BIOTA-FAPESP PROGRAM

FAPESP
SÃO PAULO RESEARCH FOUNDATION

BRAZILIAN BIODIVERSITY RESEARCH: A PROMISING FUTURE

KNOWLEDGE TO SUPPORT CONSERVATION AND SUSTAINABLE EXPLOITATION OF BIODIVERSITY

The science of biodiversity is widely recognized as a priority area for scientific research in developed countries and those in development, particularly in Brazil, home of around 20% of the world species. Therefore, it was vital to establish a coordinated research program in order to promote the advancement of scientific knowledge, education and training of highly qualified professionals, as well as the knowledge transfer to public institutions, aiming to improve mechanisms and policies for conservation, restoration and sustainable use of Brazilian biodiversity.

Created in 1999, the Biota-FAPESP program came to bridge to this gap, including, in its scope, terrestrial, freshwater and marine ecosystems, ranging from bioprospection and DNA bar-coding to landscape ecology at human dimensions of biodiversity conservation, restoration and the sustainable use.

FAPESP, São Paulo Research Foundation, is one of the main Brazilian agencies for promoting scientific research. This important Research Foundation has playing a seminal role in providing scientific funding to map and understand the biological diversity and ecosystem services. In 2009, FAPESP renewed for another 10 years the Biota program, aiming to prolong and enhance the rewards of a coordinated research investment, combining biodiversity research, personnel training, bioprospection and public-policy impact.

The Biota program brings together hundreds of scientists and students in a Virtual Biodiversity Institute. Scientific knowledge produced by the program is already being used by the state government to improve environmental conservation and/or restoration of native biodiversity and ecosystem services.

Also important for the productive sector are the discovery of new processes and the identification of new molecules of economic interest for use in pharmaceutical, cosmetics or food industries. The BIOprospecTA sub-program has been established in order to achieve such results, aggregating value and ensuring the sustainable use of native biological species from São Paulo State. A significant number of projects on bioprospection are currently underway in the BIOprospecTA program and several products and processes, originary from the São Paulo State biodiversity, are under development.

SCIENTIFIC OPPORTUNITIES IN SÃO PAULO, BRAZIL

Brazil is one of the four main emerging nations. More than 10 thousand PhD scientists are formed yearly. The country ranks 13th in the number of scientific papers published in indexed journals.

The State of São Paulo, with 40 million inhabitants and 34% of Brazil's GNP, responds for 53% of the science developed in the country. The State hosts the University of São Paulo (USP) and the State University of Campinas (Unicamp), both classified by the Times Higher Education Supplement (THES), amongst the 200 best universities in the world. Additionally, the growing State of São Paulo University (Unesp), ABC Federal University, São Carlos Federal University, the Aeronautics Technology Institute (ITA) and the National Space Research Institute (INPE) are institutions which significantly contribute to São Paulo State scientific output. The universities in the state of São Paulo have strong graduate programs: USP graduates two thousand doctorates every year, while Unicamp graduates eight hundred and Unesp, six hundred.

In addition to the three state universities and three federal universities, São Paulo has 19 research institutes and host most of Brazilian industrial R&D. São Paulo state universities have more than 10 thousand fulltime faculty members and 130 thousand students enrolled in undergraduate and graduate programs. São Paulo state scientific output corresponds to more scientific papers than any country in Latin America.

FAPESP: SUPPORT FOR RESEARCH IN SÃO PAULO

The São Paulo Research Foundation (FAPESP) promotes scientific research providing a robust program of fellowships and research grants, including support for both fundamental and applied research.

Established in 1962, the Foundation is entitled by the São Paulo State Constitution to receive 1 per cent of the State tax revenues. FAPESP has a sizable endowment and has already supported, over almost 50 years, 105,000 fellowships and scholarships and 92,000 research awards.

In 2011, FAPESP will invest US$ 556 million in fellowships and research grants. The approval rate for proposals applications ranges from 40-63 %.

OPPORTUNITIES AND CHALLENGES

One of FAPESP's goals is to broad and diversify the research system in the State of São Paulo, to strength the existing centers of excellence, in supporting their research, and to stimulate the establishment of new research centers tackling new lines of activities. The main FAPESP funding program to support such initiatives are the Thematic Projects, Young Researchers Awards, Research, Innovation and Dissemination Centers Program (CEPID), BIOEN, Biota-FAPESP, MCG and TIDIA Programs. All of these funding initiatives have in their teams, in addition to experienced scientists, young researchers as postdoctoral fellows, from Brazil and abroad. FAPESP supports more than 1,4 thousand postdoctoral fellowships nowadays.

For information on FAPESP postdoctoral fellowship program application, please contact FAPESP (www.oportunidades.fapesp.br) or the Principal Investigator of the Biota project of your interest.

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

Since 1999, the Virtual Institute of Biodiversity (http://www.biota-fapesp.net) has been studying the biodiversity of the São Paulo State. The Biota's mission is to catalogue and characterize the biodiversity of the State, defining the mechanisms for its conservation and sustainable use.

Scientists of São Paulo State public and private universities, from research institutes and NGOs participate in the Biota program. The program involves approximately 400 PhD scientists and 500 graduate students. Furthermore, there are 80 researchersfrom other Brazilian states and approximately 50 from other countries collaborating with the projects.

In 12 years, the Biota-FAPESP program has supported 134 research projects, trained 168 science Masters and 134 PhD students, produced and stored information on approximately 12,000 species and managed to link and make available data of 35 major biological collections. Scientists participating in the program have catalogued more than 1,800 new species. This effort is summarized in 760 articles published in scientific indexed journals.

Researchers involved in the Biota program have published 16 books and two atlases. In 2001, the program has launched an open-access electronic peer-reviewed journal, the Biota Neotropica (http://www.biotaneotropica.org.br), to report original research on biodiversity from Neotropical region. Currently, the journal is internationally recognized as a seminal reference on biodiversity data and it is indexed by the Zoological Record, by the International CAB, by the Open Access Journals Directory and by the Scientific Electronic Library Online (SciELO).

In 2002, the program launched a new sub-project called BIOprospecTA (http://www.bioprospecta.org.br), aiming to search for processes and products of economic interest including extracts, enzymes and pure compounds from plants, microorganisms, marine organisms and other natural sources. This initiative resulted in patents for 3 new drugs.

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

Carlos Alfredo Joly, Biology Institute / State University of Campinas (Unicamp)
Luiz Antônio Martinelli, Center of Nuclear Energy in Agriculture / University of São Paulo (USP)

FAPESP Grant 2003/12595-7 I Term: Jun 2005 to Nov 2010

Layout of the fourteen plots and the eddy covariance tower flux tower

Forest structure and floristic composition (trees with a DBH ⩾ 4,8 cm) were determined in 14 1ha permanent plots along an altitudinal gradient, from sea level (Restinga Forest) to 1.000m (Ombrophylus Dense Forest). In the case of botanical families with relevant ecological roles, such as the Leguminosae in the N cycle, a comprehensive floristic survey had included herbaceous, lianas and epiphytes. Data analysis was carried out using the FITOPAC program, and when appropriate, more detailed analyses were done using multivariate methods such as Canonical Correlation, Correspondence Analysis, PCA and PCO. The database on composition and structure of the forest have allowed the selection of species for more detailed studies including reproduction biology, seed anatomy and reserves, germination, photosynthesis and water use efficiency, N assimilation, transport and metabolism, plant populations structure and dynamics, genetic structure, forest age, key species annual average growth rates and phenology. Simultaneously, the inputs of N fixation through precipitation, biological fixation, soil mineralization and nitrification was determined, along with key parameters of N losses through denitrification and export by streams, allowing a preliminary N mass balance along the altitudinal gradient. Water and carbon balance of the forest were estimated along with the seasonal variation of this balance through the use of a micrometeorological tower and Eddy-covariance technique. The photosynthesis/respiration balance of the ecosystem was used to determine the role of the forest as a sink or source of carbon to the atmosphere.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

On average, each 1ha have approximately 1500 trees with DBH⩾4,8 cm, resulting in 21.000 trees distributed in 625 species. Overall, we have found significant differences among the four phyto physiognomies. In the Restinga Forest, we have found 84 species. This number had increased to 150 species in the Low Land ODF (50-100m) and in the Montana ODF (500 a 1000m), while the highest diversity (> 200 spp in 1ha) was registered in the Submontana ODF (100-500m). The cluster analysis, using UPGMA with Bray–Curtis similarity measure, has shown that the Restinga Forest differs significantly from all three phyto physiognomies of the Ombrophilous Dense Forest/ODF. This difference is strongly associated with soil characteristics. Restinga Forest grows in coastal plains of marine Quaternary origin, which seasonally floods and the soil is sandy, friable, non plastic and non sticky (Oxyaquic Quartzipsamments). ODF occurs in Tertiary/Quaternary, sandy-loam/sandy-clay-loam, friable, slightly plastic and sticky soils (Typic Dystrudepts/Inceptisols). Both soils are acid (pH 3.4-4.2), extremely pour in nutrients (SEB 1.5 to 22.5) and with high concentration of Al^{+++}.

The first results of floristic/phyto sociology have allowed us to choose species or group of species suitable for auto-ecology and population ecology studies. For instance, research done with melitophylous Fabaceae trees has shown that, more than 50 species of bees, use these trees as sources of energy and nutrients.

Ecosystem functioning. Trees are the main components living above ground biomass (>90%). Pteridophytes are responsible for less than 1% and palms from 1 to 5%, depending on the altitude. AGB varies from 166,08 $Mg.ha^{-1}$ (Restinga) to 235,42 $Mg.ha^{-1}$ (Submontana ODF). C and N stocks, in the under or up ground, increase along the altitudinal gradient. The underground increase may be explained by the lower temperature, which reduces the rate of decomposition and increases peat formation. These large stocks of underground C and N suggest that the Atlantic Rainforest, mainly the Montana ODF, may become a strong source of green house gases with global warming.

Data on CO_2 flux between the forest and the atmosphere shows that, during the period measured, the balance is slightly negative, i.e. the forest behaves as a carbon sink absorbing more carbon then releasing it to the atmosphere. The accurate measurement of atmospheric CO_2 flux, in the Atlantic Montane forest, is a great technical challenge, due to the steep topography. This research is funded by two other FAPESP grants (08/58120-3 and 10/50811-7), integrating FAPESP's Biota and Climate Change Program.

The multidisciplinary efforts to plan and develop this project, including mixed teams for data field and the conjoined efforts to interpret the results, have produced a significant qualitative advance in scientific knowledge on the functioning of the Brazilian Atlantic Rainforest.

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Carlos Alfredo Joly

Instituto de Biologia
Universidade Estadual de Campinas (Unicamp)

Departamento de Botânica
Caixa Postal 6109 – Barão Geraldo
CEP 13081-970 – Campinas, SP – Brasil

+55-19-3521-6166
cjoly@unicamp.br

BIOTA-FAPESP PROGRAM

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

Cláudio Gilberto Froehlich

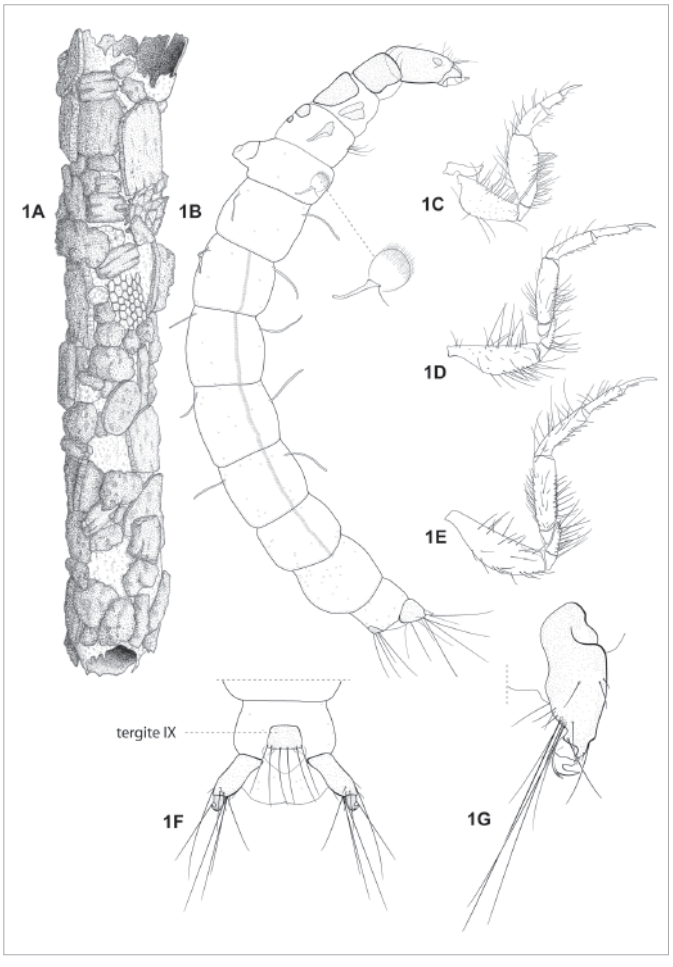
Ribeirão Preto School of Philosophy, Sciences and Literature / University of São Paulo (USP)

Main researchers: Susana Trivinho-Strixino, Alaíde Aparecida Fonseca-Gessner, Roberto da Gama Alves

FAPESP Grant 2003/10517-9 I Term: Jan 2005 to Fev 2009

The project is focused on carrying on a faunistic survey of two groups of benthic freshwater macroinvertebrates, insects and oligochaetes, with emphasis in lotic systems. A previous project had included crustaceans and mollusks, for which 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 for a better knowledge of local faunas, including ecological aspects.

For the current project, the sampled localities includes protected areas mainly in the mountains of Serra do Mar and Serra da Mantiqueira, and areas subject to impacts from anthropogenic activities. The project comprises the taxonomy of Diptera (Chironomidae and Simuliidae), Ephemeroptera, Plecoptera and Trichoptera insects and the understanding of the composition and distribution of benthic freshwater macroinvertebrates in the State of São Paulo. The ecological studies includes the evaluation of the fauna structure in spatial and temporal scales taking into account the influence of physical and chemical factors and the responses of the fauna to different anthropic impacts, in particular to different land uses.



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*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

In taxonomy, seven new genera and 39 new species of Chironomidae, one new species of Simuliidae and three new species of Plecoptera were described. New records for the state comprises one species of Chironomidae, four species of Simuliidae and four species of Ephemeroptera.

In faunistic surveys of the target taxa, insects and oligochaetes, 17 genera and 41 species of Oligochaeta were collected. The sampling of insects has comprised the orders Odonata (6 families), Ephemeroptera (6 families, 25 genera and 10 species), Plecoptera (2 families, 7 genera and 7 species), Trichoptera (11 families and 19 genera), Coleoptera (14 families and 41 genera) and Diptera (7 families). In this family, emphasis was given to the family Chironomidae (67 genera and 14 species) and in the family Simuliidae, 12 species were collected. Additional groups were also sampled, Mollusca (Bivalvia and Gastropoda), Hirudinea and Acarina.

