for conservation policies and for the understanding of the evolutionary processes in the tropics. Conservation decisions are largely dependent on precise knowledge of the geographic distribution of species. The understanding of the rate of biological evolution deeply depends of a correct association between the geographic range of species and the age of the events that may have caused these speciation processes in the past. This project uses more than 200,000 specimens of Diptera – one of the four megadiverse insect orders – collected with standardized methods along the Atlantic Forest, with emphasis in the State of São Paulo, to delimit areas of endemism of Diptera groups and to connect these areas in a hierarchy that reflects the history of geological events that resulted in the



Example of a distribution map of a single genus, *Cluzobra* (Mycetophilidae), for the Neotropical Region and with the new species collected in the study. In gray, are the hypothesized general areas of endemisms for Diptera along the Atlantic Forest. The grey areas represent the general hypothesis of areas of endemism along the Atlantic Forest for Diptera group. Differently from most species described, known only from the type-locality, the species studied in the project have species ranges known (color). There is still material from the project from central and northern states along the Atlantic Forest with material to be analysed

present biodiversity in this area. As a byproduct of the project, a Home Page is being developed with identification keys, catalogs of Neotropical families of dipterans, photographs, and information on biology and distribution maps, to be used by the general public and by researchers with different degree of expertise on Diptera systematics or conservation.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project is dealing with a collection with over 200,000 specimens belonging to more than 60 families of Diptera. The groups that have been reared below the family level resulted in the identification of species of almost 200 genera. The genera worked out resulted in the identification of almost 400 species, of which about 200 are new. The number of actual new species, however, will be far greater than this, since many families have still not been identified at the species level. General numbers, hence, point that the size of the overall Diptera diversity in the entire Neotropical region may be five times the number of species now identified in the group, of about 31,000 species. Being the most important study on Diptera diversity in the Atlantic Forest ever, the large sample along the study area covered in the project resulted in the correction of the number of areas of endemism identified for diptera groups: the seven areas proposed before were reduced to four main areas – even though subdivisions at the population level may exist. Also, the distribution of the species of many groups showed to be much more dynamic than expected, with at least marginal overlap in the distribution of species in different areas. The overlap in the Atlantic Forest, between species belonging to taxa of tropical distribution and taxa also present in Chile, Australia, and New Zealand showed the co-existence of species belonging to different temporal “layers” in the same areas. The secondary occupation of tropical terrains by species originated in temperate areas implicates in a modified understanding of the evolution of the Atlantic Forest and in new recommendation to the construction of analytical methods of biogeography. Possibly the cycles of glaciations and interglaciation occurring in South America, since the late Cenozoic, have been much more responsible for the alteration of the limits of species distribution than for speciation processes.

Very few biogeographical studies have been made with the amount of detailed original data on species distribution in the Atlantic Forest used in this project. The results generated allowed modifying different aspects of numerical methods of biogeography analyses. This includes the use of information on marginal overlap between species distribution, the subdivision of grids with geographical coordinates based on topographical accidents, the identification and separate use of allochronic taxa in biogeographic matrices, and the establishment of adequate protocols for different steps of the biogeographical study. The results of the project allow that new studies address biogeographic problems for which there is insufficient knowledge on its dipteran composition and connections. Certain areas with rather isolated spots of Atlantic Forest in eastern Mato Grosso do Sul, Goiás, central and west Bahia and Minas Gerais, and the state of Ceará have been so scarcely studied that cannot, with the information at hands, be included in general maps of species distribution. New studies can address precisely these areas, resulting in precious information for conservation purposes.

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## SEARCH FOR POTENTIAL ANTITUMORAL, ANTIOXIDANT, ANTIINFLAMMATORY, ANTIDIABETIC, ACETYLCHOLINESTERASE AND MIELOPEROXIDASE INHIBITORY NATURAL COMPOUNDS FROM CERRADO AND ATLANTIC FOREST

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*Plants and endophytic fungi-derived extracts and pure compounds*

The changing strategies for preservation and sustain the diversity in Brazil in the past few years evidences the intrinsic value of this enormous biological resource of potentially new bioactive compounds, and represents one of the greatest challenges nowadays facing the accelerated process of devastation of several Brazilian biomes. The systematic bioactivity evaluation of crude extracts and pure compounds from São Paulo state biota associated with additional information on chromatographic profile, spectrometric data and/or biological activity shall result in value-added material, which will be available for further studies. The might converge to hits or lead compounds to pharmaceutical, agroceutical, nutraceutical, cosmetics industry, which is expected to bring great contribution for conservation and sustainable economic development from the biodiversity of São Paulo state.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

As part of our initial objectives, partial insertion of data from collections of plant material and preparation of plant extracts has been performed, using the BIOprospectA databank (<https://bioprosecta.iq.unesp.br/scyllaProspecta/>).

Our bank of plant extracts, currently at Botanic Institute – IBT-SMA, Sao Paulo, has ca. 1,800 extracts from Sao Paulo State main biomes: Cerrado and Atlantic Rainforest. Expansion of this bank has not been significant over the past three years due to CGEN (MMA – Ministry of Environment) restrictions regarding collection and access to biological material. In addition, a collection of endophytic fungi extracts has been organized at NuBBE (Chemistry Institute-UNESP-Araraquara) which has been proven a rich and attractive source of bioactive natural products.

Samples from the bank of extracts have been assayed for antifungal, antioxidant, antimalarial and antitumoral activity, in addition to inhibitors of inflammation-related enzymes myeloperoxidase and cyclooxygenase, and acetylcholinesterase, which is involved in CNS related diseases. This preliminary screening indicated ca. 6% of tested samples presented bioactivity.

Modern phytochemical methodologies, including hyphenated chromatographic/spectroscopic techniques; and for bioactivity evaluation, using fast, sensitive and reproducible preliminary bioassays have been combined for conducting systematic studies, which resulted in the isolation of several bioactive compounds from selected plant species. Such screening allowed the selection of promising crude extracts, which have been chemically investigated in depth and afforded ca. 250 pure compounds. The obtained natural metabolites had their biological/pharmacological properties evaluated, which resulted in antitumor clerodane diterpenes from *Casearia sylvestris*, cytotoxic piperidine alkaloids from *Cassia leptophylla*, antioxidant glucosylxanthones from *Arrabidaea samydoides*, antifungal alkyl and benzyl sulphides from *Petiveria alliaceae*, antifungal, antibiotic and cytotoxic nor-lignans from *Styrax ferrugineus* and *S. camporum* (Styracaceae), antibiotic phenethyl fatty acid esters from *Stemodia foliosa*, cytotoxic guanidine alkaloids from *Pterogyne nitens* (Fabaceae) and *Alchornea glandulosa* (Euphorbiaceae), antioxidant flavonoids from *Nectandra grandiflora* and *Chiococca braquiata*, among others.

Such results have attracted the attention of pharmaceutical companies, to carry out co-funded research (FAPESP, FINEP) in our labs aiming the development of products containing value-added material from the Brazilian plant biodiversity.

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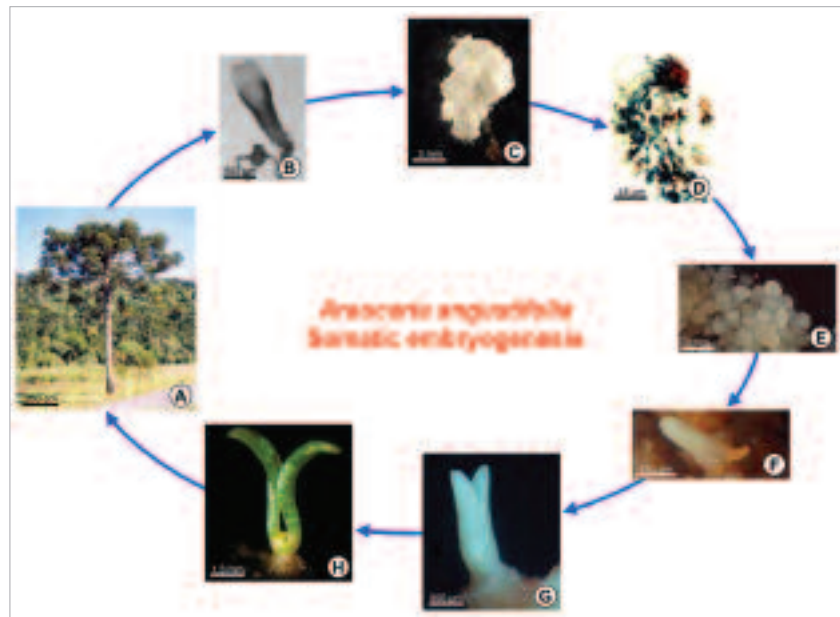
## EMBRYOGENETIC STUDIES AS A BASIS FOR STRATEGIES OF REPRODUCTION AND CONSERVATION OF TREE SPECIES

