

BIOTA-FAPESE PROGRAM

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 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 played 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

More than 16 thousand PhD scientists are formed yearly in Brazil. The country ranks 15th in the number of scientific papers published in indexed journals. The State of São Paulo, with 45 million inhabitants and 32% of Brazil's GDP, responds for 44% of the science developed in the country. The State hosts the University of São Paulo (USP), the State University of Campinas (Unicamp), State of São Paulo University (Unesp), São Paulo Federal University (Unifesp), ABC Federal University and São Carlos Federal University.

In addition to the three state universities and three federal universities, São Paulo has 34 research institutes, among them, the Aeronautics Technology Institute (ITA) and the National Space Research Institute (INPE), and host most of Brazilian industrial R&D. 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 55 years, 258,808 fellowships and research grants.

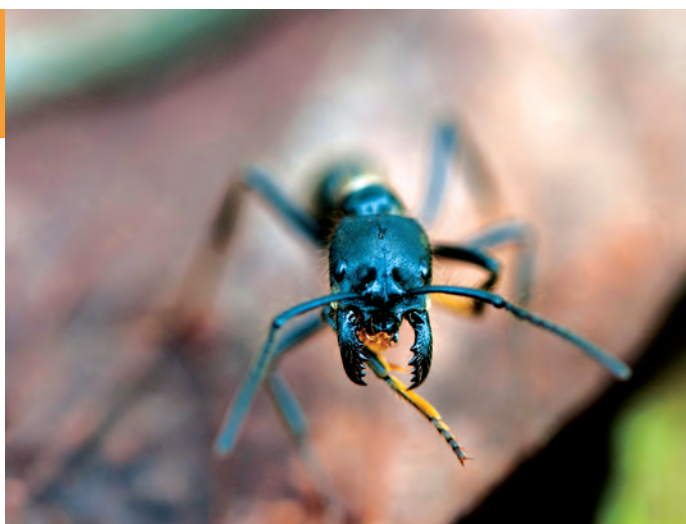
In 2016, FAPESP invested US\$ 307 million in research projects. The approval rate for proposals applications was 53%.



OPPORTUNITIES AND CHALLENGES

One of FAPESP's goals is to broaden and diversify the research system in the State of São Paulo, to strengthen 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, Climate Change 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.

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 BRAZILIAN BIODIVERSITY

Since 1999, the Virtual Institute of Biodiversity (<http://www.biota.org.br>) has been studying Brazilian biodiversity. The BIOTA's mission is to catalogue and characterize, both biologically and chemically, Brazilian biodiversity, 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. Ongoing projects of the program involve approximately 900 researchers (PhD scientists and graduate students). Furthermore, there are 150 researchers from other Brazilian states and approximately 80 from other countries collaborating with these projects.

In 18 years, the BIOTA-FAPESP program has supported 299 research projects, trained 358 science Masters and 288 PhD students, produced and stored information on approximately 25,000 species.

Scientists participating in the program have catalogued more than 3,000 new species. This effort is summarized in 2.2 mil articles published in scientific indexed journals. Researchers involved in the Biota program have published 27 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 the Neotropical region. Currently, the journal is internationally recognized as a seminal reference on biodiversity data and it is indexed by Thomson Reuters Web of Knowledge, Directory of Open Access Journals and the Scientific Electronic Library Online (SciELO) & Global Biodiversity Heritage Library/BHL.

In 2002, the program launched a new sub-project called BIOprospecTA (<http://www.bioprosecta.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 over 50 patents.

PHYLOGENETIC RECONSTRUCTION OF GASTROTRICHA BASE ON MOLECULAR AND MORPHOLOGICAL DATA

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FAPESP Process 2014/23856-0 | Term: Nov 2015 to Sep 2019



Figure 1. *Acanthodasys* sp.: Marine gastrotrichs collected from Fome beach, IlhaBela-SP. DIC photo of the specimen habitus.