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

Based on the material collected and on field observations, a series of ecological projects have been carried out. These includes the study of life cycles, body size relationships, preference for substrates, spatial distribution along streams and among streams considering multiple scales, temporal distribution and the influence of physical and chemical factors on the communities.

Another line was the study of the impact of human activities on the macroinvertebrate fauna. Different land uses including forestry, pastures, sugarcane cultivation, all affecting the fauna, in comparison with reference sites. Cultivation includes the use of inputs, such as fertilizers and biocides, that are carried down to the rivers. A study showed the presence of heavy metals in the water and their absorption by insects.

MAIN PUBLICATIONS

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Claudio Gilberto Froehlich

Faculdade de Filosofia, Ciências e Letras
de Ribeirão Preto / Universidade de São Paulo (USP)

Avenida Bandeirantes, 3900
CEP 14040-901 – Ribeirão Preto, SP – Brasil

+55-16-3602-4394
cgfroeh@usp.br

BIOTA-FAPESP PROGRAM

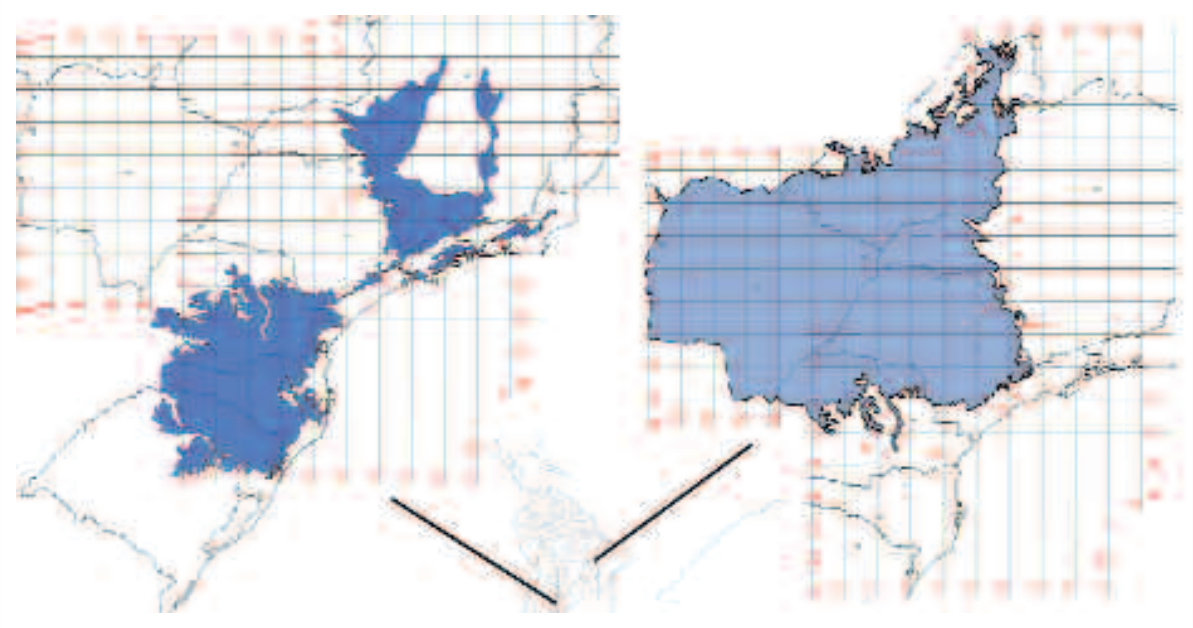
GEOGRAPHIC LIMITS AND CAUSAL AGENTS OF DIPTERA ENDEMISM IN THE ATLANTIC FOREST

Dalton de Souza Amorim

Ribeirão Preto School of Philosophy, Sciences and Literature / University of São Paulo (USP)

Main researcher: Nelson Papavero

FAPESP Grant 2003/10274-9 I Term: Dec 2004 to Nov 2009



Two of the areas of endemism obtained with the study of Diptera biodiversity in the Atlantic Forest. A. Altitude forests in southern Brazil and higher areas in southeastern Brazil. B. Semideciduous forest at the western margin of the Atlantic Forest, especially in the São Paulo, Minas Gerais, Goiás and Mato Grosso do Sul States

The mapping distribution of Brazilian plant and animal groups is in its beginning. Even in studied Brazilian areas, the endemic patterns are insufficiently known as their historical causes. This deficiency in knowledge has implications for conservation policies and for the understanding of evolutionary processes in the tropics. Conservation decisions are largely dependent on the precise knowledge of the geographical species distribution. Furthermore, the understanding of the rate of biological evolution depends on a correct association between speciation events and their causes and ages. The main goal of this project is to delimit Diptera endemic areas and to connect these areas in a hierarchical pattern reflecting the geological history resulting in the present biodiversity. Close to 400,000 Diptera specimens, collected with standardized methods along the Atlantic Forest, have been analyzed.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The analyzed insects were classified in 60 families, 300 genera and almost 700 species (being 350 new species). The number of estimated new species in the material available from the Atlantic Forest is more than 1,000, but this is still an underestimation of the real diversity in the region, since some areas were under sampled. These numbers confirm estimations that the overall Diptera diversity, in the entire Neotropical region, could be over five times the number of species identified in the group, which is around 31,000 species.

The biogeographical results has shown six major areas of endemism along the Atlantic Forest: 1) altitude forests, with elements of temperate origin, in southern Brazil and higher areas in southeastern Brazil; 2) ombrophyllous forest of mid latitudes, close to the sea, from São Paulo to Rio de Janeiro State; 3) ombrophyllous forest of lowlands in Espirito Santo State; 4) altitude forests in Espirito Santo and Minas Gerais States; 5) ombrophyllous forest from southern Bahia to Rio Grande do Norte States, at the northern range of the Atlantic Forest; 6) semi deciduous forest at the western margin of the Atlantic Forest, especially in the States of São Paulo, Minas Gerais, Goias and Mato Grosso do Sul. There is an overlap in the southern Atlantic Forest between tropical species and species of genera also present in Chile, Australia, and New Zealand.

Molecular data, and congruence with geological events point that glaciations and interglaciation cycles, occurring in South America since late Cenozoic, probably have been much more responsible for the changes in the limits of species distribution than for speciation processes. Detailed studies of the limits between the areas of endemism are now necessary to bring accuracy to the maps. Molecular studies of species along the entire range of the Atlantic Forest would help to understand evolutionary aspects, as the age of the endemism, sequence of division between the areas, and dynamic below the species level. A crucial implication of the discovery of these patterns is that most biological reserves in the Atlantic Forest are close to the sea. The biodiversity corresponding to the entire endemism of the semi deciduous forest is scarcely protected. A catastrophic loss of biodiversity may rapidly occur due to the pressure of agriculture expansion over natural environments.

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Dalton de Souza Amorim

Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto / Universidade de São Paulo (USP)

Departamento de Biologia
Avenida Bandeirantes, 3.900
CEP 14040-901 – Ribeirão Preto, SP – Brasil

+55-16-3602-3706
dsamorim@usp.br

BIOTA-FAPESP PROGRAM

CHARACTERIZATION OF NATURAL COMPOUNDS FROM CERRADO AND ATLANTIC FOREST WITH POTENTIAL ANTITUMORAL, ANTIOXIDANT, ANTI-INFLAMMATORY, ANTIFUNGAL, ANTIDIABETIC, ACETYLCHOLINESTERASE AND MYELOPEROXIDASE INHIBITORY ACTIVITIES

Dulce Helena Siqueira Silva

Araraquara Chemistry Institute / Paulista State University (Unesp)

Main researcher: Alberto José Cavalheiro

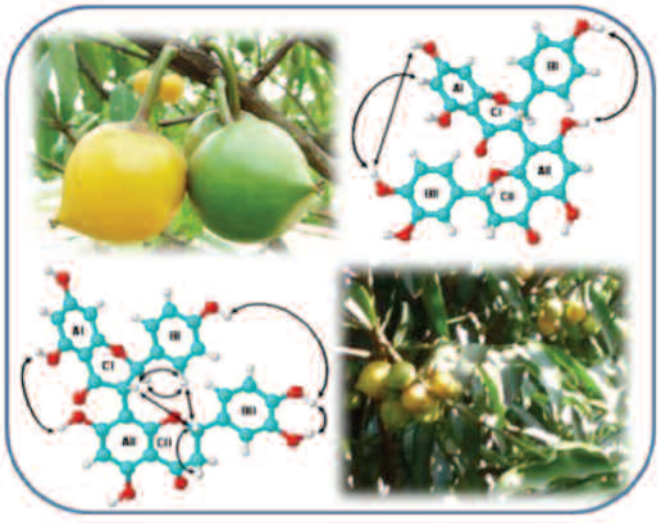
FAPESP Grant 2004/07932-7 I Term: Mar 2005 to Jul 2010

The progress on Brazilian biodiversity preservation and sustainability strategies have evidenced its intrinsic value as biological resources for new bioactive compounds, representing a big challenge nowadays facing the accelerated devastation process of several Brazilian biomes.

This project was conceived to catalogue information resulting from investigating plants and micro-organisms extracts, especially endophytic fungi, mostly concerning their chemical profiles and bioactivity towards selected targets. The collection and classification of plant and micro-organisms species were the first step, followed by extracts preparation and initial evaluation of their antioxidant, antifungal and cytotoxic activities for the selection of promising samples for further investigation.

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. These compounds might converge to hits or leads to pharmaceutical, agroceutical, nutraceutical and/or cosmetics industry, bringing contribution for conservation and sustainable development of São Paulo State biodiversity.

Lippia salviaefolia *stems and leaves, a source of pro-apoptotic flavonoids.Funari CS et al. 2010.*



Conformational analysis of chemopreventive biflavonoids from Garcinia xanthochymus. *D. C. Fernandes et al. Results presented in the 34ª. Reunião Anual da Sociedade Brasileira de Química, Florianópolis, SC, May/2011.*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our bank of plant extracts has ca. 1,800 extracts from Cerrado and Atlantic Rainforest, São Paulo State main biomes. 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. Expansion of this bank has not been significant over the past five years due to CGEN (MMA – Ministry of Environment) restrictions regarding collection and access to biological material.

Hundreds of crude extracts and semi-purified fractions have been assayed for their antifungal, cytotoxic and antioxidant activities, in addition to inhibitory or inducing properties towards selected enzymatic targets. This preliminary screening has indicated *ca.* 6% of tested samples as bioactive. Such screening has allowed the selection of promising crude extracts, which have been chemically investigated, generating *ca.* 350 pure compounds. Additionally, chemical derivatization of selected prototypes, from natural sources, has generated a series of semi-synthetic compounds with enhanced biological properties, e.g. using garllic, caffeic acid, ferulic, and protocathecuic acids as substrates.

Modern phytochemical methodologies, including hyphenated techniques: LC/MS; LC/UV/DAD; GC/MS; and fast, sensitive and reproducible preliminary bioassays, using high-throughput-screening (HTS) and metabolomics techniques, have been combined for conducting systematic studies, which resulted in the isolation of several bioactive compounds from selected plant species. Such approach led to antibacterial labdane diterpenoids from *Stemodia foliosa*, antifungal saponins from *Swartzia langsdorffii*, myeloperoxidase inhibitory flavones from *Pterogyne nitens*, anti-angiogenic, cytotoxic and trypanocidal guanidine alkaloids from Pterogyne nitens, chemopreventive anthocyanin-rich fractions from Eugenia jambolana, lipoperoxidation inhibitory flavones from Iryanthera juruensis, trypanocide chromenes from *Piper spp.*, anti-inflammatory and cholinesterase inhibitory piperidine alkaloids from *Senna spectabilis*, antifungal terpenoid glycosides from *Alibertia edulis*, DNA-protective and protease inhibitory clerodane diterpenes from *Casearia sylvestris*, chemopreventive biflavonoids from *Garcinia xanthochymus*, in addition to gallic acid from *Alchornea glandulosa* and its semi-synthetic gallate esters, which exhibited antifungal activity towards human pathogens, and tripanocidal activity.

These results have attracted the attention of pharmaceutical and cosmetic companies aiming the development of products containing value-added material from the Brazilian plant biodiversity.

MAIN PUBLICATIONS

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Dulce Helena Siqueira Silva

Instituto de Química de Araraquara
Universidade Estadual Paulista (Unesp)

Rua Francisco Degni, S/N – Caixa Postal 355
CEP 14800-900 – Araraquara, SP – Brasil

+55-16-3301-9659
dhsilva@iq.unesp.br
www.nubbe.iq.unesp.br/Portal/index.html

BIOTA-FAPESP PROGRAM

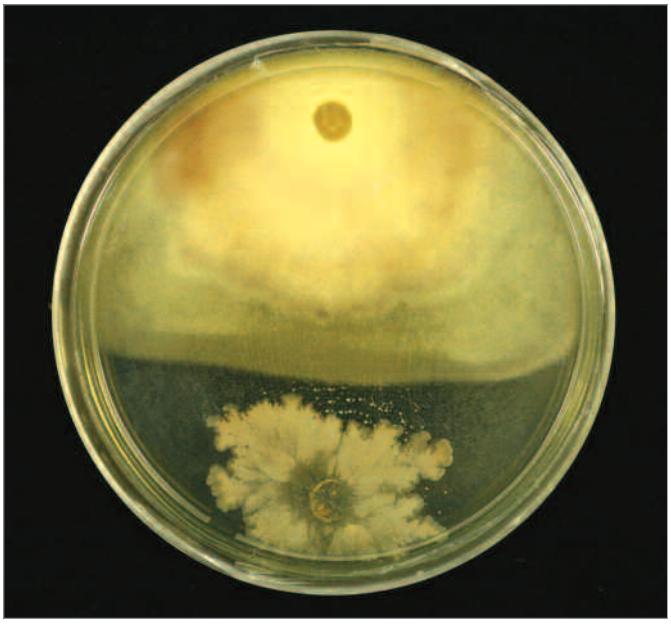
PLANT BIODIVERSITY AND SOIL ORGANISMS IN NATURAL AND IMPACTED *ARAUCARIA ANGUSTIFOLIA* ECOSYSTEMS IN THE STATE SÃO PAULO, BRAZIL

Elke Jurandy Bran Nogueira Cardoso

Higher School of Agriculture Luiz de Queiroz / University of São Paulo (USP)

FAPESP Grant 2001/05146-6 I Term: Apr 2004 to Jun 2010

Araucaria angustifolia is a plant species of great social and economic importance, and characteristic of the sub-tropical forest in Brazil. This species has been submitted to predatory exploitation and to-day is considered in danger of extinction. Therefore, new management technologies are urgently needed, with the goal of conservation and recuperation of the small remnant areas. The Araucaria ecosystem presents a high animal and plant diversity with high mutual interactions. The consequences of loosing this tree species would have a negative impact on the whole ecosystem, which would also involve a loss in the microbial diversity below the soil, impairing the functioning and sustainability of the system. The objectives of this project were to study the floristic and microbial diversity of different Araucaria forests, with special consideration of the growth promoting rhizobacteria, mycorrhizal fungi and diazotrophic bacteria, especially those in symbiosis with leguminous plants in the forest understory. A survey of the chemical and microbial attributes of these forests will contribute to select the most promising methodologies to help maintaining and to recuperate these ecosystems in the State São Paulo.