Eny lochevet Segal FLOH

Institute of Biosciences / University of São Paulo (USP)

The use of plant cell tissue and organ cultures has emerged as an important tool, when the propagation of tropical woody plants in the reforestation programs is concerned. This technique has been adopted for plants in which the massive propagation has been precluded by low productivity and or low viability of seeds, long-term seed maturation and limited vegetative propagation. In this regard, *in vitro* somatic embryogenesis has been successfully applied in production of somatic cell and viable embryos, in a morphogenetic process closely related to the natural process of zygotic embryogenesis. The major aim of this project is to investigate major physiological, biochemical and molecular changes during the somatic embryogenesis of *Araucaria angustifolia* and *Ocotea catharinensis*, two economically important woody plants in the Atlantic Forest of southern Brazil. The results would pave the way to determine a general biotechnological process required to propagate and to manage important tropical woody plants.

*Ocotea catharinensis* is an endangered native forest tree species of the Southern Brazilian Atlantic Rain Forest. Natural propagation of *O. catharinensis* throughout seeds is hampered by its recalcitrant physiology and, consequently, they cannot be stored for long periods without loss of viability. *Araucaria angustifolia* is the only native conifer of economic importance in Brazil, being the most exploited timber



Somatic embryogenesis in *A. angustifolia*.

(A) Mother tree; (B) Precotyledonary zygotic embryo used as explant; (C) embryogenic culture induced; (D) somatic pro-embryo observed in the maintenance medium; (E - H) somatic embryo on maturation treatment

source until the 1970's. Nowadays only relicts of the natural vegetation are found, representing less than 2% of the original area.

As a result of the clear-cutting form of exploitation, *A. angustifolia* and *O. catharinensis* were included in the official list of endangered Brazilian plants, under the "vulnerable" category. Therefore, it is necessary to develop technologies for the conservation and genetic improvement of these Brazilian native species.

Biotechnological tools have a large potential in breeding and biodiversity conservation programs for woody species. Biotechnology is a significant affix to the traditional tree improvement practices, and is the one that utilizes fundamental discoveries in the field of plant tissue culture for clone-forestry, gene transfer techniques, molecular biology, and genomics.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main results of this project concern to the description and analyses of different aspects of embryo development in *A. angustifolia* and *O. catharinensis*.

In order to increase the efficiency of *in vitro* embryo development, a comprehensive understanding of the biochemical and molecular events in somatic and zygotic embryo development is essential. Polyamine (PA) and nitric oxide (NO) metabolisms, as well as amino acids, plant hormones and differential protein expression, seem to be involved in the regulatory mechanisms that play important roles in certain embryo-development processes in *A. angustifolia*. Additionally, biochemical aspects during seed development have been disclosed for *A. angustifolia* and *O. catharinensis*. Some factors, such as indole-3-acetic acid, abscisic acid, polyamines, amino acid and protein contents, were studied during zygotic embryogenesis of these Brazilian species. A better understanding of biochemical alterations during zygotic embryo development, besides providing basic information on seed development, may be useful for further improvement in *A. angustifolia* and *O. catharinensis* somatic embryogenesis.

Somatic embryogenesis in *O. catharinensis*.

- (A) Mother tree; (B) Imature zygotic seed;  
(C) Isolated immature zygotic embryo used as explant;  
(D) Direct somatic embryogenesis induced from explant;  
(E) Globular somatic embryo; (F) Cotyledonary somatic embryo;  
(G-H) Somatic embryo germination



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## BIOSPHERE-ATMOSPHERE INTERACTION (PHASE 2) : CERRADO AND LAND USE CHANGES

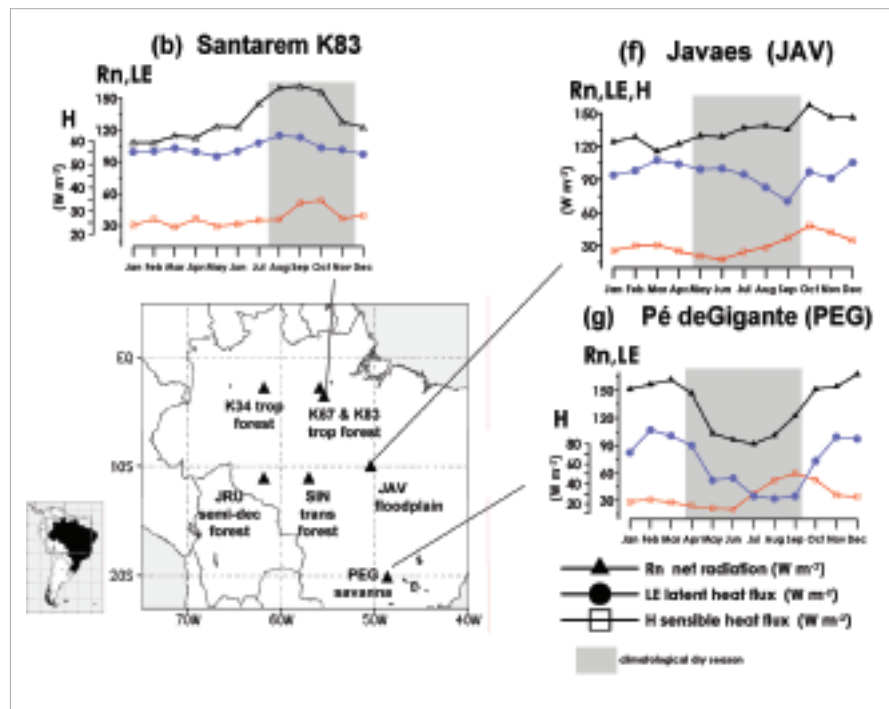
Humberto Ribeiro da ROCHA

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The project aims in investigating the functional relationships of energy, water and carbon fluxes over tropical forests, Cerrado (savanna) and agricultural ecosystems (sugar cane and eucalyptus), that includes the control of climate and environment. It is a cross-disciplinary perspective of eco-climatology and hydrology, plant physiology and geochemistry, scheduled from 2005 to 2008.

The project has a close connection with the Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA), and the team has three fronts of investigation: i) climate (climatology and land surface fluxes); ii) hydrology (hydrogeology, surface hydrology and biogeochemistry); and iii) ecology (plant physiology and ecological studies), that is coordinated to achieve the following tasks:

- 1) To monitor five experimental field sites:
  - Cerrado *sensu stricto*;
  - Ecotone (seasonally flooded savanna);
  - Tropical rain forest;
  - Agro system Sugar Cane;
  - Agro system Eucalyptus.
- 2) To measure leaf photosynthesis and water potential, according to key species and seasonality.
- 3) To measure the long term surface-atmosphere fluxes of water, energy and CO<sub>2</sub> over the field sites, using flux towers and weather stations.
- 4) To estimate the water balance for each



Source: Rocha et al. 2008, unpublished.

ecosystem on the watershed scale, using hydrogeological surveys and by measuring precipitation, evaporation and stream flow.

- 5) To characterize ecosystem functional relationships dependent on the climate and water availability, using remote sensing and field measurements.
- 6) To estimate the dynamics of C stocks in each ecosystem, and C balances in the long term by characterizing C sink or source.
- 7) To investigate how the climate variability (seasonal, intraseasonal, interannual and century-scale) controls the length and patterns of wet and dry season in the region of study.
- 8) To deploy mathematical numerical models (biosphere-hydrology-atmosphere) and field observations, to compare the productivity and water availability across the different ecosystems.