Gastrotrichs are aquatic microinvertebrates (less than 1 mm) and an important component of the benthos and phytofauna of marine and freshwater habitats. Despite the large number of specimens in different habitats, this taxon is poorly studied possibly due to the small size and fragility of their bodies. Moreover, although the taxonomic studies of the group initiated in the end of nineteenth century, currently the phylogenetic relationships within Gastrotricha are still far from being considered satisfactory. Thus, this project has different aims: a) comparative morphological analysis of external structures using techniques of scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser microscopy; b) construct a well-supported phylogenetic hypothesis of Gastrotricha based on molecular (nuclear and mitochondrial DNA) and morphological data in order to better understand the evolutionary history of the taxon; c) formally describe the gastrotrichs collected by Garraffoni et al. (2010) and Araújo et al. (2013) and only identified until genus level; d) designate and document neotypes of the 12 new species described by the Polish researcher Dr. Jacek Kisielewski, from the state of São Paulo, in the early 1990s, using microscopy with differential interference contrast optics (DIC) and SEM, e) consolidate the meiofauna collection of the Zoological Museum of the Institute of Biology, State University of Campinas (ZUEC / Unicamp). With these goals, this project will help to establish a new research group from Latin American focused on understanding the systematics, evolution and ecology of gastrotrichs and other understudied meiofaunal taxa.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Due to the fragility of the gastrotrich bodies, specimens deteriorate and most of their diagnostic characteristics vanish soon after preservation. Thus, in this first year of the present project, the main effort was dedicated to sampling distinct freshwater and marine habitats and constructing an image database of the specimens sorted in the sediments. Although most of those samplings were done in only a few sites in the states of São Paulo and Minas Gerais, we were able to find a large number of undescribed species. At least 10 morphotypes do not match with any other species described in gastrotrichs (Figs. 1 and 2). To date, two new species and one new genus were described and one of the few species previously described from Brazil had its geographic distribution expanded.



Figure 2. *Polymerurus* sp.: Freshwater gastrotrichs collected from an urban lagoon, Paulina, SP. DIC photo of the specimen habitus.

Until 35 years ago Brazilian freshwater and marine habitats were recognized as *terra incognita* for gastrotrich fauna, then two pioneering taxonomical studies were carried out by foreign researchers from samplings in the inland waters from the states of São Paulo, Mato Grosso do Sul and Pará, and along the northern coasts of the state of São Paulo. This limited sampling effort has direct impact in the knowledge of Brazilian gastrotrichs because large extensions of Brazilian inland waters and coast were never sampled before causing absence of reference collections about the taxon in Brazilian Museums. Thus,

we will continue sampling new sites in order to increase the knowledge of the biodiversity of Gastrotricha, including new investigation of the genetic diversity of the taxon.

MAIN PUBLICATIONS

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Garraffoni ARS, Di Domenico M, Hochberg R. 2016. New records of marine Gastrotricha from São Sebastião Island (Brazil) and the description of a new species. *Mar. Biodivers.* doi: <http://dx.doi.org/10.1007/s12526-016-0486-1>.

Garraffoni ARS, Di Domenico M, Amaral ACZ. 2016. Patterns of diversity in marine Gastrotricha from Southeastern Brazilian Coast is predicted by sediment textures. *Hydrobiologia*. **773**: 105-116.

Garraffoni ARS, Araújo TQ, Lourenço AP, Guidi L, Balsamo M. 2017 A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. *Syst. Biodivers.* **15(1)**: 49-62.

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BIODIVERSITY AND FUNCTIONING OF A SUBTROPICAL COASTAL ECOSYSTEM: A CONTRIBUTION TO AN INTEGRATED MANAGEMENT

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FAPESP Process 2011/50317-5 | Term: Mar 2012 to Sep 2017



The study area was Araçá Bay, located in the São Sebastião Channel, Northern Coast of the State of São Paulo (23° 49'S, 45° 24'W). The area is part of the Marine Protected Area of the Northern Coast of São Paulo State and the Alcatrazes Municipal Protected Area

The Biota-Araçá project aimed to understand the environmental, social and economic importance of a subtropical coastal ecosystem (www.biota-araca.org/; www.facebook.com/baiadoaraca). To achieve this goal, we characterized the ecosystem services provided by this environment in order to provide information that could be used in the development of proposals for the sustainability of the region. Araçá Bay, the study area of this project, is located on the North Coast of São Paulo (Brazil). The geographic and hydrographic characteristics (physical, chemical and geological parameters) of the bay, in addition to its high biodiversity, make this area a complex environment which can be used as a study model and information derived from here can be transposed to other regions.