General view of the Araucaria Forest in Campos do Jordão, SP, Brazil. Cardoso EJBN, Vasconcellos RLF, Ribeiro C, Miyauchi MYH. PGPR in Coniferous Trees. In: Dinesh K. Maheshwari. (Org.). Bacteria in Agrobiology: *Crop Ecosystems. 1 ed. Berlin: Springer, 2011.* ***1****:345-360*

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SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

PCR-DGGE analyses has shown greater similarity between bacteria community structure in the soil of native forest (NF) and in recently burnt area (RQ), than in the soil of degraded areas (RF). A multidimensional scale analysis (NMDS) with Anosim, based on bacteria amplicon profiles, has shown that these 3 Araucaria areas are different. Biolog has shown that NF has greater substrate utilization index than RF and RQ, which do not differ from one another, the same as occurring with PLFA profiles. The genetic diversity of natural populations of *A. angustifolia*, evaluated by intron *matK* SSR (simple sequence repeat, microsatellite) marker has shown high diversity indices, cross breeding rates, and a special genetic structure of up to 25m. The tree and shrub community structures were estimated in different areas of the *A. angustifolia* forests. In Campos do Jordão, the highest stratus contained 1918 individuals, and the lower stratus, 576 individuals. In Barra do Chapéu, the superior stratus contained 1879 individuals, and the inferior stratus, 915 individuals. Actinobacterial isolates were tested to find out their antagonism to the plant pathogenic fungi *Fusarium sp.* and *Armillaria sp.* in Araucaria. Twenty four out of 28 isolates were able to inhibit the production of rhizomorphs in Armillaria, while the isolate A43 was outstanding in the control of both pathogens. The same actinobacterial isolate favored spore germination of the AMF Gigaspora rosea *in vitro*. The isolate A43 stimulated growth of Pinus seedlings in the absence of the ectomycorrizal fungus, with a 100% gain of biomass in comparison to the control. A pot experiment had evaluated the effect of inoculation of the earthworm *Amynthas corticis*, AMF, and a diazotrophic bacterium isolated or growing on Araucaria seedlings. The FMA promoted growth and development of the seedlings which had presented higher contents of N and P than control samples. The fungi producing mycorrhizal associations with the orchid *Coppensia doniana* were identified as two morphotypes of the genus Ceratorhiza and one uninucleate Rhizoctonia. All three clades were successful in germinating the orchid seeds, bringing the seedlings to an advanced stage of development around 30 days. Replanted Araucaria in PETAR, and burned forest in Campos do Jordão were related to arylsulfatase, dehydrogenase, microbial biomass carbon and qMIC. Fatty acids 10Me 18:0 (actinobacteria), saturated to unsaturated fatty acids conversion rate, and metal-D-glycosides and 2-hydrobenzoic acid substract consumptions were also related to the replanted area in Petar and in Campos do Jordão. However, fatty acids 18:19c (fungi) and 16:17c (Gram+ bacteria), glucose-1-phosphate consumption, D-lactose and hydroxibutyric acid were related to the highly impacted areas. Looking for PGPR, eighteen (out of 97) isolates had produced indol-acetic acid, 27 were phosphate solubilizers, 37 produced siderophores and 83 produced phosphatases. Forty five isolates were antagonic to *Fusarium oxysporum*. Using the Fame technology and 16S rRNA sequencing, the most effective isolates belonged to Bacillaceae, Enterobacteriaceae and Pseudomonadaceae.

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Elke Jurandy Bran Nogueira Cardoso

Escola Superior de Agricultura Luiz de Queiroz (Esalq)
Universidade de São Paulo (USP)

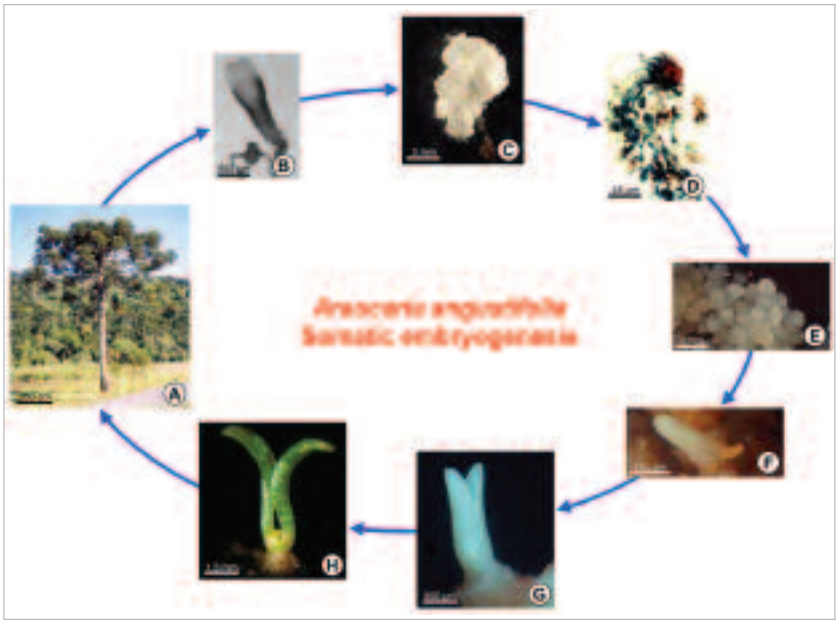
Departamento de Solos e Nutrição de Plantas
Av. Pádua Dias, 11
CEP 13418-900 – Piracicaba, SP – Brasil

+55-19-3429-4171 ramal 2143
ejbncard@esalq.usp.br

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

Eny Iochevet 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.



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*

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 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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Eny Iochevet Segal FLOH

Instituto de Biociências
Universidade de São Paulo (USP)
Rua do Matão, 277
Caixa Postal 11461 – Cidade Universitária
CEP 05422-970 – São Paulo, SP – Brasil

+55-11-3091-7556
floheny@ib.usp.br

BIOTA-FAPESP PROGRAM

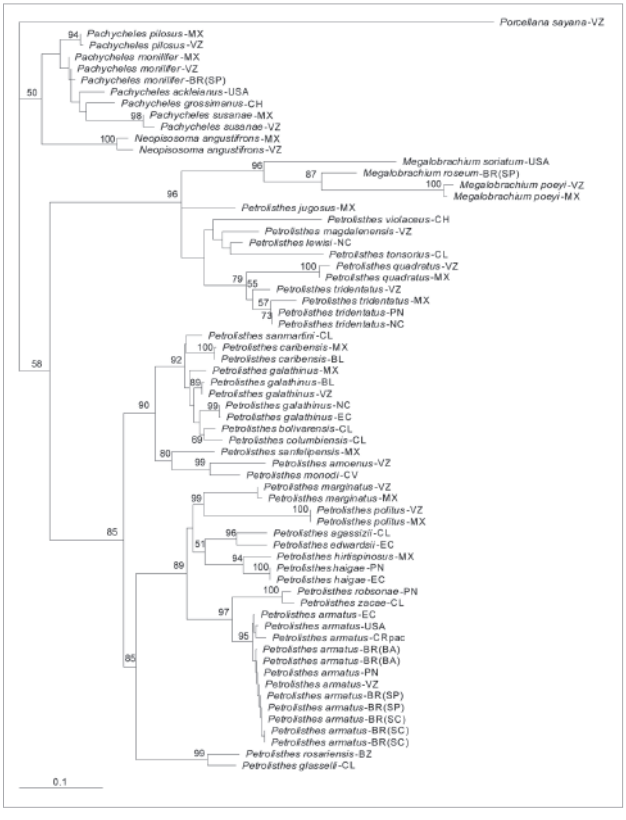
DECAPOD CRUSTACEANS: MULTIDISCIPLINARY CHARACTERIZATION OF THE STATE OF SÃO PAULO SEA COASTAL BIODIVERSITY (TAXONOMY, SPERMIOTAXONOMY, MOLECULAR BIOLOGY, POPULATION DYNAMICS)

Fernando Luis Medina Mantellato

Ribeirão Preto School of Philosophy, Sciences and Literature / University of São Paulo (USP)

Main researchers: Antonio Leão Castilho, Fernando José Zara, Rogério Caetano da Costa

FAPESP Grant 2010/50188-8 I Term: Jan 2011 to Dec 2015



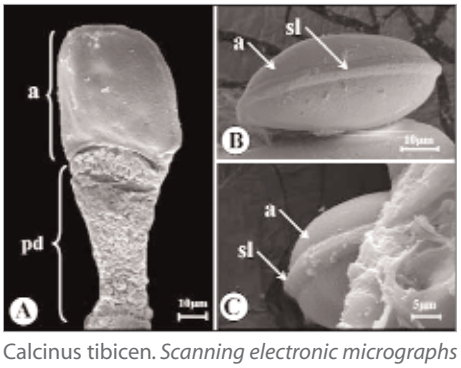
Dendrogram of the cluster analysis for species of Petrolisthes and related groups, based on a distance analysis using the minimum evolution (ME) algorithm of 16S rDNA gene sequences. Numbers below are significance values for 1000 bootstraps; values 50% are not shown. (Source: Mantelatto et al. 2011. *Zool. Stud.* **50**: 372-384)

This project searches new solutions for the analysis of some aspects of the biodiversity of the decapod crustaceans (crabs, ghost shrimps, hermit crabs, lobsters, and shrimps) of the São Paulo coast. The main objective is the optimization of molecular techniques applications (mitochondrial and nuclear DNA), morphological and ecological analyses (taxonomy, phylogeny, spermiotaxonomy, populational and reproductive dynamic) for accurate detailed taxonomic revision and identification of the biodiversity of São Paulo coast decapods crustaceous inhabitants of the estuarine, coastal and marine environments in a multidisciplinary and phylogenetic task.

The molecular genes used for taxonomy and DNA library will be integrated to the sperm/ spermatophore ultrastructure and ecological dynamic. This combination has been successfully employed in several recent studies on Crustacea taxonomy and phylogeny. Although the sperm ultrastructure has been carried out in numerous studies, the amount of knowledge on molecular systematic and sperm morphology is far from the Decapoda Brazilian biodiversity, which is estimated in more than 650 species. For instance, less than 10% of this fauna has its spermiomorphology and genetic sequences described a situation that has compromising the conduction of accurate studies on phylogeny, as well as, on the evolution of the reproductive system. Considering that about 50% of this decapod fauna is supposedly found in the São Paulo coastline, we are more than convinced that we have here a promising area to be studied and this motivated us to carry on our efforts in this project.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To achieve our goals on molecular phylogeny, DNA bar coding, spermiotaxonomy and population dynamic, the first sampling expedition along the coast of São Paulo was carried out in a less known area in terms of decapod diversity, i.e. the fauna of São Paulo southern region (Cananeia, Ilha Comprida and Iguape). As a preliminary result, we have obtained at least five new records of decapod for São Paulo region, including an alien species, and one new species, of snapping shrimp. In addition, during the sampling, the key species and genera of some taxa of all decapod groups were obtained and have already been processed in all three directions of our aims. In terms of molecular perspectives, new sequences have been obtained and deposited at the Genbank and are still in current analysis of phylogeny. Regarding spermiotaxonomy, samples have been processed for transmission and scanning electron microscopy and different regions of the male reproductive system were chosen for both sperm and spermatophore analysis. On population dynamic, our preliminary results evidences interesting differences in terms of population profile of some commercial penaidean shrimps when compared with others at the northern area of São Paulo State.



Calcinus tibicen. *Scanning electronic micrographs of spermatophores. A, General morphology of spermatophore, showing ampulla (a) and peduncle (pd). B, C, Detail of ampulla (a) and its suture line (sl). (Source: Amadio & Mantelatto 2009.* J. Crust. Biol. ***29****: 466-475)*

Due the high complexity of our results, comparative studies with specimens from other Brazilian regions and outside of national boundaries will be essential to complete the major objectives.

Finally, the combination of all these efforts brought up to date the species of decapod crustaceans of the São Paulo coast, endorsed for a genomic library (DNA-bar coding) and a scientific collection of reference with samples available on-line.

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Fernando Luis Medina Mantelatto

Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto / Universidade de São Paulo (USP)

Departamento de Biologia
Avenida Bandeirantes, 3.900
CEP 14040-901 – Ribeirão Preto, SP – Brasil

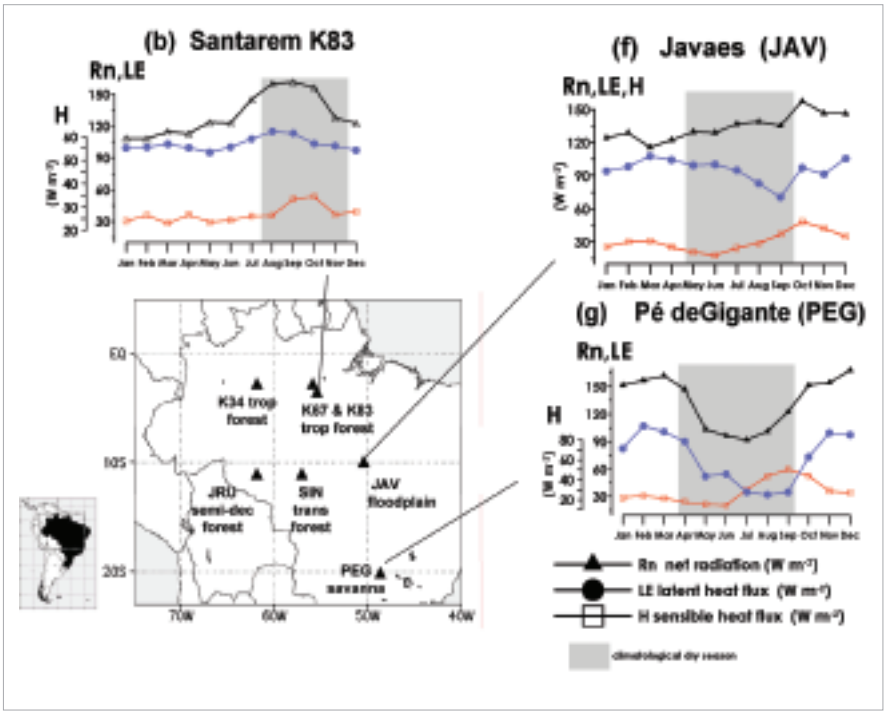
+55-16-3602-3656
flmantel@usp.br

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

Humberto Ribeiro da ROCHA

Institute of Astronomy, Geophysics and Atmospheric Science / University of São Paulo (USP)

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.