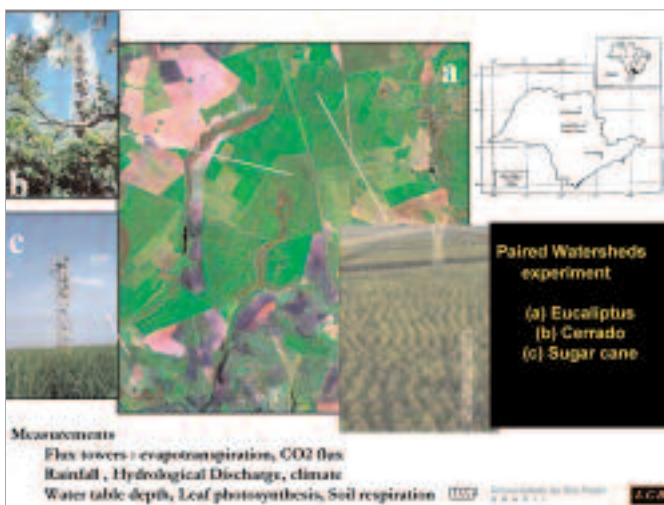
## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Four field sites with flux towers and stream gages are ran, three of them in northern Sao Paulo state (Cerrado and paired sites of sugar cane and eucalyptus), and the floodplain (savanna) site in Tocantins state. A species inventory and a set of 6 parcels were defined in the Cerrado site, where leaf photosynthesis and litter fall were measured.

*Anadenanthera falcata* Speng was a dominant species, with larger photosynthetic potentials. Photosynthetic rates show different patterns of the species, although the seasonality is similar and well defined between them.

In the forest-savanna transition in Brazil, the mean annual sensible heat flux at all sites varied from 20 to 38 Wm<sup>-2</sup> and was generally lower in the wet season and higher in the late dry season, consistent with seasonal variations of net radiation and soil moisture. At the sites where the dry season length does not exceed 4 months and annual precipitation is above 1900 mm, the monthly net radiation progressively increased along the dry season and was positively correlated with evaporation, that increased to as much as 4 mm d<sup>-1</sup> (Manaus, Santarem and Rondonia). In contrast, those ecosystems with less precipitation, below 1700 mm, and longer dry season, as in Mato Grosso, Tocantins and Sao Paulo, showed clear evidence of reduced evaporation in the dry season, to 2,5 mm d<sup>-1</sup> in the transitional forests and to 1 mm d<sup>-1</sup> in the Cerrado. The later sites showed characteristics of savanna, where the reduction of evaporation is more pronounced associated with longer dry season lengths and larger annual amplitude of air temperature. The patterns of seasonal evaporation and energy partitioning we have discussed is key to identifying two functionally different type of sites, that help to explain the complexity of functioning of tropical forests, savannas, and the transitional ecosystems that exist in between them.

Using the EVI (Enhanced Vegetation Index), photosynthesis increased during the late 2005 drought, which suggests that the resilience of the ecosystem might be higher than expected.



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## EVOLUTION OF THE SOUTHEASTERN BRAZILIAN REPTILE FAUNA FROM CRETACEOUS: PALEONTOLOGY, PHYLOGENY AND BIOGEOGRAPHY

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This main objective of this project is to study the Brazilian southeastern reptile fauna from the upper Cretaceous to present time, allowing the characterization of expansion and retraction pulses that took place in the past 70 million years, and responsible for the present configuration of the reptile biodiversity. The improvement of knowledge about these processes will help in the recognition of parameters leading to the loss of biodiversity (in different temporal scales) in southeastern Brazil and ultimately contributing to the establishment of conservation strategies and sustainable use of natural resources.

In order to develop our study, it is necessary to improve and organize our knowledge of the reptile diversity through geological time. This survey will allow cross-checking of data of extant and past faunas in a vertical axis (chronostratigraphic axis). The project possesses five major lines of scientific activities: i) a survey of the reptile diversity in the state of Sao Paulo from the upper Cretaceous to Recent through the elaboration of a database including specimens in natural history collections; ii) Inventories of living and fossil reptiles in the biomes and fossil basins of southeastern Brazil; iii) Elaboration of phylogenetic studies of key extant and extinct tax; iv) Combine data on the distribution of fossil and living tax with phylogenies and identified timeframes; v) Strengthen collections and study groups of vertebrate paleontology in the state of Sao Paulo.

The Cretaceous and Tertiary will be



*A draw of an alive Najash rionegrina, a snake with feet in the superior cretaceous in Argentina, described in Nature, 2006, by the grant coordinator and a colaborator from Argentina*

surveyed in the following basins: 1) Bauru basin (Upper Cretaceous); 2) Itaboraí basin (Paleocene) and Taubaté basin (Eocene - Oligocene). The extant reptile fauna will be sampled in localities within the two major biomes of the southeast: the Atlantic Forest and the Cerrado. Other regions of Brazil will be surveyed in order to provide a comparative background for the reptile diversity pattern found in the Brazilian southeast.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The team conducted 50 field expeditions which resulted in significant collections of fossil and recent Amphibians and Reptiles in more than 20 localities in Southeastern Brazil. Several new taxa were collected and are presently under study.

Results from research and field work can be summarized as follows:

- 1) elaboration of commented lists of snakes from the Atlantic Forest and reptiles from the State of Sao Paulo;
- 2) normalization of the scientific collections of recent and fossil Amphibians and Reptiles of the Zoology Museum of the Sao Paulo University;
- 3) discovery of a new Lower Cretaceous fossil locality in the Sanfranciscan basin, with remains of Dinosaurs and other tetrapods (the first record of fossil dinosaurs in this basin);
- 4) elaboration of a molecular phylogeny of Neotropical Xenodontine snakes;
- 5) conduction of a total evidenced analysis of the higher-level affinities of snakes;
- 6) description of several fossil crocodiles and a snake from the Upper Cretaceous of Brazil, and several amphibians and Reptiles from the Atlantic forest, Cerrado and Amazonian biomes.

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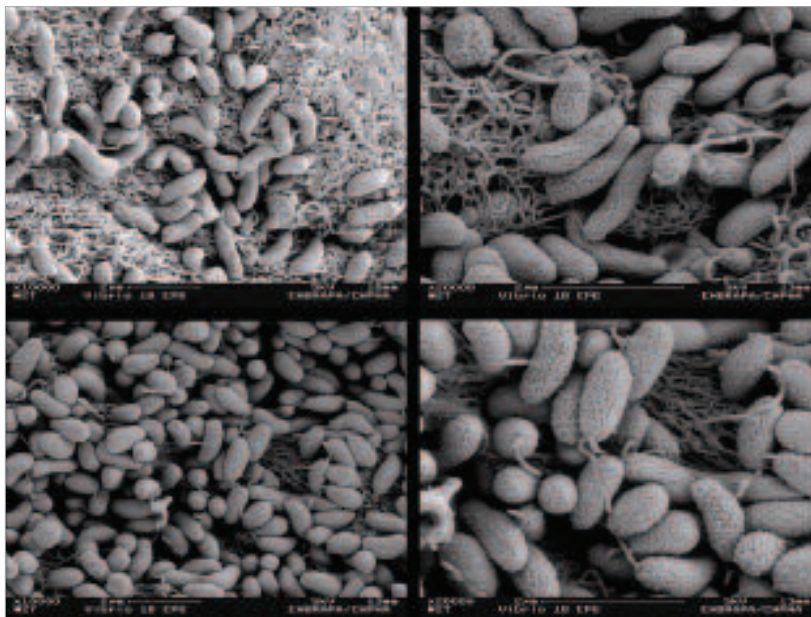
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## BIODIVERSITY AND FUNCTIONAL ACTIVITIES OF MICROORGANISMS FROM MANGROVE OF SÃO PAULO STATE

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Mangrove communities are recognized as highly productive ecosystems that provide large quantities of organic matter to adjacent coastal water in the form of detritus. This ecosystem is rich in organic matter, in general are nutrient-deficient, especially of nitrogen and phosphorus. Microbial activity is responsible for major nutrients transformations within a mangrove ecosystem. So it is open up new areas of biotechnological explorations, which drive the necessity to isolate and culture these organisms. The biochemical versatility and diversity of rare microorganisms represent an enormous variety of genes that are still unknown. The objectives of this project were: to study the biological diversity of microorganisms from sediments, rhizosphere and endophytes, especially, cyanobacterias, bacteria, fungi, actinobacterias and archeobacteria and to study the molecular diversity present within a species and genus. In this study, so far, about 2,100 bacteria, 56 actinobacteria, 850 fungi and 27 cyanobacteria were isolated from sediments, phiosphere and rhizosphere of red, white and brown mangroves. 53 endophytic bacteria, isolated from *Rhizophora mangle*, were able to fix atmospheric nitrogen. Most of these diazotrophic bacteria were identified as *Bacillus spp.*, *Pseudomonas spp.* and *Vibrio sp.* Some of the cyanobacteria strains