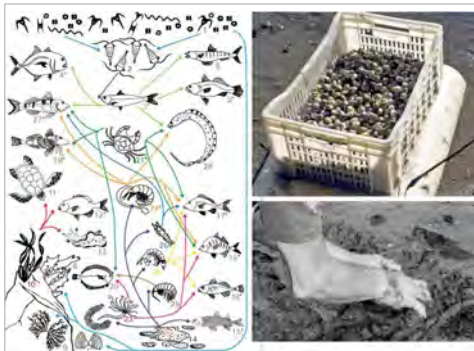
During the development of the project, data collection and analysis occurred simultaneously in the different habitats of the bay, which allowed us to investigate this region using an integrated approach. For this, the project was structured in research modules: 1- Planktonic System; 2- Nektonic System; 3- Benthic System; 4- Mangrove System; 5- Hydrodynamics; 6- Sediment Dynamics; 7- Trophic Interactions; 8- Fisheries Assessment; 9- Identification and Valuation of Ecosystem Services; 10- Integrated Management; 11- Ecological Modeling; and 12- Management and Data Sharing.

The Biota-Araçá project benefited from the integration of different areas of knowledge and a large number of researchers with different backgrounds and from different research institutions. This allowed us to better understand the current state of the area and its ecological, social, economic and political importance, thereby allowing a dialogue between science and decision makers. The project also provided training for a great number of young researchers, and helped improve the production of knowledge and the scientific competence of the State of São Paulo for studies on biodiversity, conservation and marine management.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Araçá Bay is exposed to various impacts such as irregular constructions, sewage disposal and activities of the Port of São Sebastião and the Almirante Barroso Oil Terminal (TEBAR). The bay is therefore a reflection of the conflicts that affect several coastal regions in Brazil and worldwide.

The results obtained in the different research modules mapped the different habitats in Araçá Bay, and showed the composition and structure of their assemblages. Additionally, we managed to better understand the local trophic food web. Several works have been and are being produced about the biodiversity and functioning of the bay (more than 70 articles in indexed scientific journals have already been published). The results reveal Araçá Bay as an ecosystem of



*Trophic web of Araçá Bay. The arrows indicate the direction of the food flow. Species marked with an asterisk are used for human consumption (left). The clam *Anomalocardia brasiliiana* is abundant and largely consumed by the local population, accounting for 38% of the weight of capture fisheries Araçá Bay*

high species richness and diversity, with more than 1400 taxa identified. Among these, more than 300 were first recorded at the bay, and 50 are new species to science in addition to the description of new genera and a new family.

The elaboration of trophic web models showed that the main energy sources of the Araçá Bay food web are planktonic and sediment organic matter, and microphytobenthos. We attempt to predict impacts of the expansion of the Port of São Sebastião (an ongoing issue) and found that the shading expected by the expansion of the port would deeply reduce the total primary production (algae and mangrove) in the bay. This would result in a decrease of more than 70% of the total biomass in less than 20 years, impairing the sustainability of the food web and completely modifying the ecosystem.

The characterization of the ecosystem services offered by the environment allowed us to evaluate the socioeconomic importance of the bay in a local context. Finally, a better understanding of the social processes that permeate the region enabled us to elaborate management and conservation proposals that resulted in a "Local Plan for Sustainable Development of Araçá Bay", a work done together with the local community.

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DIMENSIONS OF MARINE LIFE – PATTERNS AND PROCESS OF DIVERSIFICATIONS IN PLANKTONIC AND BENTHIC CNIDARIANS

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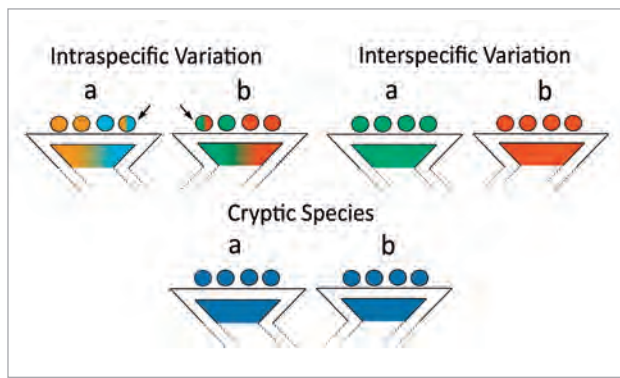


Figure 1. Levels of morphological variation, including absence of variation (cryptic species). Clades indicate different lineages and colors and shades represent the phenotype in current time, and the circles represent individuals. (From Cunha et al 2016, ODE, 16 D.O.I. 10.1007/s13127-016-0276-4).