Source: Rocha et al. 2008, unpublished.

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:

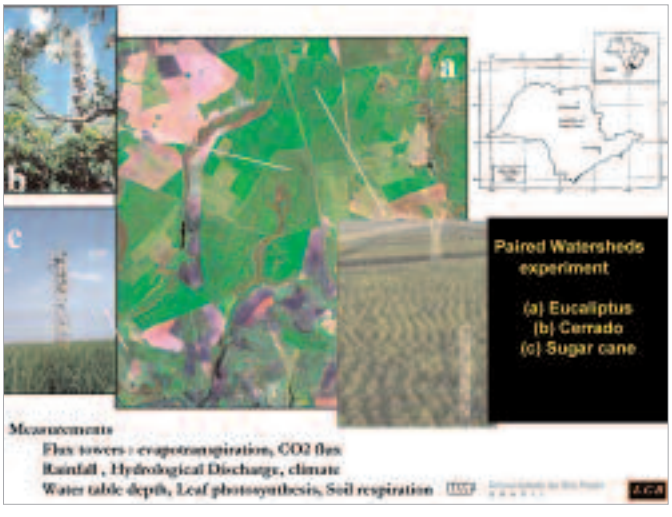
1) To monitor five experimental field sites:
   Cerrado *sensu stricto*;
   Ecotone (seasonally flooded savanna);
   Tropical rain forest;
   Agro system Sugar Cane;
   Agro system Eucalyptus.
2) To measure leaf photosynthesis and water potential, according to key species and seasonality.
3) To measure the long term surface-atmosphere fluxes of water, energy and CO_2 over the field sites, using flux towers and weather stations.
4) To estimate the water balance for each ecosystem on the watershed scale, using hidrogeological 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-2 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-1 (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-1 in the transitional forests and to 1 mm d-1 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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Humberto Ribeiro da ROCHA

Instituto de Astronomia, Geofísica e Ciências Atmosféricas / Universidade de São Paulo (USP)
Rua do Matão, 1226 – Cidade Universitária
CEP 05508-090 - São Paulo, SP - Brasil

+55-11-3091-4713
humberto@model.iag.usp.br

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

Hussam El Dine ZAHER
Zoology Museum / University of São Paulo (USP)

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.

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

The Cretaceous and Tertiary will be 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 taxes were collected and are presently under study.

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

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 Sanfransciscan basin, with remains of Dinosaurs and other tetra pods (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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Hussam El Dine ZAHER

Museu de Zoologia
Universidade de São Paulo (USP)
Avenida Nazaré, 481 – Ipiranga
CEP 04263-000 – São Paulo, SP – Brasil

+55-11-6165-8090
hzaher@usp.br

BIOTA-FAPESP PROGRAM

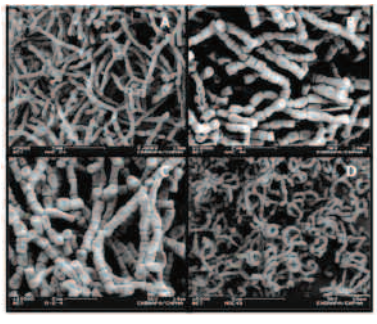
BIODIVERSITY AND FUNCTIONAL ACTIVITIES OF MANGROVES MICROORGANISMS

Itamar Soares de Melo

Brazilian Agricultural Research Corporation (Embrapa)

Main researcher: João Lucio de Azevedo

FAPESP Grant 2004/13910-6 I Term: May 2006 to Jun 2011



Scanning electron micrographs of new strains of Streptomyces isolated from mangroves (Canova, 2009)

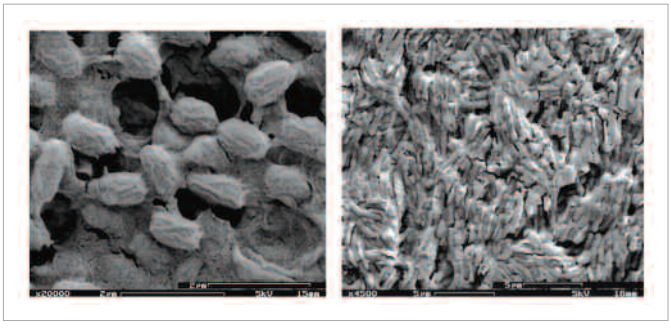
Mangrove is a transition coastal ecosystem situated between terrestrial and marine environments under the influence of tidal regime and defined by the dense growth of shrubs and trees. The mangrove plant roots architecture trap the sediments, contributing to shoreline stabilization through consolidation of unstable mineral sediments and peat formation, acting as a sink for nutrients and metals. The Atlantic Brazilian coastline has 7,408 km and the mangrove forest is distributed in 6,786 km (25,000 km^2), representing the second largest area of mangroves in the world. The Sao Paulo State coastline retains approximately 231 km^2 of mangrove ecosystem. The main vascular plants colonizing the mangroves in São Paulo State belong to the genera *Avicennia, Rhizophora* and *Laguncularia* and their leaf surfaces (phyllospheres) can provide appropriate conditions for colonization by microorganisms. The mangroves are recognized as highly productive ecosystems providing large quantities of organic matter, in the form of detritus to adjacent coastal water. Although this ecosystem is rich in organic matter, it is in general nutrient-deficient, especially in nitrogen and phosphorus. Microbial activity is responsible for major nutrients transformations within a mangrove ecosystem. In this context, mangroves represent untapped resources for biotechnological exploration, which drives 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 have been to study the biological diversity of microorganisms from sediments, rhizosphere, phyllosphere and endophytes, especially, Cyanobacteria, Bacteria, Fungi, Actinobacteria and Archeobacteria. So far, this study has covered about 2,100 bacteria, 56 actinobacteria, 850 fungi (most of them endophytes) and more of 56 cyanobacteria from sediments, phyllosphere and rhizosphere of red, white and black mangroves.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The bacterial diversity present in sediments of a well-preserved mangrove in Ilha do Cardoso, located in the extreme south of Sao Paulo State coastline, has revealed a bacterial community dominated by Alphaproteobacteria (representing 40.36%), Gammaproteobacteria (19.28%) and Acid bacteria (27.71%), while minor components of the assemblage were affiliated to Betaproteobacteria, Deltaproteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Profiles of specific DGGE gels have shown that both dominant ('universal' Bacteria and Alphaproteobacteria) and low-density bacterial communities (Betaproteobacteria and Actinobacteria) are responsive to shifts in environmental factors. A number of free-living bacteria strains, obtained from tissues of *R. mangle*, has shown capacity to grow in N_2-free conditions and also has shown nitrogenase activity (C_2H_2 reduction).

To our knowledge, there is no information about epiphytic cyanobacterial genera that colonize mangrove leaves of vascular plants. The results obtained have shown that *Rhizophora mangle* and *Laguncularia racemosa* phyllospheres harbor a homogeneous cyanobacterial population. Concerning the group abundance, a predominance of sequences affiliated to the order Nostocales and Oscillatoriales was observed, with a remarkable number of sequences similar to *Symphyonemopsis* strain (order Nostocales). The project has studied the presence of actinobacteria in sediments and has evaluated the potential in producing secondary metabolites. The most found genera in this environment are *Streptomyces* (Figure 1). Here, we present a survey on the bacterial community from the rhizosphere of *R. mangle*, exploiting the ability of these bacteria to produce endoglucanase and to form biofilms in response to the increase in the saline conditions (Figure 2). The highest endoglucanase producers have revealed a more intense biolfim structure when cultivated in higher NaCl concentrations. Moreover, the NaCl has modulated the endoglycolytic activity. The salinity of the medium has shown to be an important factor to modulate the ecological behavior of *Bacillus* in mangroves, resulting in free-living or biofilm-immersed cells in the rhizosphere of *R. mangle*.

Effect of salinity on biofilm formation by B. subtilis. Observe the mucilage formed in response to high concentration of NaCl (15%) (B), in comparison to the treatment with 3% of NaCl (A) (Sa, 2008)



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Itamar Soares de Melo

Empresa Brasileira de Pesquisa Agropecuária
Embrapa Meio Ambiente

Rodovia SP-340, Km 127,5
Caixa Postal 69, Tanquinho Velho
CEP 13820-000 – Jaguariúna, SP – Brasil

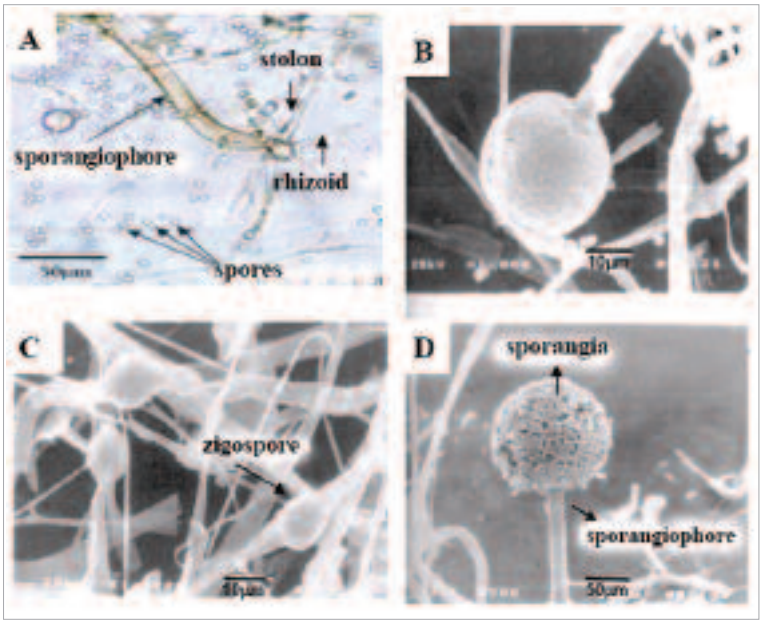
+55-19-3867-8765
itamar@cnpma.embrapa.br
www.cnpma.embrapa.br/site_projeto_manguezais

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

Jairo Kenupp BASTOS

Ribeirão Preto School of Pharmaceutical Sciences / University of São Paulo (USP)

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;



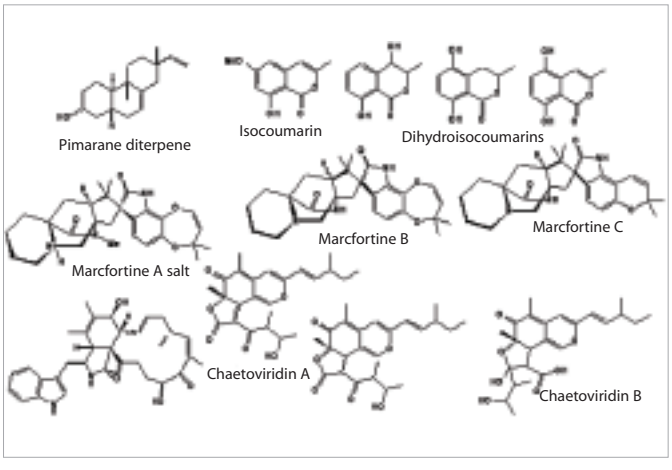
Rhizopus microsporus var. rhyzopodiformis.
A- Photonic microscopy; B, C and D- Microscopy electron scanning

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

Faculdade de Ciências Farmacêuticas
de Ribeirão Preto / Universidade de São Paulo (USP)
Avenida do Café S/N – Monte Alegre
CEP 14040-903 – Ribeirão Preto, SP – Brasil

+55-16-360-2416
jkbastos@fcfrp.usp.br

SOCIO-ENVIRONMENTAL CHANGES IN THE STATE OF SÃO PAULO: PERSPECTIVES FOR CONSERVATION

Luciano Martins Verdade

Center of Nuclear Energy in Agriculture / University of São Paulo (USP)

Main research: Antonio R. Almeida Jr.

FAPESP Grant 2006/60954-4 I Term: Aug 2008 to Jul 2012

The state of São Paulo has suffered dramatic environmental changes since 1850 with a consistent destruction of most of the original ecosystems. As consequences, progressive loss of water and soil qualities, as well as crescent contamination of wildlife by pesticides have been promoted. Hopefully, there are no documented case of local extinction of wildlife and plants in São Paulo State, what stresses possible adaption processes to anthropogenic environments. This suggests the inclusion of agro ecosystems in the context of conservational biology. The present study proposes to evaluate the socio economical and cultural aspects causing the changes in the land use in São Paulo during the 20th Century, and also how such changes have affected the ecosystems capacity to produce environmental services essential to life maintenance.

*Mixed footsteps of a puma (*Puma concolor*) and a human on an agricultural landscape in the state of São Paulo (Source: Lisboa MAM & Prado BH.* Fazenda das Areias e Fazenda da Conquista: história, memória e cultura. Instituto Florestal. *São Paulo* – in press*)*

Puma crossing an eucalyptus plantation in the state of São Paulo (Source: Lisboa MAM & Prado BH. Fazenda das Areias e Fazenda da Conquista: história, memória e cultura. Instituto Florestal. *São Paulo* – in press*)*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The preliminary results of the projects can be summarized as follows:

Conceptual basis improvement: temporal dimensions can be eventually more relevant than geographical dimensions to determine the current patterns of biodiversity abundance and distribution in agricultural landscapes. In addition, agroecosystems (i.e., the matrices of agricultural landscapes) should not be considered as natural "non-habitats", since they have resident species. On the other hand, agricultural activities are possibly the main anthropogenic pressure on biodiversity conservation due to pollution, introduction of exotic species and the destruction of natural ecosystems. The ,-diversity of an agricultural landscape can be considered a direct measure of its conservation value. In order to increase it, agricultural practices should be less invasive.

Technological/methodological development: We are working to develop multi-taxa systematized sampling procedures in long-term biodiversity monitoring programs, and molecular markers to identify, the species, the individuals of mammal terrestrial carnivores and their familiar lineages in microgeographic scale. We are also improving the use of stable isotopes of C and N in order to better understand the trophic structure of agricultural landscapes. The outcomes of this project will allow, for the first time, a broad view of wildlife adaptation to agricultural landscapes in terms of the use of space and feeding ecology by the species. We are also developing and validating models of water use for urban and agricultural purposes, as well as models to estimate biomass of native vegetation in altered environments such as in restoration areas.

Institutional articulation and improvement: This project is deeply related with the impacts the current proposed change in the Brazilian Forest Code might have on biodiversity. The dubious role of the media on this and other environmental issues have been pointed by this project. In addition, our results suggest a necessary interdisciplinary approach for the conflict between agriculture and conservation including the improvement of the related institutions in terms of research, education, extension, public policy and law enforcement.