*Endophytic strains of Vibrio sp. isolated from red mangrove*

isolated produced secondary metabolites against *Micrococcus luteus*, and some of them, identified as *Phormidium*, *Synechococcus* and *Leptolyngbya* synthesized polyketides. Evidence for the *in vitro* antibiosis of 86 fungi was demonstrated. Organic extracts from five strains were inhibitory to the human fungal pathogens, *Aspergillus fumigatus* and *Candida albicans*. Two fungal strains completely inhibited the growth of *Trypanossoma cruzi*. Bioassay studies showed that compounds produced by two *Streptomyces* strains, isolated from rhizosphere of red mangrove, had antifungal activity against *Pythium*, an important plant pathogen in hydroponic system. In greenhouse conditions, these strains reduced the disease in cucumber. These actinobacteria may be useful for biocontrol and other applications.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In this research, it was found a great diversity of fungi and bacteria from mangrove sediments. So far, 2.100 bacteria, 850 fungi, 56 actinobacteria and 27 cyanobacteria were isolated. At the same time, the project investigates the non-cultured diversity of bacterial communities. ARDRA (Amplified Ribosomal DNA Restriction Analysis) revealed the presence of 10 ribotypes, further identified in the following genera: *Vibrio*, *Listonella*, *Aeromonas*, *Microbacterium*, *Dermabacter*, *Brevibacterium*, *Paenibacillus*, *Staphylococcus*, *Kurthia*, *Bacillus*, *Nesteronkonio*, *Kytococcus*, *Kocuria* and *Rothia*. Additionally, DGGE technique applied to access non-culture bacteria determined that Alphaproteobacteria responded to seasonal variation, while Betaproteobacteria communities were different in the summer and winter.

The biodiversity of cellulolytic bacteria was also studied. Mangrove leaves and wood are mainly of lignocellulose components that are degradable by microorganisms, and bacteria are responsible for most of the carbon flux in tropical mangrove sediments. Cellulolytic activity was found in 31 rhizobacteria that produced high amount of endoglucanase (1,4- $\beta$ -D-glucan). The confirmation of endoglucanase activities was proved by amplification of the *EglA* gene. The production of cellulase was restricted to the genera *Bacillus* and *Paenibacillus*. In high concentration of salt the strains produced significant amount of biofilms suggesting the function of this polymer as mechanisms of tolerance to saline habitats.

In this study, 53 endophytic bacteria strains, most of them belonging to the genera *Bacillus* and *Pseudomonas* and *Vibrio* were isolated from *Rhizophora mangle*. All strains, isolated in selective nitrogen free broth medium, showed the potential in fixing N<sub>2</sub>. This is the first report of endophytic strains of *Vibrio*. These results indicate that endophytic bacteria from mangrove may provide an advantage as nitrogen – fixer agents.

Bacterial strains, isolated from contaminated mangrove of Bertioga, São Paulo, produced biosurfactants that reduced the surface tension. One *Pseudomonas stutzeri* strain which produced water/oil emulsifications was able to grow in the presence of petroleum and its derivatives.

Bioprospection studies are in course involving microorganisms from mangrove. Some fungal strains produced antifungal antibiotics against *Trichophyton rubrum*, *Candida albicans*, *Aspergillus fumigates* and *Cryptococcus neoformans*. Extracts of two fungi also were effective against *Trypanosoma cruzi*. Some cyanobacteria, identified as *Phormidium*, *Synechococcus* and *Leptolyngbya*, amplified the PKS gene (polyketide synthase). These antibiotics were active against *Micrococcus luteus*.

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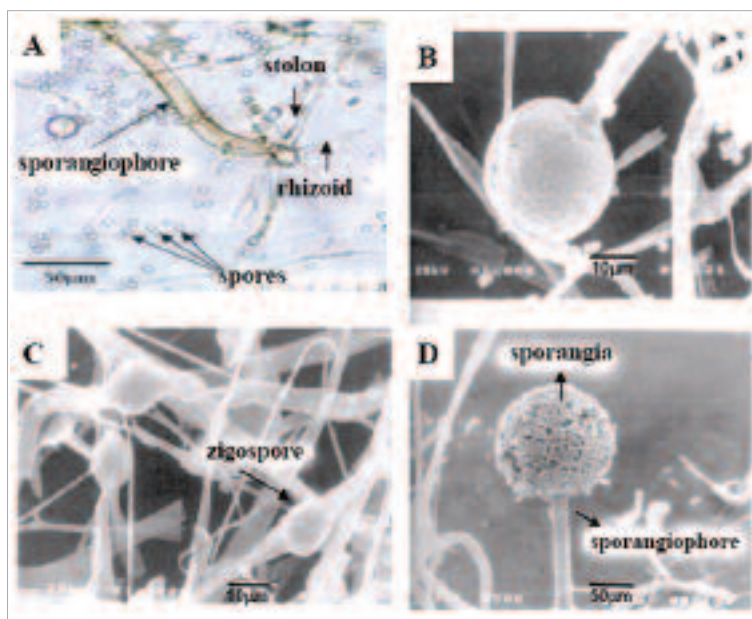
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## BIOPROSPECTION IN FUNGI: THE SEARCH OF LEAD COMPOUNDS FOR DRUG DESIGN AND ENZYMES FOR PHARMACEUTICAL AND INDUSTRIAL APPLICATIONS

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Fungi have been less explored than plants in bioprospecting programs, especially the endophytes, which remain a promising and practically unexplored source of interesting bioactive natural products. One of the major advantages of working with microorganisms in a natural products discovery program, is the possibility to screen a vast number of cultures on a small scale, before identifying the microorganisms, while retaining a high probability of success in reproducing both the activity and the metabolite production on a large-scale fermentation. Another advantage is that manipulations, in culture conditions, may lead to a wider range of compounds produced by fungi than it would be available from traditional production of extracts from plants. In this project we propose to exploit the chemical and biological potential of endophytic and soil fungi collected in São Paulo State in three approaches: i) obtainment of crude extracts from microbial cultures to be evaluated in antimicrobial assays performed in our labs and other bioassays of the network, and subsequent fractioning of most promising extracts aiming to obtain new leads for drug development;



*Rhizopus microsporus* var. *rhizopodiformis*.

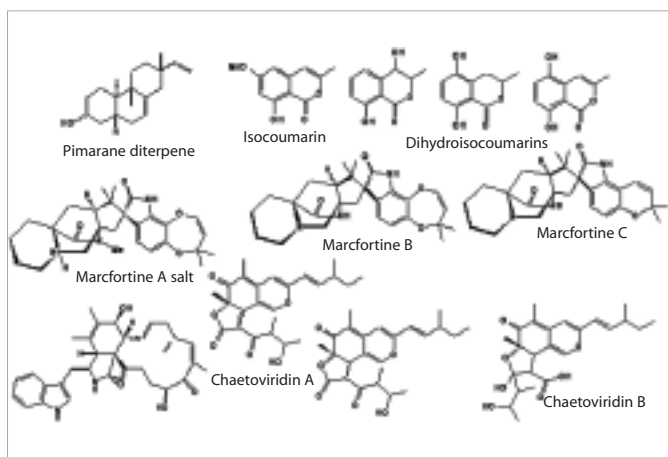
A- Photonic microscopy; B, C and D- Microscopy electron scanning

ii) obtainment of products from biotransformation reactions of bioactive natural products previously isolated in our labs (grandisin, budlein A and tagitinin C) and evaluation of their biological activities in bioassays of the network, and iii) production of enzymes, which might be useful for industrial processes (xylanase, glucose oxidase, phytase, amylase, acid and alkaline phosphatases) and as new pharmaceuticals (L-asparaginase). The proposed antimicrobial screening, as the first one, may be an indication of a wider range of bioactivity.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The collection of soil and plant samples were undertaken for the isolation and identification of fungi strains, many of which were identified either by the staff of the Federal University of Pernambuco or by molecular biology (rDNA sequence). The group has selected fungi with potential for the production of enzymes of interest, such as L- asparaginase, xylanases, pectinases and others. From soil and endophytic fungi, it was isolated more than 30 secondary metabolites. The biotransformation of grandisin and budlein was also successful. The obtained results demonstrated the potential of the isolated fungi for the production of enzymes and secondary metabolites bearing the desired biological activities.