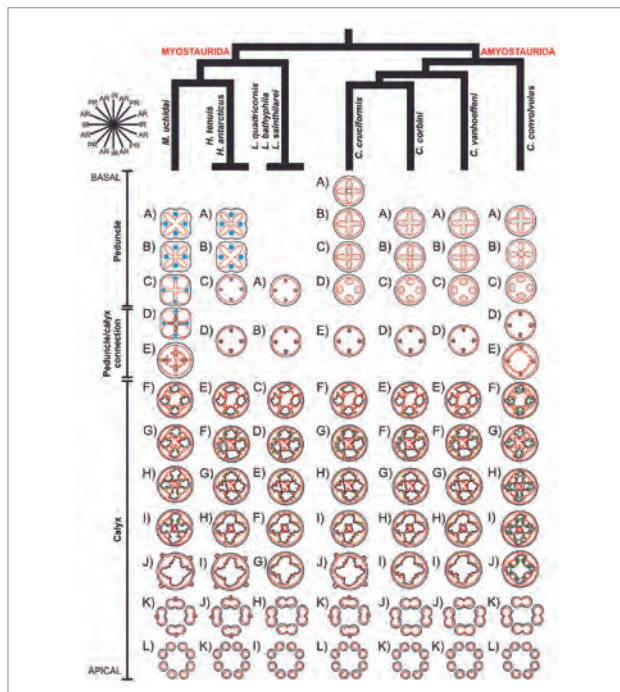


Figure 2. General body plan of different species and their phylogenetic relationship (From Miranda et al 2016, PeerJ, 4:e2594, D.O.I.: 10.7717/peerj.2594).

In order to understand marine diversification, and ultimately promote its conservation, it is clear that an integrative approach of adequate spatial, temporal, and taxonomic coverage must be developed. Marine biodiversity has been modulated by historical and recent events resulting from micro and macroevolutionary variables that act synergistically and are equally important. For instance, the assessment of historical areas of endemism and past biogeographical barriers (inferred from paleoceanographic records) provides information on the historical affinities of the biota. On the other hand, results from population genetics and phylogeographic studies, when correlated with relevant abiotic and biotic variables, provide data on the dynamics and patterns of recent communities. Consequently, both approaches may explain unique patterns of isolated communities and populations. Data on species richness is crucial since it provides estimates of phylogenetic communities that can be based on a multitude of data, obtained from sources that range from phylogenomics to population ecology to niche modeling. In the same way, assessment of introduced species and their impact on native communities, life history data, biotic interactions or experimental ecology, ecophysiological studies, and additional features, have also been investigated.