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Luciano Martins Verdade

Centro de Energia Nuclear na Agricultura
Universidade de São Paulo

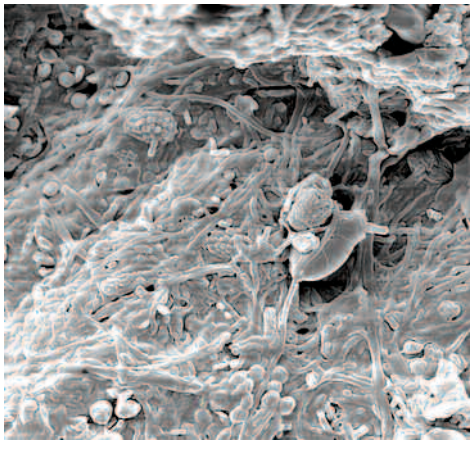
Laboratório de Ecologia Isotópica
Caixa Postal 96
CEP 13400-970 – Piracicaba, SP – Brasil

+55-19-3429-4070
lmverddae@usp.br

MICROBIAL DIVERSITY IN THE PHYLLOSPHERE AND SOIL OF THE ATLANTIC FOREST

Márcio Rodrigues Lambais
Higher School of Agriculture Luiz de Queiroz / University of São Paulo (USP)
Main researcher: Marli de Fátima Fiore
FAPESP Grant 2008/50824-1 I Term: Dec 2009 to Nov 2013

In a previous work, we have observed that the phyllosphere of different plant species, select for distinct bacterial communities, and that each phyllosphere may harbor a substantial number of unknown bacterial species. Our estimates point from 2 to 13 million of new bacterial species in the canopy of the Atlantic Forest. In another work, developed in a 10 ha permanent plot at the Carlos Botelho State Park, we have observed that the bacterial communities in the phyllosphere of plants phylogenetically closer, were more similar to each other than in the phyllosphere of plants phylogenetically more distant. Also relevant is the fact that, even considering the spatial variability of the bacterial community structure in the phyllosphere of plants of the same species at different geographical positions, such variation is smaller than that observed among individuals of different species. Our data suggest that the bacterial populations in the phyllosphere are selected by the plant species, and that each plant species harbor a unique bacterial community in its phyllosphere. The analyses of the bacterial community associated to the bark of the same plant species reveled low bacterial species richness, with the dominance of few genera, as compared to the phyllosphere, and community structures defined by the plant species as well. In contrast, the soil under the canopy of the tree species sampled showed bacterial communities with higher species richness, as compared to phyllosphere and bark, with lower spatial variability and less dependency on the plant species. In general, our data points to a new paradigm in microbial ecology: the microbial diversity associated to the plant surfaces may be as high as the diversity in the soil, considered the environment with the highest known microbial diversity. Understanding the functional roles of these microorganisms in the phyllosphere and the soil of the Atlantic Forest is not a trivial task, even though the advances in the analytical techniques have significantly contributed to that. Hence, establishing a relationship between phylogeny and metabolic function is crucial for determining which microbial groups are essential for ecosystem sustainability, in particular for the C and N cycles. In the proposed project, we aim to evaluate the bacterial and fungi diversity in the phyllosphere and soil, respectively, in four 10 ha permanent plots of the Biota program, at Cardoso Island State Park (High Restinga Forest), Carlos Botelho State Park (Dense Ombrophylous Forest) and Assis Ecological Station (“Cerradão”), and their possible relationships with biochemical processes relevant for the functionality of forest ecosystems, using metaproteomics. In addition, we aim to quantify the biological nitrogen fixation and the diversity of diazothrophs in 1 ha permanent plots of a Dense Ombrophylous Forest at the Serra do Mar State Park.



Microbial biofilm covering the leaf surface

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have confirmed, using pyrosequencing, that the bacterial community structure in the phyllosphere of plants of the same species are more similar to each other than to plants of different species. Additionally, we have observed that the bacterial community structure in the phyllosphere of phylogeneticaly related plants are more similar, suggesting that bacterial communities co-evolved with their plant hosts. In addition, we have observed that the bacterial communities in the phyllosphere of M. robusta show geographical patterns of organization, with similarity in community structure decreasing with geographical distances. The bacterial community structure in the phyllosphere is also affected by organic volatile compounds released by the leaves, which might be important determinants of bacterial survival in such environment. Analyzing the meta proteome of the microbial community in the phyllosphere of several plant species, we have identified several microbial proteins putatively involved in transport, biofilm formation, stress responses, anti-oxidative responses, and nitrogen fixation. Biological nitrogen fixation has been measured in the phyllosphere and dermosphere of several plant species and seems to contribute with a significant part of N input in the Atlantic Forest.

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Márcio Rodrigues Lambais

Escola Superior de Agricultura Luiz de Queiroz (Esalq)
Universidade de São Paulo (USP)

Departamento de Ciência do Solo
Av. Pádua Dias, 11
CEP 13418-900 – Piracicaba, SP – Brasil

+55-19-3417-2107
mlambais@esalq.usp.br

DIVERSITY OF RED MACROALGAE (RHODOPHYTA) OF SÃO PAULO STATE, BRAZIL, BASED ON BARCODING, MORPHOLOGY AND GEOGRAPHIC DISTRIBUTION (RHODO-SP)

Mariana Cabral de Oliveira

Institute of Biosciences / University of São Paulo (USP)

Main researchers: Mutue T. Fujii, Orlando Necchi Jr.

FAPESP Grant 2007/51270-7 I Term: Apr 2008 to Aug 2012

Praia do Costa, Ubatuba, SP, illustrating one of the collection sites in the marine environment (Photos M.C. Oliveira)

In this project we propose the screening of the red macro algae (Rhodophyta) biodiversity for the State of São Paulo (Brazil), which includes more than 50% of the diversity known for this group in the country. For that, the DNA bar-coding technique will be used supplemented with morphological and geographical distribution. These data will be integrated to the Biota database. Studies on the biodiversity of marine algae from the State of São Paulo have been carried out since 1950; however, this knowledge is based on morphological data being sporadically supplemented with molecular data. The taxonomic identification of red algae is notoriously difficult due to: (i) a relatively simple morphology and anatomy, which are convergent in many species, (ii) phenotypic plasticity and (iii) complex life-cycles with heteromorphic stages. The comparisons of DNA sequences have been fundamental for biodiversity studies and for the inference of the relationships among the different groups of organisms. The DNA bar-coding technique generates a great amount of data in relatively short time. These data, organized and available in databanks, can be used for many different types of research, including biodiversity screenings, conservation, the detection of cryptic and exotic species, development of DNA probes for various applications, taxonomy and phylogenetic studies, ecophysiology, forensics and others.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

So far over 1,000 specimens were collected, and 906 sequences have been generated for five different molecular markers: 324 *cox*1, 160 rbcL, 34 SSU rDNA, 150 UPA and 238 *cox*2. Our analyses indicate that *cox*1 barcode region is a suitable marker for the delineation of Rhodophyta species, supporting its use as a DNA barcode and also in identifying cryptic species and phenotypic plasticity. Moreover, once there is a reliable database of sequences available for comparison, the sequencing of *cox*1 is an easy and relatively rapid tool for species identification of red algae. With the same purpose, we are also sequencing another UPA barcode marker. This second marker has shown to be a little more conserved than the *cox*1, however, it is easily amplified and sequenced. Other markers for phylogenetic analysis have also been obtained (*rbc*L and SSU) for selected species. These sequences and the morphological analyses are being deposited in the Bold database. With the obtained data, new records and species are being detected, as well as the potential introduction of exotic ones. The project also aims to list the species and their distribution in the state, as well as the production of a photographic guide.

Botryocladia bahamensis (scale 0-0.3-cm), material collected in Laje de Santos, SP (Photo R. Rocha, Jorge)

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Mariana Cabral de Oliveira

Instituto de Biociências
Universidade de São Paulo (USP)
Departamento de Botânica
Rua do Matão, travessa 14, 321, Butantã
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-7630
mcdolive@usp.br
http://www.ib.usp.br/rhodosp

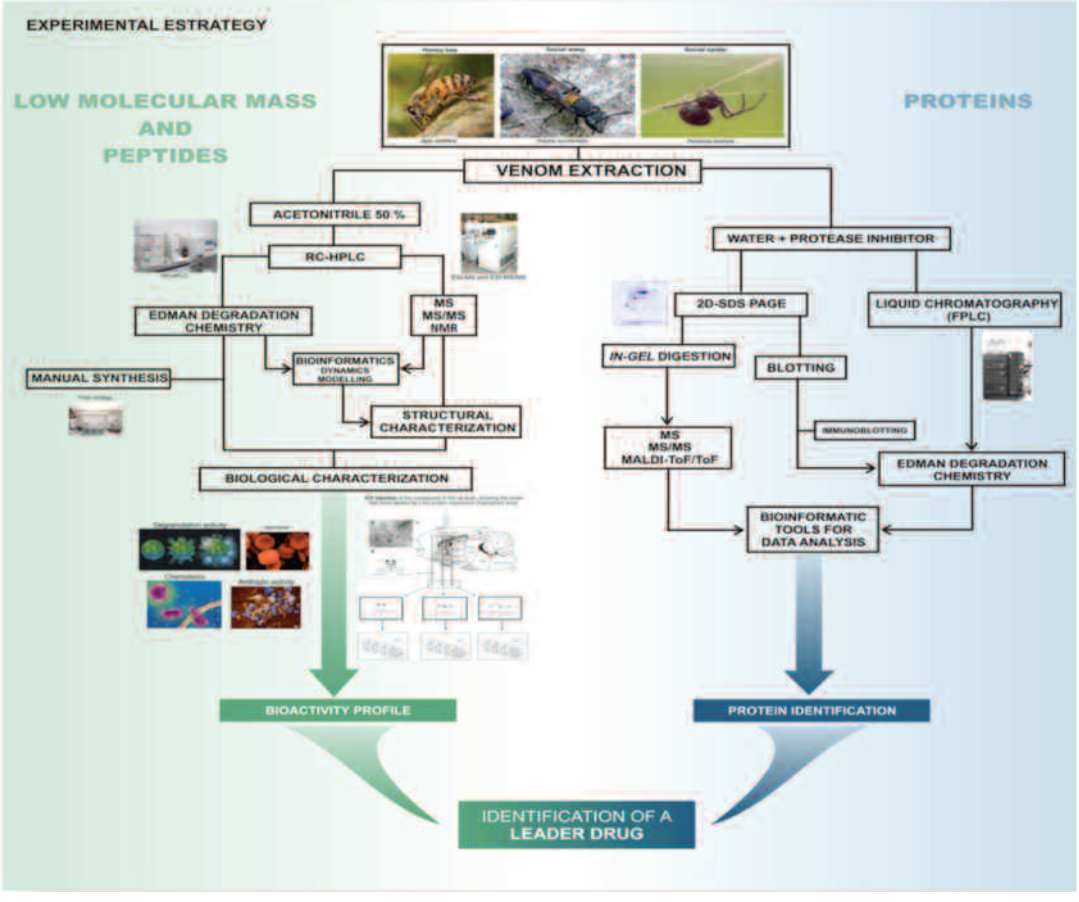
BIOTA-FAPESP PROGRAM

BIOPROSPECTION OF THE FAUNA OF BRAZILIAN ARTHROPODS IN THE SEARCH FOR LEADER DRUGS FOR THE RATIONAL DEVELOPMENT OF NOVEL PHARMACEUTICALS AND PESTICIDES

Mario Sérgio Palma

Institute of Biosciences / Paulista State University (Unesp)

FAPESP Grant 2006/57122-7 I Term: Dec 2007 to Nov 2011



Flow chart of bioprospection strategies of the fauna of Arthropods of São Paulo State

The main objectives of this thematic project are: i) the identification and ii) synthesis of abundant low molecular mass compounds from toxic secretions of spiders and social insects (Hymenoptera) presenting neuroactive actions; iii) pharmacological and physiological assays of these compounds for neurotoxicity/neuroprotection; iv) the screening for polycationic peptides in the venoms of social Hymenoptera, assigning their amino acid sequence and determining their secondary structure; v) peptides synthesis and peptides screening for antibiosis, pain/analgesy and inflammation and vi) the characterization of interactions between antibiotic peptides with natural /synthetic membranes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The use of metabolomic approaches, specially the footprint profile of the defensive secretions from wasps and spiders, has allowed the identification of low molecular compounds with neurotoxic functions. Novel natural products were also detected in the footprint profile, most of them with molecular structures elucidated by spectroscopic techniques (HRMS, LC-MS, MS^n and NMR). A large fraction of these compounds had their synthesis route developed, and the synthetic compounds were submitted to a wide range of neurotoxicity and neuroprotective assays (open field behavioral assays, electrophysiology and neuropharmacological tests). More than one-hundred of novel acylpolyaminetoxin structures, from orb-web-spider venoms, were elucidated, as well as twelve alkylindole alkaloid toxins from spider´s web and venoms, and two organ metallic compounds from the oily droplets of Nephilinae spiders´web. A neurotoxic histaminyl glucoside, presenting blocking activity against different types of ion channel receptors was identified in the venom of some species of social wasps. In addition to this, alkaloid toxins, such as piperidine derivatives were isolated from the venom of the banana-spider (*Phoneutria nigriventer*). Several of these compounds proved to be potent neuro-protective agents in experimental assays of epilepsy, with a great potential to become models for the development of new neuropharmaceutical drugs. In parallel to these investigations, a great family of polycationic peptides have been detected in the venom from social wasps through LC-ESI-IT-TOF-MS, and sequenced by using mass spectrometric analysis under CID conditions. These peptides were manually synthesized on solid-phase, purified and its secondary structure analyzed by spectroscopic techniques (circular dichroism, fluorescence, FT-IR, and NMR). The peptides were submitted to a wide range of biological assays, including antibiosis, analgesic effect, anti-hypertensive action, anti-inflammatory action, and anti-proliferative effect and their interactions with membranes (natural and synthetic) were evaluated by 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. Since some of these peptides are involved with the mast cell exocytosis, it was developed an analytical platform combining affinity chromatography with immobilized peptides, as ligands, and membrane proteolipossomes, and proteomic analysis for the bioprospection of G-protein coupled receptors. Five different protein receptors were identified, allowing the expansion of the current knowledge about the mechanisms of mast cell activation induced by the polycationic peptides.