Chemical structures of isolated  
compounds from studied fungi



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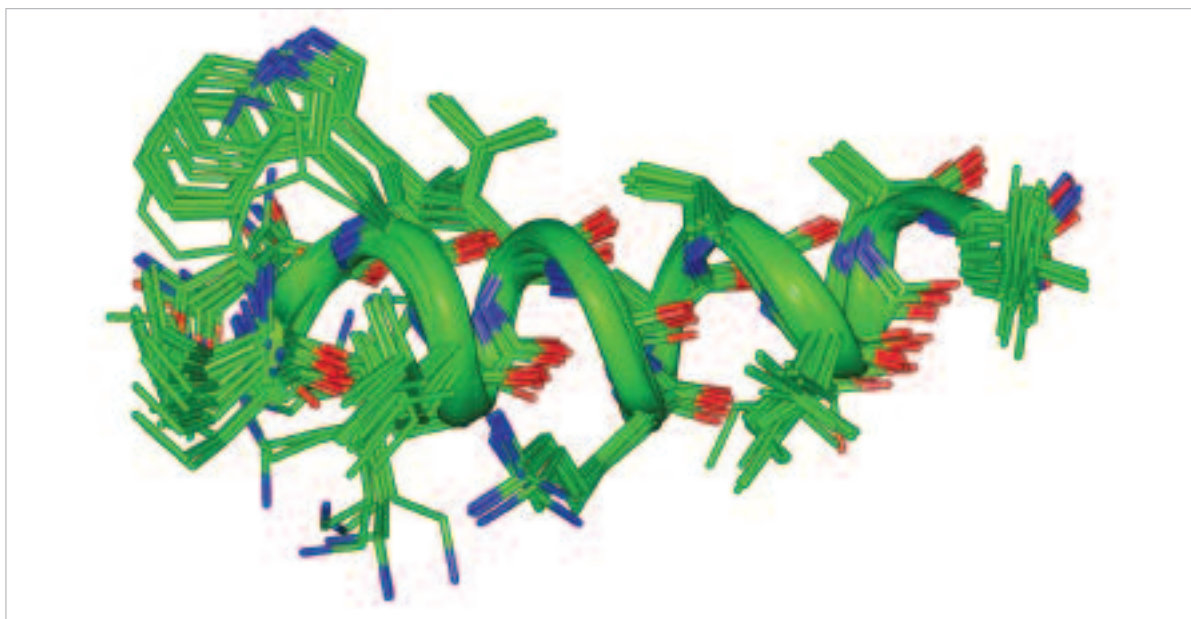
### Jairo Kenupp BASTOS

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## BIOPROSPECTION OF THE BRAZILIAN ARTHROPODS FAUNA SEARCHING FOR LEADER DRUGS FOR RATIONAL DEVELOPMENT OF NOVEL PHARMACEUTICALS AND PESTICIDES

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*Natural peptides are isolated from different biological sources, purified, sequenced and submitted to investigations of structure and activity. Their secondary structures are studied by using NMR, circular dichroism, molecular modeling and simulations of molecular dynamics. The figure above shows the secondary structure of an antimicrobial peptide determined by NMR analysis. A series of different conformations are overlapped to each other*

Recently, the low molecular mass compounds from animal origin have been subject of interest by pharmaceutical and agrichemical companies. The Arthropods are considered a source of potentially important novel molecules, offering notable properties such as: high efficiency, low probability of development of microbial resistance, limited toxicity and low immunogenicity to men. In order to bioprospect these compounds, in the Arthropod fauna from São Paulo State, the main objectives of the present project are: (i) to identify the most abundant low molecular mass compounds from the toxic secretions of spiders and social Hymenoptera presenting neuroactive actions, (ii) to elucidate their molecular structures, (iii) to synthesize and (iv) to submit them to pharmacological and

physiological bioassay screenings. Also the polycationic peptides will be investigated, as well as their sequence, structure and for some of these components, their target-receptors will be identified. The elucidation of chemical structures in general will be performed by using a series of spectroscopic techniques. When necessary, the elucidated chemical structures will be synthesized and used for functional characterization. The biological characterization of the neuroactive compounds, will include the classical neuropharmacology approach, immunohistochemistry and electrophysiology methods. The investigation of the action mechanism of the polycationic peptides will focus traditional protocols of pharmacology for pain, analgesy and inflammation and we will also investigate the antibiotic action of these peptides. The promising compounds, presenting some specific potential application at level of therapeutic use, which may be used as models for future drug development, will be submitted to an intensive investigation about structure/ activity relationship for a future rational development of novel drugs.



## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Several new natural products have been isolated from wasps and spiders secretions, most of them had their molecular structures elucidated by spectroscopic techniques (Nuclear Magnetic Resonance, Mass Spectrometry). A large fraction of these compounds had their synthesis route developed, and the synthetic compounds have been submitted to a wide range of neurotoxicity and neuroprotective assays (with different electrophysiological and neuropharmacological approaches). More than one-hundred of new acylpolyaminetoxin structures from spider venoms have been resolved, twelve alkylindole alkaloid toxins were elucidated from spider webs, and two organometallic compounds had their chemical structure determined from aerial web-spider body secretions. A defensive Spiro glycoside compound, and new analogs of histamine, presenting blocking activity against different types of ion channel receptors, were identified in the venom of some species of social wasps. Several of these natural compounds of animal origin proved to be potent neuroprotective agents, some of them with anti-epileptic activity (even in animal models), with a great potential to become models for the development of new neuropharmaceutical drugs. Some of these compounds showed selective insecticide activity against some arthropods and can become models for the development of new insecticides.

In parallel to these investigations, a great family of polycationic peptides have been isolated and sequenced from the venom of social wasps. The peptides are studied concerning their secondary structures using spectroscopic techniques (circular dichroism, fluorescence, FT-IR, and NMR) and submitted to a wide range of biological assays, including antibiosis, analgesic effect, anti-hypertensive action, anti-inflammatory action, and anti-proliferative effect (against tumor cells strains). The interactions between peptides and membranes (natural and synthetic) are evaluated through the combination of spectrometric and biophysics techniques. Some of these peptides were identified as strong antibiotics against pathogenic bacteria, while other peptides have been identified as selective ligands of some sub-types of G-proteins. Therefore we are currently working on the development of an analytical platform, where it will be possible to combine affinity chromatography and proteomic analysis for the bioprospection of G-protein coupled receptors.

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## NATIVE AND CULTIVATED PASSIFLORAS IN BRAZIL. PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATIONS CONCERNING POPULAR USES AND DEVELOPMENT OF LOCAL PHYTOMEDICINES

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The rational and scientific use of medicinal plants from the Brazilian rainforest is currently considered an important scientific and therapeutical alternative, mainly by the great pharmaceutical industries. The genus *Passiflora*, *Passifloraceae*, commonly used by the folks in tropical and subtropical regions has already been proven to have potential therapeutical activities. The species *Passiflora incarnate* has been used in many countries as anxiolytic, sedative and antispasmodic in Brazil. Many phytotherapies (medicines) with *Passiflora* extracts can be found in the market. However, since no therapeutical activity could yet be suitably proven, there are no scientific data of its efficacy. The present work aims at isolating and biomonitoring the active substances of the extracts of the species. Eventually the species with proven pharmacological activity will be characterized considering their macroscopic aspects, and the contents of their bioactive substances will also be determined.



*Passiflora* species: *P. edulis*

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In a preliminary screening, the hydroalcoholic extracts of *P. alata*, *P. edulis* and *P. coccinea* (aerial parts) presented anxiolytic-like and anticonvulsant activities in mice. Possible effects of all extracts on the spontaneous motor activity in mice were not excluded. Tested on experimentally-induced gastric ulcers in mice, the extract of *P. alata* reduced the ethanol-induced gastric ulcers in rats. The antiulcer activity of the extracts of the aerial parts of *P. edulis*, *P. coccinea* and *P. nitida* also presented antiulcer activities in the same animal model. Tested at 0.5, 1.0 and 2 g/kg, p.o., the extract of *P. incarnata* (aerial parts) obtained from commercial sources had not presented antisecretory gastric effect in pylorus-ligated mice, and was ineffective on gastric ulcers induced by 75% ethanol, cold-restrained stress or non-steroidal anti-inflammatory agents (NSAIDs). At equal doses, the extract of *P. edulis* (fruits) protected the gastric mucosa against gastric ulcers induced by cold-restraint stress and NSAIDs, but not ethanol. The results indicated that the anxiolytic-like and anticonvulsant activities detected for the *Passiflora* extracts, may be related to the presence of flavonoids in these species. These compounds have been shown to present benzodiazepine-like activity. The antiulcer activity of *P. alata* and *P. edulis* was unrelated to inhibition of gastric acid secretion, and possibly associated with its central depressant activity. The photochemical composition of fractions (hexane, dichloromethane, ethyl acetate, water) derived from freeze-dried extracts by successive partitioning, has been performed. The aerial parts of the species with proven pharmacological activity have been characterized considering their morphologic and anatomic aspects.