This research program correlates micro and macroevolution, integrating developmental biology, life cycles, speciation, phylogeography / population genetics, ecological niche modeling, phylogeny / taxonomy and biogeography / faunal studies. The goal is to traverse the spatial and temporal dimensions of marine life, investigating patterns and processes at nested biological levels through the study of planktonic and benthic marine invertebrates, focusing on cnidarians, a group with wide distribution and highly variable life cycles and developmental trajectories.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This research program is a continuation and extension of a previous thematic project, named "Biodiversity, evolution, endemism and conservation of Medusozoa from the Southwestern Atlantic Ocean". Among the project achievements, one of the most important is the significant increase in the faunal knowledge of the Atlantic and Pacific coasts of South America, culminating with an extensive monograph of the medusozoans of the subcontinent, including ~1,000 species. The study also proposed 35 new taxa, from new species to new subclasses, all based on solid phylogenetic contexts and several resulted from extensive taxonomic revisions that contributed to describe and redescribe the anatomy, morphology, morphometry and cnidome of innumerable extant and fossil taxa. We added data of natural history and ecology for over thirty taxa, many of them completely unknown hitherto, including native and introduced species. Biogeographic and phylogeographic inferences were used in theoretical and actual analysis for different methods and covering extensive areas of the southernmost regions of South America, the whole Antarctica, and Atlantic Ocean (especially deep sea data). These patterns of endemism were correlated with putative paleoceanographic barriers, which eventually allowed us to hypothesize on the ages of endemic biotas. Besides revealing several cryptic taxa, the phylogeographic approach also provided data to understand the recent population dynamics of the medusozoans and it has shown that there was remarkable convergence in the genetic structures of populations across species with different life cycles. Evolutionary results were based on several different phylogenetic inferences, including the most extensive data sets produced for medusozoan hitherto, like for the Leptothecata, Proboscoida and Staurozoa, among others. These patterns were used to infer and discuss evolutionary processes related to the life cycle and ecology of extant and fossil taxa. Some peculiar systems were also analyzed like venomomics (including proteomics, transcriptomics and genomics), biomechanics and functional anatomy, all under an evolutionary perspective.

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COMMUNITY STRUCTURE AND DYNAMICS IN COASTAL STREAMS OF THE ATLANTIC FOREST: THE ITANHAÉM RIVER BASIN

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FAPESP Process 2012/51511-2 | Term: Dec 2013 to May 2017



Figure 1. Stand of the aquatic macrophyte *Nymphaea rudgeana* in Preto River, Itanhaém – SP – Brazil). Photo by Antonio Camargo.



Figure 2. Itanhaém River, Itanhaém, SP, Brazil. Photo by Antonio Camargo.

The factors governing the spatial structure and temporal dynamics of biodiversity involve a complex mix of ecological, biogeographic and evolutionary processes. The relative influence of these processes is at the heart of the current debate on biodiversity research. Since nature is dynamic, studies that consider sampling over time, including several generations of populations, are highly desirable and necessary for a better understanding of biodiversity patterns. Rivers and streams are discrete areas embedded in a terrestrial landscape - the watershed. Environmental changes in watersheds are very dynamic and occur at different time scales, and have both natural and anthropogenic causes. Among the natural causes, changes can occur due to seasonality with aquatic environments presenting different characteristics between summers and winters, for example. However, changes may occur at longer time scales due to rainy and dry annual cycles due to El Niño and La Niña. As for the anthropic changes, these occur in the long term, due, for example, to agricultural and urban expansion, deforestation, but also to sewage collection and treatment and reforestation. We have observed that these situations have been occurring in the last 20 years in Itanhaém, south coast of São Paulo. The selection of this river basin as a unit to develop ecological studies was motivated by the diversity of physiographic characteristics, diversity of use and occupation of the soil (conservation, agricultural and urban areas) and diversity of water types (white, clear, black and brackish). In addition, a geo-referenced database has allowed us to monitor and map changes in basin land use, especially in relation to the emergence of new anthropogenic impacts. Although this project has as its central theme the long-term ecological study of the Itanhaém river basin, it is composed of subprojects with independent objectives and themes. In general, this project attempted to answer questions such as: 1) How is the variation between communities in space and time maintained? 2) What generates this variation? 3) Are the species with wide distribution in the basin also species with great environmental tolerance? The answers to these questions are fundamental for the understanding of the functioning of these communities as well as the design of biomonitoring programs.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Until the beginning of 2017, our main findings are: 1) Stream macroinvertebrate beta diversity patterns can be studied in watersheds like this by using five subsamples per stream. This allows one to reduce subsampling and invest in making the spatial extent of the study larger by including more streams; 2) Phylogenies and traits provide distinct insights about the historical and contemporary assembly of aquatic insect communities. We suggest that both trait and phylogenetic approaches to community ecology should be kept in the ecologist toolbox, but phylogenetic distances should not be used as proxies of traits differences. Although the phylogenetic structure reveals processes operating at the evolutionary scale, only specific traits explained local processes operating in our communities; 3) Dispersal limitation, rather than species sorting, was the main driver for phylogenetic beta diversity in the stream macroinvertebrates. We suggest that life-history strategies and mainly voltinism drive the distance decay of similarity in the insect communities examined; 4) To estimate fish population size along a 1000 m extension in streams with high accuracy and precision we would need to sample at least half of this extension and ensure at least 50% of detection probability; 5) Rithron fishes are mainly structured by habitat selection while potamon fishes are structured by dispersal in downstream areas; 6) Systematic sampling provides good cost effective design to monitor fish diversities in these streams as it provides more precise estimates with smaller sample sizes. However, combining stratified sampling across sub-basin with systematic sampling along the environmental variation axis allows monitoring all range of variation in environmental characteristics and cover the entire basin area. Twelve sites are a reasonable sample size as it allows defining three sites per sub-basin and can be accomplish during a one-week of fieldwork; 7) The greater the distance from the river mouth, the lower is algal biomass. There is, thus, a longitudinal biomass gradient in the head-to-mouth direction, according to the River Continuum concept; 8) Abiotic stress (low nutrient availability) is responsible for the absence of *S. alterniflora* in the upper estuary and that the competition between the two species is responsible for the absence of *C. americanum* in the lower estuary; 9) Invasive species reduced the presence of rooted-submerged species, whereas native species facilitated the occurrence of rooted-submerged and free-submerged species. Thus, African signal-grass was able to change the composition of the macrophyte assemblage and can represent a threat to native communities of tropical freshwater ecosystems.