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Mario Sérgio Palma

Instituto de Biociências de Rio Claro
Universidade Estadual Paulista (Unesp)

Avenida 24A, 1515, Bela Vista
CEP 13506-900 – Rio Claro, SP – Brasil

+55-19-3526-4163
mspalma@rc.unesp.br

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

Massayoshi YOSHIDA
Chemistry Institute / University of São Paulo (USP)

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 phytotherapics (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. Eventualy 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 anxyolitic-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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Massayoshi YOSHIDA

Instituto de Química
Universidade de São Paulo (USP)
Departamento de Química Fundamental
Avenida Prof. Lineu Prestes, 748, Bloco 11, Térreo
Caixa Postal 26077 – Cidade Universitária
CEP 69075-351 – São Paulo, SP – Brasil

+55-11-3091-3875
myoshida@iq.usp.br

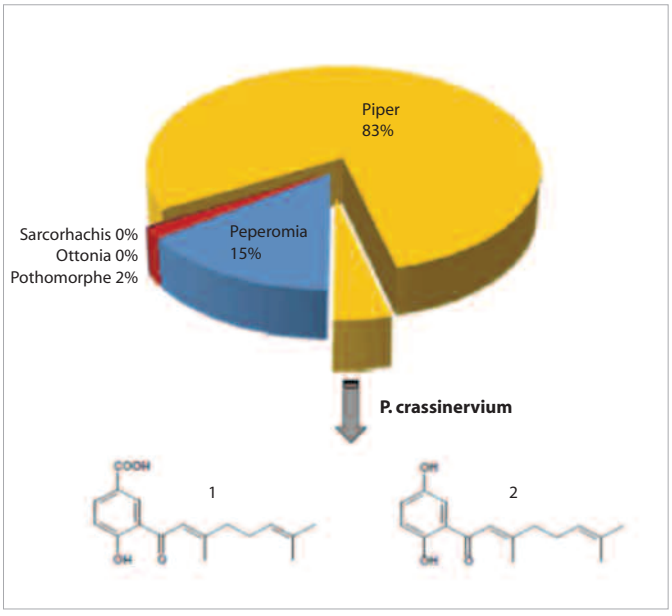
MOLECULAR DIVERSITY OF ANGIOSPERMS

Massuo Jorge Kato

Chemistry Institute / University of São Paulo (USP)

Main researcher: Eny Iochevet Segal Floh

FAPESP Grant 2009/51850-9 I Term: May 2010 to Apr 2015



Distribution of Piperaceae members considering the report of some biological activity for their isolated compounds. The abundance of Piper crassinervium *is highlighted, and the structure of the geranylated bioactive compounds, 4-hydroxy-3-E-(30,70-dimethyl-10-oxo-20,60 -octadienyl)-benzoic acid (1) and 1,4-dihydroxy-2-E-(3,7´´-dimethyl-10 -oxo-20,60-octadienyl)-benzene (2) is presented (Lopez* et al.*, 2011)*

This project is a multi- and interdisciplinary study of Angiosperms biodiversity. We propose to unravel the factors behind the Angiosperms metabolic diversification through systems biology approaches, describing the chemical variability resulting from the plants interactions with associated flora and fauna. Piperales plant species were chosen to be tested as models based on their diversity richness, ease of propagation, and the perspective to integrate the research with groups working with systematic, cytogenetic, molecular phylogeny, ecology, bioprospecting and metabolome studies.

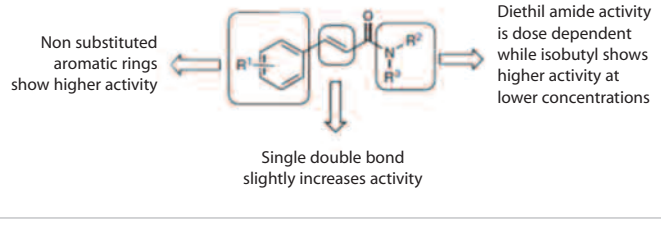
The phylogenetic studies will be based on molecular markers (Internal Transcriber Space, *matK* and others) and the robustness of the phylogenetic trees will be tested by gene sequences of secondary metabolites (PKS, TYDC, prenyltransferases, dirigent protein and involved in lignification process) and by metabolome (mass spectrometry or NMR followed by multivariate analysis). The study of genetic variability will be supported by microsatellites, SSR and other genetic marker analysis. Possible ecophysiological functions of the major class of secondary compounds are expected to be inferred by antifungal and insecticide bioassays, biosynthetic regulation pattern and also by phylogenetic positioning. The establishment of a network is an initiative to integrate germoplasm conservation, ecology, chemistry and bioactivity, as well as, evolutionary studies as a model to investigate the biodiversity in the tropics.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The initial activities of the project have involved intensive sampling of *Piper* and *Peperomia* species for fingerprinting and DNA analysis. To date, the phylogenetic analysis has been carried out based on *Internal Transcriber Spacer* (ITS) sequences and the phylogenetic tree was examined for the presence of major classes of secondary compounds. Some clades, highlighting phenylpropanoid derivatives, were further investigated according to the genetic diversity and chemical variability. The chemometric data, based on NMR and/or ESIMS, has discriminated groups producing tetrahydrofuran lignans and dihydrobenzofuran neolignanas. Metabolomic analysis has also been applied to scrutinize extracts in order to determine similarities among Piper species and also as preliminary criteria to address further characterization of major secondary compounds.

The electro spray ionization mass spectrometry analysis of the principal component of the crude extracts, and ^{1}H NMR data highlighted species producing lignans (*P. solmsianum*), neolignans (*P. regnellii*), chromenes (*P. gaudichaudianum*) and amides (*P. tuberculatum*). Nevertheless, the analysis performed on seedlings organs of the species producing lignans/neolignans and chromenes have indicated the production of dillapiole and apiole, while, extracts of adults and seedlings of amide-producing species (*P. tuberculatum, P. reticulatum* and *P. amalago*) have shown a similar profile, indicating some specific control during development. This approach will be further evaluated in terms of expression of biosynthetic enzymes and their regulatory process.

Preliminary structure-activity relationship for antifungal amide. (Marques et al.*, 2010)*



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Massuo Jorge Kato

Instituto de Química
Universidade de São Paulo (USP)

Departamento de Química Fundamental
Av. Prof. Lineu Prestes, 748
Bloco 11T – Cidade Universitária
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-1886
majokato@iq.usp.br

BIOTA-FAPESP PROGRAM

DEVELOPMENT OF A PLATFORM FOR *IN VIVO* AND *IN VITRO* METABOLISM STUDIES WITH NATURAL PRODUCTS, A DEMAND FOR A SYSTEM OF PRE-CLINIC EXPERIMENTS

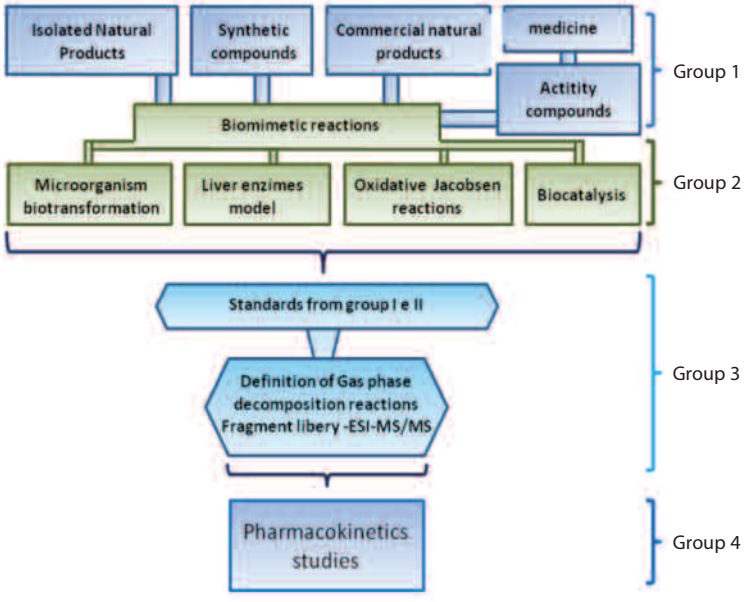
Norberto Peporine Lopes

Ribeirão Preto School of Pharmaceutical Sciences / University of São Paulo (USP)

Main researcher: Mônica Tallarico Pupo

FAPESP Grant 2009/51812-0 I Term: Jan 2010 to Dec 2013

Several actions for the development of bioactive natural products have been taken at national and state level, in majority ones that led to the identification of substances with therapeutic potential. A prerequisite for clinical and compound stability studies is the chemical characterization of active targets and also the elucidation of possible metabolites. In this context, the project aims the establishment of a working platform that envisions supporting pre-clinical studies, hereby generating four big working groups. Since the platform model still is somewhat uncommon the team size may oscillate during project execution, having involved in this first year seventeen members. The possibility of a variable group size occurs in function of the demand and opportunity of identifying a potentially active compound as well as having it in sufficient quantity for studies, which finally is the limiting factor for different works. Furthermore, the groups exhibit diverse characteristics concerning their publication potential and speed of obtaining results, which makes the global analysis a little different.

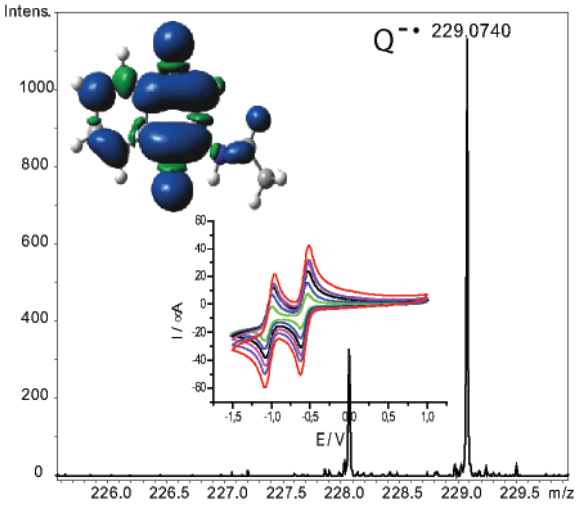


Group 1 (8 articles): Selection of bioactive natural and synthetic products

Group 2 (6 articles): *In vitro* metabolism studies of selected compounds in chemical and biological models. Obtainment of potential metabolites in larger scale

Group 3 (5 articles): Comprehension of decomposition reactions in gas phase by mass spectrometry (supported by theoretic calculations) from selected compounds and their derivatives, envisioning the creation of an ESI fragmentation spectra library

Group 4 (1 article): Application of the fragmentation models and obtained references for the development of analytical methods in in vitro and in vivo studies for absorption, kinetic disposition and also biotransformation of natural products

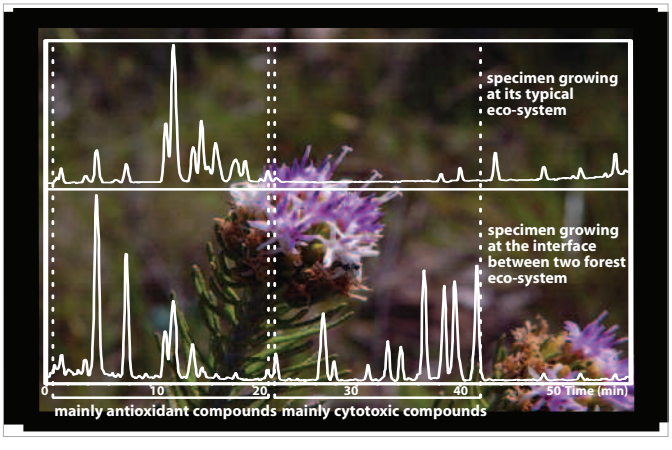


Generation of naphthoquinone radical anions by electrospray ionization: comparison between solution and gas-phase chemistry

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The selected active natural products were submitted to biomimetic studies, in which the Jacobsen's catalyst was introduced instead of the more common metalloporphyrins, achieving for biomimetic reactions very high yields. In two cases, the yields of catabolized active compounds exceeded 90%, which is extremely significant. In two cases, the main products obtained by biomimetic reactions were the same as observed in the microsomal metabolism. This enabled the perspective of producing phase one metabolites for further pharmacokinetic analysis. The fragmentation studies in gas phase allowed the definition of three complete pathways from three classes of natural products. The first pharmacokinetic pilot study has clarified the elimination mechanism and half life time of the alkaloid piperine. Initial results has shown the viability of the proposal and has generated the expectation of better understanding the absorption, distribution and metabolization mechanism for selected natural products.

Differential metabolic and biological profiles of Lychnophora ericoides Mart. *(Asteraceae) from different localities in the Brazilian "campos rupestres"*



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Norberto Peporine Lopes

Faculdade de Ciências Farmacêuticas de Ribeirão Preto / Universidade de São Paulo (USP)

Departamento de Física e Química
Avenida do Café, s/n, Monte Alegre
CEP 14040-903 – Ribeirão Preto, SP – Brasil

+55-16-3602-4707
npelopes@fcfrp.usp.br

BIOTA-FAPESP PROGRAM

FAUNA AND FLORA OF REMNANT FOREST FRAGMENTS IN THE SÃO PAULO STATE NORTHWEST REGION

Orlando Necchi Junior

São José do Rio Preto Institute of Biosciences, Language Studies and Exact Sciences / Paulista State University (Unesp)

Main researchers: Denise de Cerqueira Rossa Feres, Neusa Luis Henrique Zanini Branco, Taroda Ranga, Reinaldo José Fazzio Feres

FAPESP Grant 2004/04820-3 I Term: Dec 2005 to Nov 2010

The fragmentation of forest habitats is an evident and growing process in tropical regions. The understanding of these 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 must not be reverted without actions of ecological management. Despite this impact, it is a region of relevant species richness and that, paradoxically, have received little attention concerning to the study of its biodiversity.

Aspects of forest fragments in the São Paulo State northwest region (Photos: João Marcos Rosa, Agência Nitro)

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, to be incorporated with distinct matrices (sugar cane and orange plantations, pastures, urban areas, etc) and sizes. The objectives of the project includes: 1) to prepare an environmental diagnostic to propose further actions for environmental conservation; 2) to have a sketch on the possible effects of forest fragmentation for population dynamics and physiology of animal and plant species; 3) to indicate the relevance of forest fragments for the maintenance of regional biodiversity; 4) to evaluate the importance of forest fragments as a reservoir of species 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 more meaningful considering that it is expected provide essential subsides for future studies on conservation/maintenance of this valuable biological patrimony.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Fourteen groups of animals, plants and fungi were surveyed in 18 fragments of semi deciduous seasonal forest, resulting in the identification of almost 2,000 species. Only 205 species (11.8%) occurs in more than half of the fragments, whereas only eight species (0.5%) occurs in all sampled fragments. Consequently, the similarity in species composition between the fragments was very low and the species composition shows a strong pattern of nesting when the forest fragments were ordered in terms of species richness. The species richness is directly related to the area of the forest fragments. The results has shown that, despite the fact that large fragments holds higher species richness than the smaller fragments, species distribution among the fragments is more heterogeneous than expected, resulting mainly from stochastic events due to fragmentation process than to the characteristics of forest fragments. Thus, the conservation of biodiversity in the northwest region of São Paulo State should not involve only the preservation of the largest or the best preserved fragments, since the species are distributed into fragments of different sizes and degrees of conservation.

Aspects of forest fragments in the São Paulo State northwest region (Photos: João Marcos Rosa, Agência Nitro)

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Lemes-Silva NM, Branco LHZ, Necchi Jr. O. 2010. Corticolous green algae from tropical forest remnants in the northwest region of São Paulo State, Brazil. *Revista Brasileira de Botânica.* **33**: 215-226.