*Passiflora  
alata*

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## FAUNA AND FLORA OF REMNANT FOREST FRAGMENTS IN NORTHWESTERN SÃO PAULO STATE: BASIS FOR BIODIVERSITY CONSERVATION STUDIES

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Above, *D. nanus*; and below, *H. raniceps*

The fragmentation of forest habitats is an evident and growing process in tropical regions. The understanding of such changes is essential to avoid important losses in biodiversity, as well as to manage the sustainable use of the remnant biodiversity. A perfect and astonishing example refers to the vegetation from northwestern São Paulo State, composed by semi-deciduous seasonal forest and savanna. The remaining forested areas are nowadays restricted to 9% of the original forest coverage and have been replaced by pastures, several types of agricultural plantations and urban areas. Such impact characterizes this region as the most deforested and fragmented in São Paulo State. In addition, it has the lowest number of conservation units, resulting in a condition that it will not be apparently reverted without actions of ecological management. Despite such impact, it is a region of relevant species richness and that, paradoxically, have received little attention concerning to the study of its biodiversity.

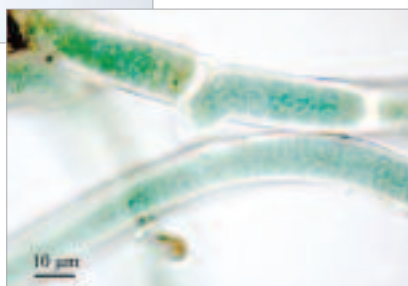
This investigation is proposed to survey several taxonomic groups (higher plants, pteridophytes, bryophytes, algae, fungi, aquatic and terrestrial invertebrates and vertebrates). Eighteen forest fragments will be sampled in the region, trying to incorporate those with distinct matrices (sugar cane and orange plantations, pastures, urban areas, etc) and sizes. The project aims are: i) to prepare an environmental diagnostic that will represent a sound basis to propose further actions for environmental conservation in the region; ii) to have a sketch on the possible effects of forest fragmentation to population dynamics and physiology animal and plant species; iii) to indicate the relevance of forest fragments or the maintenance of regional biodiversity; iv) evaluate the importance of forest fragments as a reservoir of specie with potential capacity in the colonization of deforested areas and in agricultural pest control.

The project will effectively contribute to enlarge the knowledge on biological diversity in the northwest region of the State. The relevance of this study is even more meaningful, considering that it will provide essential subsidies for future studies aiming at organizing strategies to conservation/maintenance of this valuable biological patrimony, which is tending, at the present rhythm, to a considerable decrease in short period of time.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Based on the objectives of the project, we defined two classes of fragment size: small fragments, with area between 50 and 150 hectares; and large fragments, with areas greater than 200 hectares. The limits of the north-east region of the state of São Paulo, as defined in this project, includes entirely the Units for the Management of Hydric Resources (UGRHs) of the Turvo-Grande and São José dos Dourados and parts of the UGRHs of the Baixo Pardo, Baixo Tietê and Tietê-Batalha. This area includes the administrative regions of São José do Rio Preto, Araçatuba, and part of the administrative regions of Bauru (northern portion) and Ribeirão Preto (western portion). Forty seven forest fragments were visited and, 18 being selected to be included as sample areas for the project. The sample scheme consisted of nine large fragments (designated from G1 to G9) and nine small ones (designated from S1 to S9), divided in two sets of nine fragments, to be sampled during one year (one set in 2007/2008 and another in 2008/2009). Of the 18 fragments, two (one large – G9, and one small, S4) are being sampled for two years, so as to allow the evaluation of representativity and repeatability of each sampling. In this way, in each year a set of 10 fragments will be sampled, totaling 20 samplings. Each set will be sampled, as a rule, twice a year: in the dry season (June to August) and in the rainy season (December, January and February).

The principal results achieved up to the present, can be summarized in the number of species of the groups of animals and plants studied, considering each sub-project: 1) cryptogams - 12 lotic macroalgae, 14 aerophytic algae, 20 polyploid basidiomycetes; 2) phanerogams – 215 trees, 75 lianas and 45 shrubs; 3) aquatic invertebrates: 45 zooplankton and 35 aquatic insects; 4) terrestrial invertebrates: 167 plant mites; 4.400 insects (Hymenoptera); 5) aquatic vertebrates: 36 fish; 6) terrestrial vertebrates: 32 amphibians; 138 reptiles (Squamata); 121 birds and 44 mammals.



Above, *Lyngbya cf. truncicola*; and on right, *Hassallia sp.*

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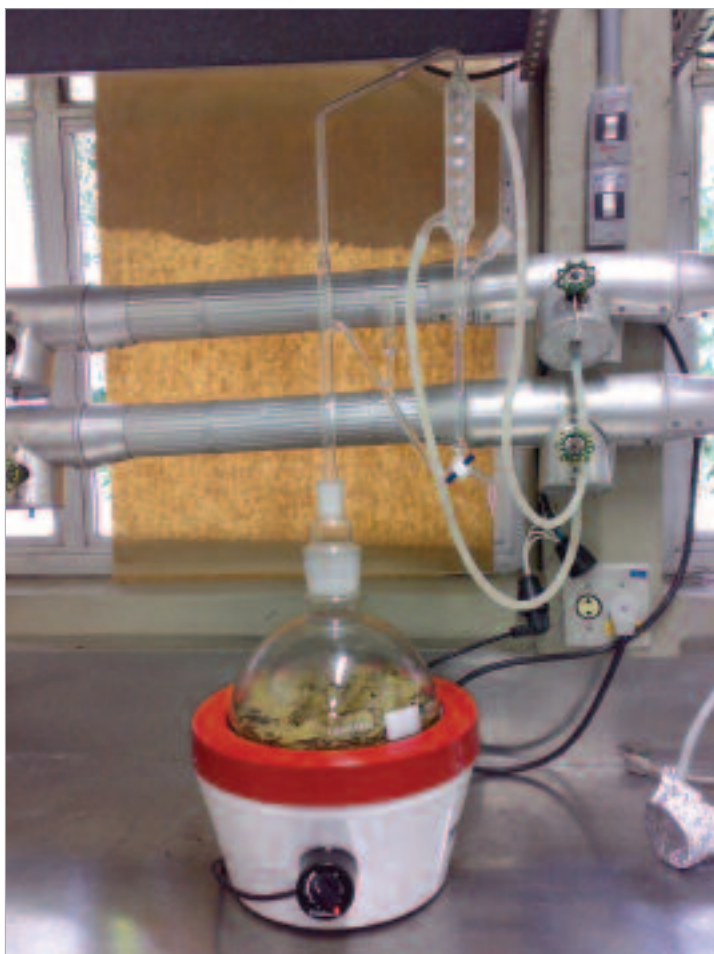
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## ATLANTIC FOREST AROMATIC IN SÃO PAULO STATE: CHEMICAL COMPOSITION OF VOLATILE OILS AND BIOLOGICAL ACTIVITY ANALYSIS

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The aims of this proposal will contribute with the knowledge of the chemical constitution of volatile oils found in native species of the Atlantic Rain Forest as well as verifying the putative pharmacological activity of these oils (antibacterial, antifungal, anti-inflammatory and antitumour activities). The species whose oils presented constituents interesting for the aroma, or pharmaceutical industries, will be selected for posterior studies of *in vivo* and *in vitro* propagation aiming at the sustainable use and the maintenance of germoplasm. The main goal of this proposal is to contribute for the sustainability of the biome, pointing its economic potential in accordance with the current demands. The search for a sustainable development has influenced either the process of selection of potential species or the methods of production of raw material. The employment of natural products in the cosmetic industries or in Phytomedicine is limited by the scale of the collection and the sustainable use of the forest. In the great majority of the cases, the industry looks for new cultivars that will guarantee the raw materials high production. In this way, the development of the biotechnology, particularly genetic engineering, facilitates the culture of medicinal and aromatic plants far off its natural habitat, contributing to evolve from extractive to cultivated.