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BIODIVERSITY AND ECOSYSTEM FUNCTIONING IN DEGRADED AND RECOVERING AMAZONIAN AND ATLANTIC FORESTS

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FAPESP Process 2012/51872-5 | Term: Aug 2013 to Dec 2017

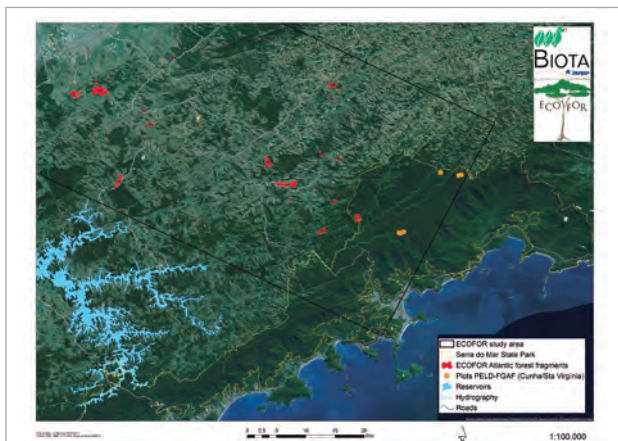


Figure 1. ECOFOR study area in the Atlantic Forest. The orange dots show the position of the 1 ha Permanent Plots within the Serra do Mar State Park, the red areas show size, shape and position of the fragments studied.

The ECOFOR Project (<http://ecofor.hmtf.info/>) is jointly supported by the British NERC/Natural Environment Research Council and FAPESP. Within NERC the project is part of the Human-Modified Tropical Forests/HMTF (<http://www.nerc.ac.uk/research/funded/programmes/forests/>) and within FAPESP it is part of the BIOTA/FAPESP Program (www.biota.org.br).

There is growing consensus that the combined impact of degradation processes such as logging and fire on forest biodiversity and carbon stocks is of comparable magnitude to deforestation. In areas where no intact forests remain, these heavily modified ecosystems are the last refuges for many endemic species. Yet we still have a limited understanding of how these disturbed forests are functioning, their ability to provide critical ecosystem services, and the prospects for long-term biodiversity persistence.