Orlando Necchi Junior

Instituto de Biociências, Letras e Ciências Exatas
de São José do Rio Preto
Universidade Estadual Paulista (Unesp)

Rua Cristóvão Colombo, 2265 – Jardim Nazareth
CEP 15054-000 – São José do Rio Preto, SP – Brasil

+55-17-3221-2406
orlando@ibilce.unesp.br

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

Paulo Roberto Hrihorowitsch MORENO
Chemistry Institute / University of São Paulo (USP)

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

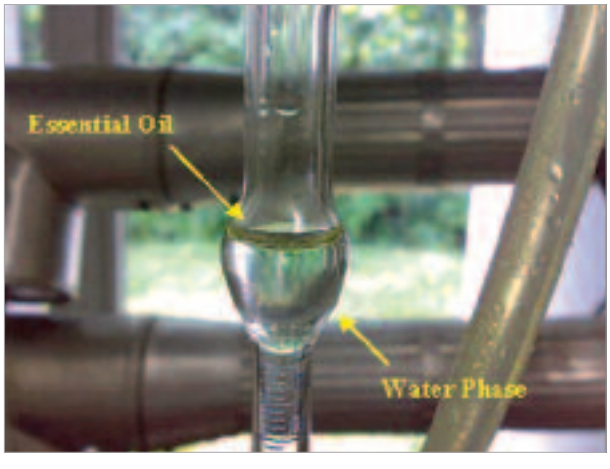
The Atlantic Rain Forest in São Paulo has a great diversity of essential oil-producing plant species. Lauraceae and Myrtaceae, both recognized producers of volatile oils, are also listed among the four most important families in the floristic composition of the São Paulo Atlantic Rain Forest. During 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 Clevenger apparatus



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Paulo Roberto Hrihorowitsch MORENO

Instituto de Química
Universidade de São Paulo (USP)
Avenida Prof. Lineu Prestes, 748, Bloco11, Térreo
Caixa Postal 26077 – Cidade Universitária
CEP 05508-000 – São Paulo, SP – Brasil

+55-11-3091-3875
prmoreno@iq.usp.br

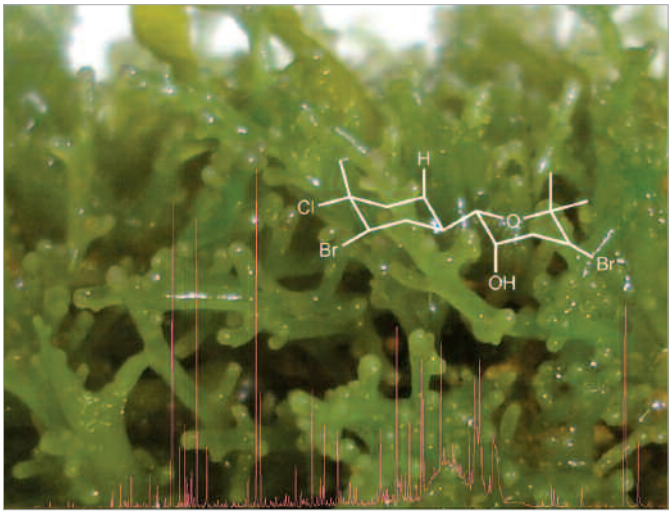
BIOPROSPECTION AND USE OF ALGAL BIOMASS AS A SOURCE OF METABOLITES OF ECONOMICAL IMPACT

Pio Colepicolo Neto

Chemistry Institute / University of São Paulo (USP)

Main researchers: Eliane Marinho Soriano, Mutue Toyota Fujji, Paulo Horta, Norberto P. Lopes, Marcia Mesko, Nair Yokaya, Ernani Pinto

FAPESP Grant 2010/50193-1 I Term: Aug 2010 to Jul 2015



Picture of Laurencia catarinensis *and a CG/MS chromatogram of volatile compounds (photo by Erika Stein)*

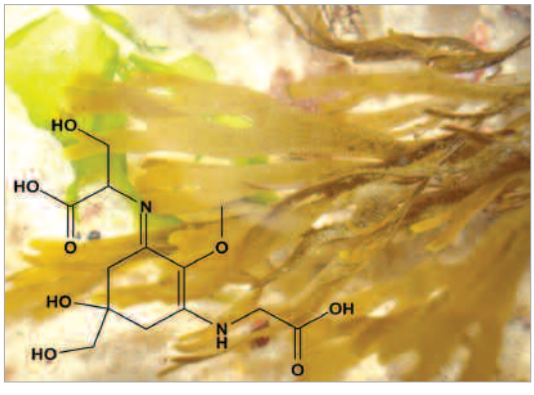
In the marine environment, algae are one of the largest groups in terms of diversity. In order to survive in the highly competitive aquatic environment, marine algae have developed defense strategies that rely on a tremendous diversity of compounds produced by several different metabolic pathways, which makes algae particularly promising as the source of novel biochemically-active compounds, in addition to essential compounds for human nutrition. Macro algae play a vital role in the maintenance of the marine equilibrium, in the preservation of the biodiversity and in the mitigation of the global warming. Due to growing of its economical importance there is an increasing need for improved techniques of the isolation of algae extracts, fractions or pure compounds, novel strategies for bioprospection and for rapid screening of extracts and bioactive fractions. In addition, the microalgae have enormous potential for the development of biotechnology and, as such, constitute an important strategic natural resource for the country. Since the expanding market for algal-based products brings enclosed the risk of natural populations over-harvesting, further improvements in the controlled cultivation, harvesting and conservation of algae will be necessary to allow the sustainable large-scale production of algae and algal-derived products avoiding further damages to marine environments. The long term goal of this project is to isolate biological active substances from macro algae of the São Paulo seacoast. Algae and algal products also have an economic impact in several other sectors, such as in the aquaculture, in the pharmaceutical, nutraceutical, biomedicine, veterinary medicine, cosmetic industries and in the public health. The biological activity includes antioxidant, antibiotic, anticancer, antifungal, anti-inflammatory and UV blocking compounds. In addition, the macro algae biodiversity will be described and its sustainable cultivation, for bioremediation use, will be tested.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The development of new algal anti-cancer drugs represents one of the least explored frontiers in medicinal chemistry. In this regard, the diversity of macro algae can be viewed as a largely untapped natural resource. As preliminary reported, we have described a comparative study on the cytotoxic properties of extracts obtained from macro algae. Four samples initially investigated have shown significant levels of toxicity towards a model tumor cell line (human uterine sarcoma, MES-SA). The highest levels of cytotoxicity were typically associated with non-polar (hexane) algal extracts, while the lowest levels of cytotoxicity were found with the corresponding polar (methanol) extracts.

Qualitative and quantitative studies of mycosporine-like amino acids (MAAs) in three species *Gracilaria birdiae*, *G. domingensis* and *G. tenuistipitata* were performed. A simple and efficient extraction procedure based on ethanol was used. HPLC, UV and mass spectrometry experiments revealed different profiles between extracts obtained from one species cultivated in the laboratory (*G. tenuistipitata*) and two species collected in their natural environment (*G. birdiae* and *G. domingensis*). The levels detected in the latter two species were approximately 150 times higher than in the species cultivated in vitro. This study revealed that *G. birdiae* and *G. domingensis* present potential sources for economical exploration of MAAs.

The absorption efficiency and kinetic parameters (V_{max}, K_s and V_{max}:K_s) of the seaweed *Gracilaria cervicornis* for NH_4^+, NO_3- and PO_4^{3-} nutrients were evaluated. Absorption efficiency was measured by monitoring nutrient concentrations in culture media. Absorption efficiencies for this alga were greater in treatments with lower concentrations, as evidenced by a reduction of NH_4^+, NO_3- and PO_4^{3-}. Kinetic parameters has shown that *G. cervicornis* exhibits greater ability to take up high concentrations of NH_4^+ and low concentrations of PO_4^{3-}. These results suggest that *G. cervicornis* has good absorption capacity for the nutrients tested and may be a promising candidate as a bioremediation of eutrophized environments.



Picture of genus Dictyota (Dictyotaceae-Phaoephyta) and the strucuture of MAA isolated from marine macroalgae (photo by Jean-Paul Soriano)

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Pio Colepicolo Neto

Instituto de Química
Universidade de São Paulo (USP)

Departamento de Bioquímica
Avenida Prof. Lineu Prestes, 748 – Caixa Postal 26077
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-2170
piocolep@iq.usp.br

BIOTA-FAPESP PROGRAM

DISCOVERY AND DEVELOPMENT OF POTENTIAL CHEMOTHERAPEUTIC AGENTS FROM MARINE INVERTEBRATES AND ASSOCIATED MICROORGANISMS

Roberto Gomes de Souza Berlinck

São Carlos Chemistry Institute / University of São Paulo (USP)

Main researchers: Clarice Weis Arns, Lara Duraes Sette

FAPESP Grant 2010/50190-2 I Term: Mar 2011 to Feb 2013

Ascidian Didemnum sp.

The aim of this project is the chemical investigation of biologically active crude extracts obtained from marine invertebrates and marine microorganisms collected in different regions of the Brazilian coastline. Extracts of marine invertebrates active as cytotoxic and antituberculosis, as well as with antimicrobial activity against both antibiotic-sensitive and antibiotic-resistant human pathogenic microbes and of inhibition of specific enzymes implied in the life cycle of pathogenic protozoa, will be subjected to dereplication by LC-PDA-MS before a chromatographic fractionation toward the isolation of biologically active compounds. Marine fungi and bacteria isolated from marine invertebrates will be grown in optimized artificial media in order to produce secondary metabolites. Extracts from marine microbes growth media will be subjected to the same above mentioned bioassays. A new set of bioassays related to the inhibition or stimulation of chemical mediators implied in immunomodulation processes will be applied to the whole crude extract collection in order to search for active extracts. Dereplication by LC-PDAMS analysis in order to get information about crude extracts chemical profiles will provide basis for the isolation of novel bioactive compounds. Pure chemical entitites obtained from marine invertebrates and microorganisms will be identified and evaluated in the bioassays in which the original crude extract was active, aiming to obtain information on their mechanism of action.

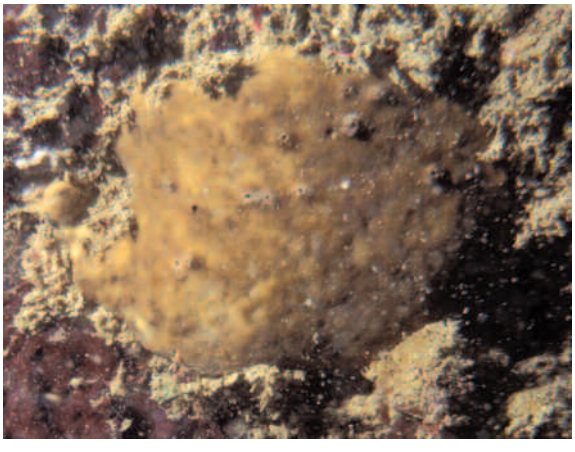
SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have screened > 1500 crude extracts from both marine invertebrates and microorganisms in cytotoxicity, anti-Leishmania, antimicrobial and anti-inflammatory bioassays. Several active extracts were investigated. Pure compounds have been isolated from both marine invertebrate and microbes. These include polycyclic bis-piperidine alkaloids from Haplosclerid sponges that displayed cytotoxic and anti-Leishmanial activities, cytotoxic alkaloids from a nudibranch of the genus Tambja, anti-Leishmanial and anti-neuroinflammatory polyketides from the marine sponge *Plakortis angulospiculatus*, anti-bacterial modified diketopiperazines from two ascidians of the genus Didemnum and anti-tuberculosis polybrominated tyrosine derivatives from marine sponges. The investigation of marine-derived fungi culture media led to the isolation of several bioactive metabolites, mostly with antibiotic activity.

Aiming to overcome the low yield production of secondary metabolites by marine-derived fungal strains, we have developed a method for the optimization of secondary metabolites production in culture media. Consequently, it has been possible to isolate and identify minor metabolites produced in tiny amounts under standard culture conditions. The method is currently being improved and further explored in the production of bioactive natural products by marine-derived fungal strains.

The exploitation of marine biological sources of natural products has been very productive, and showed promising results towards the finding of new lead compounds for drug discovery. Although the compounds so far isolated are structurally too complex to be considered as drug leads, the finding of novel bioactive compounds from the São Paulo state and Brazilian coastline biodiversity shows the importance of a continuous search for such chemical entities with a potential for the development of new drug candidates.

Sponge Plakortis angulospiculatus



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Roberto Gomes de Souza Berlinck

Instituto de Química de São Carlos
Universidade de São Paulo (USP)

Avenida Trabalhador São-carlense, 400
Caixa Postal 780
CEP 13560-970 – São Carlos, SP – Brasil

+55-16-3373-9954
rgsberlinck@iqsc.usp.br

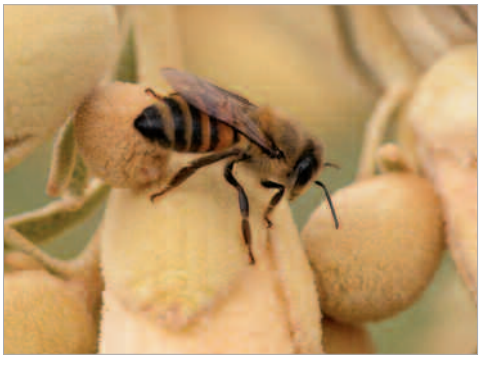
THE STUDY OF SECRETORY STRUCTURES IN PLANT SPECIES FROM BRAZILIAN CERRADO THROUGH STRUCTURAL, CHEMICAL AND ECOLOGICAL APPROACHES

Silvia Rodrigues Machado

Botucatu Biosciences Institute / Paulista State University (Unesp)

Main researcher: Luiz Claudio Di Stasi

FAPESP Grant 2008/55434-7 I Term: Nov 2009 to Oct 2013



Above, Ananas Ananassoides, *and below,* Zeyheria montana

The plant secretory structures play essential role in the production of secondary metabolites with several functions on biological interactions. Its study might give help in the analyses of plant communities' structure, providing information on defense and attraction mechanisms on plant-animal interactions.

The current project objectives to study the different aspects of the secretion system in the cerrado native plant species belonging to Apocynaceae, Bignoniaceae, Bromeliaceae, Fabaceae, Malpighiaceae and Rubiaceae families.