*Clevenger apparatus for essential oil extraction by steam distillation*

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

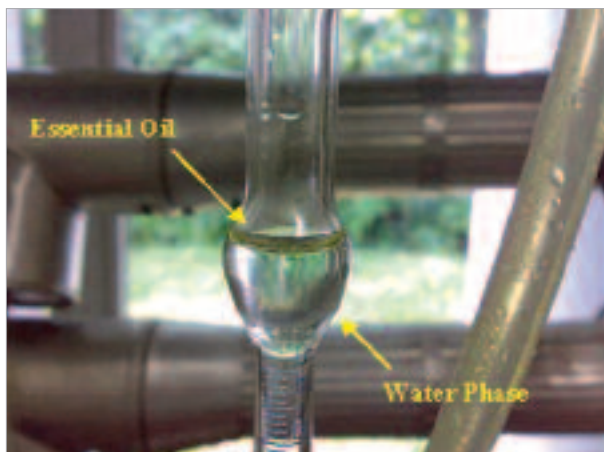
The Atlantic Rain Forest in São Paulo has a great diversity of essential oil-producing plant species. Lauraceae and Myrtaceae, both recognized producers of volatile oils, are also listed among the four most important families in the floristic composition of the São Paulo Atlantic Rain Forest. During the fieldwork, 111 specimens were collected in different conservation units, leading to 170 essential oil samples that were analyzed regarding their chemical composition and biological activity.

In the antimicrobial assay, all the oils tested presented a high growth inhibition for *Staphylococcus aureus*, probably in an unspecific way. Regarding the fungi, 22 samples presented one or more compounds with inhibitory activity against at least one of the tested strains. Accordingly with the results obtained so far, six samples presented promising results in the *in vitro* anti-inflammatory assay. In the cytotoxicity assay with tumor cells, 42 samples (71%) were lethal to at least one of the cancer cells strains.

Seasonality studies with Myrtaceae species suggested that variations observed in the essential oil yield and composition are associated with the changes from dry to wet season, mainly in the constituent proportions rather than the presence/absence of certain compounds.

Germination studies carried out with six species of *Eugenia* (Myrtaceae) demonstrated a remarked decrease in the germination percentage for all the species when the seeds were dried. Practically no germination was observed when water content reached values near 15%. A direct relationship between water potential and reduction of water content was observed. Seeds of three *Eugenia* species were cut through several methods and germination levels in all cases were higher than 100%, confirming their regeneration ability and the potential to increase the production of seedlings from a same seed lot.

Detail of the oil extraction in the Cleverenger apparatus



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## CONSERVATION AND SUSTAINABLE USE OF CERRADO AND ATLANTIC FOREST DIVERSITY: CHEMICAL DIVERSITY AND POTENTIAL DRUGS PROSPECTION – PHASE II

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The main goal of this thematic project is to search lead molecules from plant species and endophytic fungi occurring in the State of São Paulo, especially in the Cerrado and Atlantic Forest. The project was conceived to identify antioxidant, anti-inflammatory, antifungal, anticancer, antimalarial and acetylcholinesterase inhibition lead compounds from plant species of these biomes. With a strong multi-disciplinary relationship, this is the second phase of the bioprospecting program, initiated four years ago, when Biota-FAPEESP was created as a permanent FAPEESP's Program. Besides the main goals on drug discovery, biodiversity conservation, and sustainable economic growth, this project also is supported on advanced natural product chemistry knowledge such as: i) to evaluate, correlate and develop chemical methodologies to study intra- and inter-specific variability in some selected bioactive species; ii) to develop micropropagation and cell cultures of rare plants producing active compounds; iii) to study biosynthetic pathways and particularly determine the activity of phenyltransferases, terpene cyclases and cytochrome P-450 oxido-reductases for kinetic studies and further proteomic and functional analysis. This should lead to the cloning of genes that eventually can be used for recombinatorial biosynthesis of heterologous overexpression in other plants, to conduct studies on structure activity relationships (SAR) of some lead compounds previously identified and to sustain and maintain the virtual database (data mine) already initiated in phase I.



*Seedlings of Senna spectabilis*



## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Around 800 species belonging to 87 families of plants were collected. All stored extracts (ca. 1,700) were subjected to antifungal, antimalarial, antitumoral, and acetylcholinesterase inhibition bioassays aiming to select bioactive extracts/fraction for further guided-fractionation. *In vivo* test for assessment of activity in the central nervous system, have been performed with some pure compounds. From all species assayed, ca. of 5% showed promising activity in one of the targets tested, and were selected for guided-fractionation and detailed evaluation of the toxicological and pharmacological properties. More than 150 compounds of plants and ca. of 100 from endophytic fungi were isolated and tested for confirmation of activity, in the trials already described. Four plant species biosynthesize secondary metabolites with potential for further investment as prototypes. The main goal of this project is looking for bioactive compounds. Two semi-synthetic derivatives of alkaloids isolated from plants were selected as prototypes of acetylcholinesterase inhibitors, and it is in pre-clinical trials aiming to reach the pharmaceutical chain to Alzheimer drugs (national and international patent protected). This pharmaceutical innovation has been licensed for a national pharmaceutical industry, and the patent deposited in USA, Japan, China, and Europe. Two species of plants with potential for cosmetic products have been also licensed, with contract of royalties already defined and agreed upon between the Paulista State University (Unesp), Federal University of Rio de Janeiro (UFRJ) and FAPESP. Two species of plants showed potential as herbal medicines, and toxicology studies are on advanced stage. It is important to emphasize that besides the main goal of this project, which is dedicated in searching for new natural products as lead molecule, the state-of-art of natural product chemistry is being developed taking in account the last advances on phytochemistry and molecular biology. In addition, with the standardized methodology to screen large amount of plant species, our lab gained good experience to carry out dereplication methodology, which is fundamental for the success of bioprospecting. Studies of biosynthesis involving isolation of key enzymes in the metabolic process of pharmacological substances of interest is a gap in the national phytochemical research, and has been achieved with success. The isolation, synthesis and function of biological peptides of plants are unprecedented, and the partial results are very promising.

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## BIODIVERSITY AND SUSTAINABLE USE OF POLLINATORS, WITH EMPHASIS ON BEES

Vera Lúcia Imperatriz FONSECA

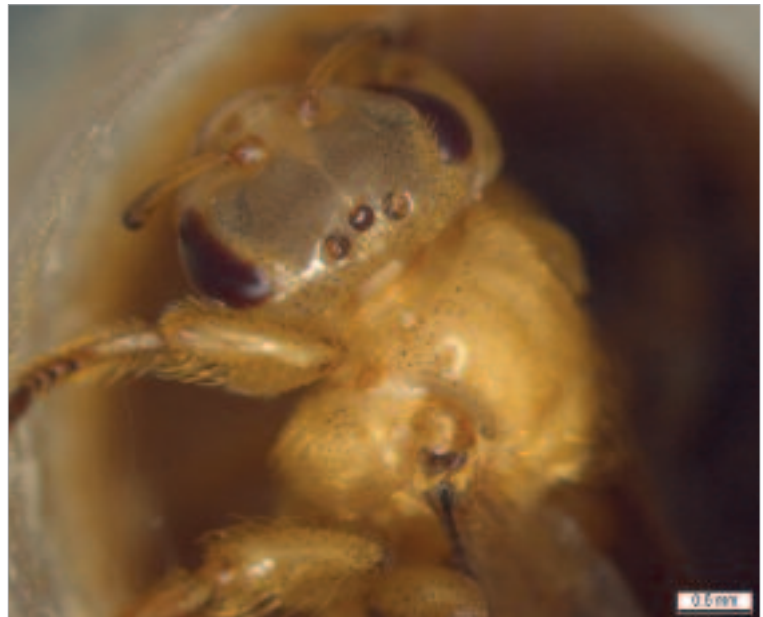
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The Convention of Biological Diversity and the United Nations Millennium Assessment Program ([www.millenniumassessment.org](http://www.millenniumassessment.org)) considers environmental services provided by pollinators, with emphasis on bees, as a priority for the 21<sup>st</sup> century. Conservation and sustainable use of pollinators helps to ensure food security, sustainable agriculture and wild plant conservation.

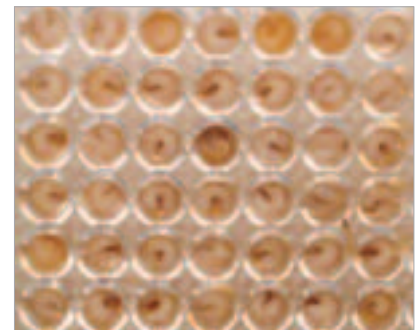
This project has four components: i) evaluation of the status of the interactions between bees and plants; ii) population dynamics of native bees, using molecular tools; iii) problems that limit the use of bees in agriculture; iv) information Technology tools for research and extension in ecosystem services (with emphasis on bees as pollinators).