The analyses of plant secretory structures are being performed by light and electron microscope. *In situ* detection of main substance categories, present in the secretory tissues and cells, as well as histochemical, citochemical and imuno-histochemical tests, ultra structural citochemistry and biochemical analyses have been employed. The chemical profile of the secretion will be analyzed using thin-layer chromatography and gas chromatography-mass spectrometry (GC/MS). The function of the substances produced by external secretory glands, on plant-animal interactions, has been evaluated through field observations and experiments. We also look forward to contribute with important information on the economical potential of bioactive products and its sustainable utilization. In addition, our results can help to the strategies definition of conservation and management of biological diversity in the Cerrado at the Sao Paulo State, a fragmented landscape.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The major advances obtained with the development of the project, to date, are as follows:

i) *Tabernaemontana catharinensis* has been used as a model for ontogenesis, development, anatomy and ultra structure studies, from embryo to adult plants, with and without experimental damages;

ii) Mellithophilous and ornithophilous species were chosen to compare the distribution, morphology, ultra structure and histochemistry of floral glands and their chemical secretion profile. Analysis of microorganisms associated to floral nectar has been performed;

iii) Floral biology, flower morphology, nectar structure and secretion of *Ananas ananassoides* were evaluated. Nectar dynamics experiments were performed to verify the pattern of secretion during the whole anthesis;

iv) Osmophores and floral anatomy of nocturnal pollinated legume species, canals and cavities of oil, oil-resin of *Copaifera spp.* and tanin producer structures in *Dimorphandra mollis* and *Stryphnodendron adstringens* have been studied;

v) Floral biology, phenology and flower morphology have been evaluated. Anatomy and sub cellular structure of extra floral nectaries and sepal, petal and connective glands have been analyzed;

vi) Populations of 21 species of Rubiaceae were identified in cerrado and semi deciduous forest for phenology and periodicity to collect material for secretion studies. Herbivory, bacteria nodules, anatomy and ultra structure have been evaluated in vegetative apex and leaves of all species;

vii) Experimental analyses have been performed in order to verify the influence of temperature and light conditions on the development of secretory system of *Copaifera langsdorffii* (Leguminosae).

viii) Considering that several and specific messages send by plant VOCs could be modulated by the selection through mutualisms, as well as antagonists, this sub-project aims to evaluate the VOCs composition in relation to florivores and pollinators richness and behavior. Chemical analysis of *Bauhinia rufa VOCs* will be performed by Gas Chromatography/ Mass spectrometer (GC/MS);

ix) Experiments have been designed aiming to evaluate the influence of biotic and abiotic changes, on the functioning of floral glands and consequently on the interaction with pollinators and reproductive success of cerrado species;

x) Floral nectar secretion and nectary structure are going to be evaluated in two genera of Asteraceae, one basal and the other derived, both very common in disturbed areas of cerrado. Anatomy and ultra structure of nectary, besides field experiments on nectar secretion, and records on floral visitors, have been performed.

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Silvia Rodrigues Machado

Instituto de Biociências de Botucatu
Universidade Estadual Paulista (Unesp)

Departamento de Botânica
Distrito de Rubião Jr., s/nº – Caixa Postal 510
CEP 18618-970 – Botucatu, SP – Brasil

+55-14-3811-6053
smachado@ibb.unesp.br

BIOTA-FAPESP PROGRAM

THE CONSERVATION AND SUSTAINABLE USE OF BRAZILIAN CERRADO AND ATLANTIC FOREST FOR POTENTIAL DRUGS PROSPECTION

Vanderlan da Silva Bolzani

Araraquara Chemistry Institute / Paulista State University (Unesp)

Main researcher: Maria Claudia Marx Young

FAPESP Grant 2003/02176-7 I Term: Nov 2004 to Sep 2010

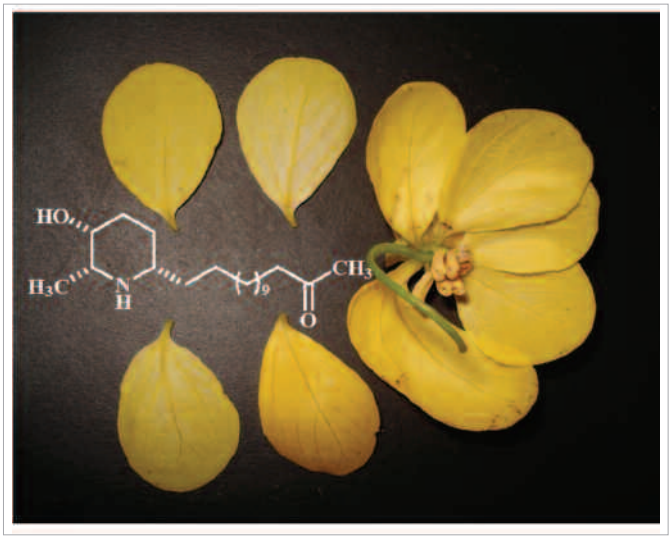


Photo: Marcos Pivatto

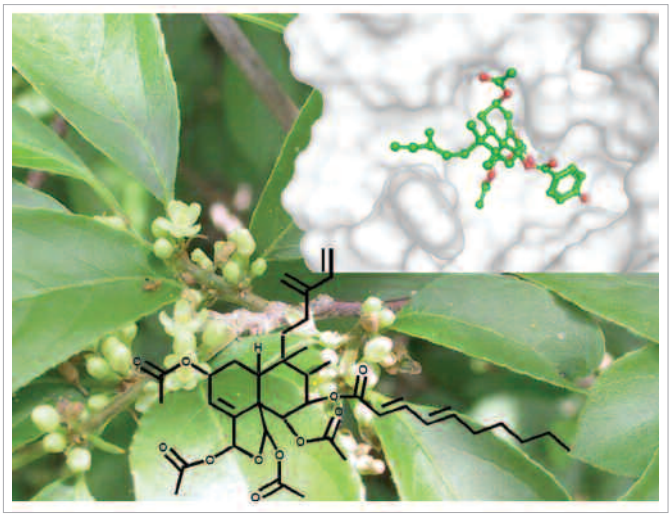
The main goal of this project is to search lead molecules from plant species and endophytic fungi occurring in the State of São Paulo, especially in the Cerrado and Atlantic Forest. The main interested compounds include antioxidant, anti-inflammatory, antifungal, anticancer, antimalarial and antiacetylcholinesterase. Besides drug discovery, biodiversity conservation, and sustainable economic growth, this project also supports the advances in 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 micro propagation and cell cultures of rare plants producing active compounds and (iii) to study biosynthetic pathways and particularly to 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 for heterologous over expression in other plants, to conduct studies on structure activity relationships (SAR) of lead compounds previously identified and to sustain and maintain the virtual database already initiated in phase I.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The first bioprospecting efforts have resulted in a storage bank of 2,000 plant extracts and more than 150 isolates of endophytic fungi, most of these from bioactive plant species belonging to approximately 88 different families. These plant species are mostly represented by Angiosperms occurring in Cerrado and Atlantic Forest (the two major biomes of São Paulo State). All the plant material samplings were performed in function of the restricted polices from CGEM.

All stored extracts (ca 774 from Cerrado plants and 1226 from Atlantic Forest) were screened with *Cladosporium cladosporioides* and *C. sphaerospermum*. From these, 800 extracts inhibited fungi growth, (nystatin 0.1mg/mL as positive control), indicating a great potential to find antifungal compounds (Graphic 1). Acetylcholinesterase (AchE) thin-layer chromatography assay (TLC) method was used to screen several extracts, and after the development of this test in an appropriate solvent, the presence of active compounds related to the reference of AchE inhibition (galanthamine as positive control) were detected in ca. 354 extracts, representing 17.7 percent of the total extracts (Graphic 2). The potential anticancer activity of extracts was tested against a panel of cell lines using *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The preliminary antifungal screening, with human pathogen fungi, was tested against the strains *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. neoformans* showed promising activity. From pure compounds assayed, 50 percent has shown promising activity, and these compounds are being tested in additional enzymatic and receptors bioassays. Although all plant extracts and endophytic fungi still have to be bioassayed in other assays, these results have indicated that bioprospection is a promising tool for exploring Brazilian biodiversity.

Photo: Alberto Cavalheiro



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Vanderlan da Silva Bolzani

Instituto de Química de Araraquara
Universidade Estadual Paulista (Unesp)

Departamento de Química Orgânica
Rua Francisco Degni, S/N – Caixa Postal 355
CEP 14800-900 – Araraquara, SP – Brasil

+55-16-3301-9660
bolzaniv@iq.unesp.br
http://www.nubbe.iq.unesp.br

BIOTA-FAPESP PROGRAM

BIODIVERSITY AND SUSTAINABLE USE OF POLLINATORS, WITH EMPHASIS ON BEES

Vera Lúcia Imperatriz Fonseca

Institute of Biosciences / University of São Paulo (USP)

Main researchers: Antônio Mauro Saraiva, Lionel S. Gonçalves, Maria Cristina Arias

FAPESP Grant 2004/15801-0 I Term: May 2006 to May 2010

The Convention of Biological Diversity and the United Nations Millennium Assessment Program considers environmental services provided by pollinators as a priority for the 21st century. Conservation and sustainable use of pollinators helps to ensure food security, sustainable agriculture and biodiversity conservation.

The main focus of this four-year project is to study the biodiversity and conservation of bees, as well as their sustainable use as pollinators. To address these questions, we have been using molecular tools, bee surveys, trap nests, baits, pollinic analysis, biodiversity informatics for species distributions, ecological modeling, automatic identification of species through wing venation, artificial diets for colonies improvement, *in vitro* queen rearing and experiments in pollination.

Queen and workers of Melipona scutellaris. *Oldroyd BP, Beekman M. Intergenerational reproductive parasitism in a stingless bee.* Molecular Ecology. ***18****: 3958-3960*

Stingless bee, Melipona fasciculata *(Apidae), collecting pollen by vibration in eggplant (*Solanum melongena *L., Solanaceae). Nunes-Silva P, Hnrcir M, Imperatriz-Fonseca VL. 2010. A polinização por vibração.* Oecologia Australis. ***14****: 140-151*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Solitary bees were sampled in remnants of Atlantic Rainforest by trap nest and baits methods and the pollinic analysis has allowing the knowledge improvement on floral resources used by females to feed their offspring. The molecular analysis has brought new insights on the conservation *status*, as showed by the genetic variability, gene flow and the patterns of dispersion, mainly for the orchid bee populations.

Concerning the social bees, our current knowledge about the biology of stingless bees has a pronounced improvement. We highlight the study of small managed populations of stingless bees, where, despite a great reduction in the genetic variability, the genetically impoverished population can be successfully bred, providing useful guidelines for stingless bee breeding and conservation efforts. Indeed, we have provided genetic evidence showing that: 1) upon loss of the mother queen, many colonies are invaded by unrelated queens that fly in from unrelated hives nearby and 2) some reproductive workers greatly outlive all other workers and lay male eggs for a long time. These very innovative studies opened a new area of investigation, and showed how powerful and fundamental are the molecular studies to address basic biological questions.

The next important results with stingless bees were the development of a methodology to rear *in vitro* queens. It allows the production of large amount of colonies in laboratory conditions, in order to provide the market with bees to be used in greenhouse pollination. To address this question, we showed that eight Melipona species are very good candidates for pollination of agricultural crops, since they are very effective in buzz pollination and are efficient pollinators for several crops. All these results summarizes that the breeding of stingless bee species, in large scale, is therefore of great importance for the use in agriculture, as well as, for the conservation of natural biodiversity in the tropics.

Biodiversity informatics tools were developed and used throughout the project. A monitoring system was developed to allow data acquisition inside the colonies to study thermoregulation and flight activity. Wireless sensor networks technology was also studied aiming at the development of a new generation of monitoring systems. We demonstrated the importance of geometric morph metrics based on wing features to identify bee genera, species, subspecies and populations, opening the possibility to track the geographical origin of the bees. We used the species distribution modeling and geographical system information tools in order to analyze the influence of different factors that act in the geographical distribution of Neotropical bees and plants presenting obligatory interactions. Also, our data has shown a general increasing in the models' accuracy and has appointed future scenarios projection, considering climate changes.

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Vera Lúcia Imperatriz Fonseca

Instituto de Biociências
Universidade de São Paulo (USP)

Departamento de Ecologia
Rua do Matão, travessa 14, 321, Butantã
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-7533
vlifonse@ib.usp.br
http://www.webbee.org.br/bioabelha

BIOTA-FAPESP PROGRAM

STANDARDIZED EXTRACTS FOR THE TREATMENT OF CHRONIC DISEASES

Wagner Vilegas

Araraquara Chemistry Institute / Paulista State University (Unesp)

FAPESP Grant 2009/52237-9 I Term: Apr 2011 to Mar 2016

The increase in human life expectancy has required, correspondingly, development of drugs to treat chronic diseases affecting the digestive tract (gastric and duodenal ulcers, ulcerative colitis and Crohn's disease), diabetes, cancer and other inflammatory diseases. The safety use of standardized herbal drugs has shown promising results for treating these diseases and this project aims to study Brazilian plant species that may be used in the treatment of human chronic diseases.

Recently, the Brazilian Health Ministry published a list of 71 plant species as potential sources for herbal medicines. Besides its intrinsic importance, this group is not sufficient enough to meet the needs of governmental health plans, including the National Policy on Herbal and Medicinal Plants. In addition, there are serious deficiencies in the correct chemical characterization of raw vegetables, as well as the evaluation of their pharmacological and toxicological activities, essential steps to ensure efficacy and safety of herbal medicines.

The current project proposes to prepare plant extracts according to pharmacopoeia standards and their analysis through pharmacological and toxicological assays. The most promising extracts have been standardized according to international procedures.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The chemical profile of the investigated plant species has shown the presence of flavonoids, catechins, tannins, phenolic acids, saponins, alkaloids, terpenes and aliphatic molecules. Many of the biological results can be correlated to the chemical structures of the plant constituents. Plants containing phenol derivatives have shown significant antioxidant activity, whereas those containing flavonoids, catechins and tannins exhibit antiulcer activity, sometimes resulting in mutagenicity. Extracts containing nonpolar molecules, such as terpenes, shows significant anti-inflammatory activity.

It is important to point out that our experiments have demonstrated that some phytopreparations displays high variation and low stability regarding their chemical composition. This result might impair the use of these extracts as phytomedicines.

In general, the assays with the selected species have confirmed the initial ethno pharmacological survey, with several promising results. However, some species not used in the folk medicine, has shown higher activity than those popularly consumed. On the other hand, some species popularly used has shown pronounced toxicity and mutagenicity, suggesting caution regarding its indiscriminate use as phytomedicines.

MAIN PUBLICATIONS

Luiz-Ferreira A, de Almeida ACA, Cola M, Barbastefano V, de Almeida ABA, Batista LM, Farias-Silva E, Pellizzon CH, Hiruma-Lima CA, Santos LC, Vilegas W, Brito ARS. 2010. Mechanisms of the gastric antiulcerogenic activity of *Anacardium humile* St. Hil on ethanol-induced acute gastric mucosal injury in rats. *Molecules.* **15**: 7153-7166.

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Wagner Vilegas

Instituto de Química de Araraquara
Universidade de São Paulo (USP)

Departamento de Química Orgânica
Rua Prof. Francisco Degni, 55
Caixa Postal 355 – Quintandinha
CEP 14801-970 – Araraquara, SP – Brasil

+55-16-3301-9668
vilegasw@gmail.com

FAPESP
SÃO PAULO RESEARCH FOUNDATION

RUA PIO XI, 1500, ALTO DA LAPA
CEP: 05468-901 - SÃO PAULO - SP - BRASIL
FONE: 55.11.3838-4000