Improvement of the knowledge base on bees focuses on reproductive aspects (individual and colonial), swarming, nest founding, colony growth, protein diets for colony feeding, sex allocation, automatic monitoring of colony growth and flight activity and studies of bees on flowers. Trap nests will be used to study solitary bee diversity in habitat fragments. Nogueira-Neto meliponaries in São Simão (SP), Luisiania (Goiás) and Xapuri (Acre) will be intensively studied, as well as stingless bees nests found on the university campuses in São Paulo and Ribeirão Preto will be used, as control.

A field station will be established in Mossoró (Rio Grande do Norte), where



*Above, in vitro reared queen and on right, in vitro reared larvi*



colony absconding is very frequent (30% to 50% of colonies in apiaries). A new program for beekeeping development was established at this location, monitoring absconding and correlated environmental conditions. Artificial diets and foraging behavior will also be studied, including efficiency on some crops, including sunflower and melon. This research will also include a study of Africanized bee biology and dynamics, using morphometrics to help understand their migratory behavior.

Amongst the social bees, stingless bees (Meliponini) are very representative in tropical areas and could be used as crop pollinators, as well as for wild flower pollination.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have developed a system for automatic identification of Meliponini species to determine their areas of occurrence, the study of swarming activity with trap nests for stingless bees, rearing queens *in vitro* and the effects on inbreeding, through studies of meliponaries and regional mating, analyzing sex ratios, queen supersedure and colony development. Molecular studies are also under development in a stingless bees beekeeping project, to determine if diploid males develop, with a good possibility of a new method for detecting them in nature by analyzing adult bees. *Melipona scutellaris* is a key species with great potential for beekeeping and pollination; an intensive study of this species is under development.

Research is being done on improving stingless bees beekeeping, with focus on potentially useful species, including *Scaptotrigona depilis*, *Nannotrigona testaceicornis*, *Melipona bicolor*, *Melipona quadrifasciata*, *M. rufiventris*, *M. scutellaris*, *Schwarziana quadripunctata* and *Tetragonisca angustula*.

The conflict between workers and queens to produce sexuals is also under investigation in stineless bees (Meliponini), through analysis of the maternity of the males with molecular tools (microsatellites). Colony conflict also includes queen production by the colonies. Producing enough queens is a limitation for large-scale breeding of stingless bees. Because of this, rearing queens *in vitro* is being investigated, with very good results until now. These queens have been studied, and they perform appropriate adult activities. This is a very relevant point of this project.

We are also working on a data bank of bees that are in CEPANN collections with around 40,000 bees, representative of São Paulo state and other areas where research of the Biosciences Institute bee lab has been done. A XLM schema was developed for the plant-pollinator relationships. This schema can be consulted and is under development in the site of Taxonomic Database Working group (TDWG) in <http://wiki.tdwg.org/twiki/bin/view/DarwinCore/InteractionExtension>. We will include 17,000 records of bees on flowers available for bees deposited in the CEPANN collection.

Another resource is the study of bee nests. Trap nests are a standard method for evaluating solitary bees (under development in part 3 of this project). Trap nests for Meliponini bees are also being studied. We are recording the swarming frequency and behavior of some species that use our trap nests, the size of new populations and how successful they are. A survey of trees as a nest resource in Brazil is under development, as well as lists of trees used by stingless bees for nesting to help orient environmental restoration. To facilitate these studies, a software was developed for the pocket PC platform. Field data recording and transfer to computers are facilitated with this Trap nest scouter.

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## SUSTAINABLE USE OF THE BRAZILIAN BIODIVERSITY: CHEMICAL AND PHARMACOLOGICAL PROSPECTION ON HIGHER PLANTS

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The apparent incompatibility between chemical and pharmacological investigation of a plant specie can be solved with the strong determination by dealing rationally with the problem.

The research groups on Natural Products Chemistry from the Chemistry Institute (IQ-Araraquara/Unesp), on Pharmacology, from Institute of Biosciences (IB/Unicamp) and Institute of Biosciences (IB-Botucatu/ Unesp) and on Biological Sciences, from School of Pharmaceutical Sciences (FCF-Araraquara/Unesp) have started collaboration some years ago, and have already produced a significative amount of work, with relevant results on the investigation of plant species with anti-ulcer, anti-oxidant, analgesic, anti-inflammatory and antimicrobial activities.

These results arose from an approach that includes ethno botanical and ethno pharmacological research, pharmacological, microbiologic and mutagenic assays with crude extracts or infusions, chemical screening to determine new chemical classes of compounds, isolation and structural determination of compounds and use the compounds or enriched fractions to determine the possible pharmacological action mechanisms involved with the detected properties.

This project deals with the integrated chemical and pharmacological investigation of plant extracts, including species that composes the bioma Cerrado, a savannah like vegetation, of the State Sao Paulo, comparatively to the Bioma Cerrado of other Brazilian States, like the State of Tocantins, which it is also under our investigation.



*An Anacardium tree and in detail, it's flowers*

Plant extracts are among the most attractive sources for developing new drugs and have been shown to produce promising results in the treatment of several diseases. The Brazilian Cerrado is one of highest biogeography regions of the world and also the most threatened. It includes several thousands of native vascular plants species grouped in hundreds of families. Many of these plants are commonly used as natural remedies by people living in these areas to treat many illnesses. An ethnopharmacological inventory made at this region is the starting point of our project, which involves the chemical and pharmacological investigation of extracts and infusions of ca. 30 plant species belonging to the genera *Alchornea*, *Anacardium*, *Ananas*, *Byrsonima*, *Davilla*, *Guapira*, *Indigofera*, *Miconia*, *Mouriri*, *Neea*, *Qualea* and *Strychnus*. Fractionment of these plant extracts will be followed by structural determination of the secondary metabolites, as well as the establishment of the qualitative and quantitative chromatographic fingerprint.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To perform the phytochemical step we used chromatographic techniques, mainly those for the analysis of polar substances (GPC, XAD2, DCCC, HSCC, HPLC, etc). To determine the structure of the isolated compounds we used modern spectrometric methods (NMR, IR, UV, MS).

To evaluate the biological activities, the possibility of toxicity and genotoxicity effects of each specie were determined. Simultaneously, we evaluated the activity of the extracts and pure substances (or enriched fractions) against different experimental models of peptic ulcer disease which operate by distinct mechanism of ulcerogenesis in man. The analgesic and anti-inflammatory activities were examined by using of chemical and thermal pain models and the classic inflammatory assays in rats or in mice. Through these models, we quickly evaluated the presence or absence of these activities. The antimicrobial activity was assayed against Gram positive and Gram negative bacteria, and also on the *Mycobacterium* genus, with emphasis on the etiological agent of tuberculosis, *M. tuberculosis*. The determination of the antiulcerogenic mechanisms was investigated through the effect of the isolated substances (or enriched fractions) on specific receptors, enzymes and substances produced in response to the gastric lesion, such as the expression of the new epidermic growing factor. Simultaneously, the antioxidant activity of extracts/substances was also evaluated, mainly those related to the possible mechanisms of the antiulcerogenic activity. Furthermore, assays for the detection of mucus, prostaglandins, somatostatin, gastrin and others involved with mechanisms of gastric secretion were also be evaluated. Assays against *Helicobacter pylori*, the most important bacterial pathogen of humans involved in the pathogenesis of peptic ulcer disease, were also performed. Finally, assays to detect the production of NO, H<sub>2</sub>O<sub>2</sub> and TNF were performed with extracts, substances and enriched fractions to evaluate their potential immunostimulating activity.

The compounds isolated and identified were catechins, flavonoids, saponins, terpenes, steroids, alkaloids, phenolic compounds and proanthocyanidins.

The results indicated plant species with promising activities, like antiulcer, anti-inflammatory, immunomodulatory, antimicrobial, antioxidant, anti tuberculosis and antitumor, most of them with no acute toxicity and no mutagenicity. This approach led to a better understanding of the biological activities observed in crude extracts and enriched fractions. Additional experiments are in progress to further evaluate the activity of the isolated compounds and also to investigate the mechanisms of action related to the biological activities observed.

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