In both the Atlantic and the Amazon Forests, we are assessing changes in biodiversity and ecosystem functioning across gradients of human-modification, comparing intact primary forests with those that have been logged, burned or are regenerating after clear-felling. As far as possible we have balanced our design in the Atlantic Forest and the Amazon, providing a strong basis for comparative work with respect to both ecological findings and policy development. In the Amazon we have been closely monitoring disturbances in forest in and around the FLONA do Tapajós, near Santarém, while in the Atlantic Forest we are studying areas in and around the Serra do Mar State Park, mainly Nucleus Santa Virgínia. We are assessing biodiversity as well as undertaking detailed measurements of forest recovery and carbon cycling. These assessments are accompanied by a thorough evaluation of soil conditions and the main leaf traits of the dominant plant species in the plots. This is the largest carbon monitoring of its kind in disturbed tropical forests, and it is unique in linking plant physiology with ecosystem functioning. Through our permanent plot network, we will be able to understand the main ecophysiological characteristics of the species that dominate forest recovery, providing important insights into the resilience of disturbed Amazonian and Atlantic forests.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Results so far have shown that human-induced disturbance have greater impact than previously thought on both carbon stocks and biodiversity: for example, every year the Brazilian Amazon loses to both logging and fire an amount of carbon that corresponds to 40% of that lost annually to deforestation, and this figure remains unaccounted for in the national greenhouse gases inventory. In terms of biodiversity, we found that selective logging, fire and edge effects are reducing the conservation value of vast swathes of primary forest. In the Amazon for example, we show that a typical landscape with 80% of forest cover – therefore meeting the maximum required under the Brazilian Forest Code – will only hold around 50% of its species. The additional loss of 30% of species is due to disturbance. These results, and the many more to come, are shedding new light on the issue of forest degradation.

In the Atlantic Forest region forest fragmentation is much older, dates from the early 19 century when coffee was the main driver of forest slash. Later these degraded areas were transformed in pasture, and from 1995 onwards pasture is being replaced by *Eucalyptus spp* for paper mill. Nevertheless in the 16 fragments studied we registered approximately 350 tree species from over 60 families. In the 1ha Permanent Plots within the Serra do Mar State Park we are monitoring 12,000 trees for carbon changes, and for 6 plots we have 3 years data on CWD and fine roots (data available in the ForestPlots database <http://www.forestplots.net>). Leaf traits – such as Specific leaf mass, Leaf Carbon & Nitrogen, Leaf Lignin, Phenols and Tannins - have been studied for 600 individuals from 26 species. Bird traits indicate that from the perspective of many species the Brazilian Atlantic Forest does not constitute a large connected meta-population with free movement of individuals. For the hydroclimatology of the region we have data on hydrological patterns, ecosystem services of climate regulation, spatial and temporal variability of temperature and humidity, restoration of water spring surrounding vegetation as control of water production.

We hope that, both in the Amazon and Atlantic forests, these new findings can influence forest management and conservation policies, incentivizing measures that protect forest condition as well as forest extent.

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DIVERSITY AND CONSERVATION OF BRAZILIAN AMPHIBIANS

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FAPESP Process 2013/50741-7 | Term: Apr 2014 to Mar 2019



*Figure 1. Female of *Fritziana tonimi* transporting eggs. This marsupial tree frog was described recently from the Atlantic forest of Espírito Santo state, as a result of the project. Photo by João L. Gasparini.*

The degradation of natural ecosystems by human action has generated an unprecedented crisis in the biota of the planet. Besides the environmental deterioration caused by man, through fragmentation, degradation, or complete destruction of ecosystems, there are other serious problems, such as the introduction of exotic species, diseases, pollution / contamination, climate change, and the synergistic interaction of these factors. Among the organisms most affected are the amphibians, which are facing serious population declines, local extinctions or even species extinctions. Alongside this framework of loss of diversity, the number of new species described is increasing in tropical regions as a result of investments in faunal surveys. The Brazilian official list of threatened amphibians has just been published and the picture in it is alarming, with an increase in the number of threatened species of the order of 150%. About 20 new species of amphibians have been described each year in Brazil, but it is difficult to determine today what will be the approximate final number, given the uncertainties and the vast areas of the country not yet prospected. Therefore, there is both a need for a better understanding of our diversity of amphibians, not completely described yet, as well as a need to better understand the roles played by the different factors that threaten Brazilian species. This project proposes approaches to evaluate these two major issues.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

For the evaluation of the Brazilian diversity of amphibians, our first goal in this project, we described 17 new species of amphibians after two years of studies (Figure 1), and produced articles on anurofaunistic surveys, morphology, cytogenetic, phylogeny, and phylogeography. Besides the evaluation of diversity, these articles focus on understanding the processes of evolution and diversification in this vertebrate group. We published several articles on the natural history, behavior, bioacoustics, and ecology of amphibians that, in addition to their academic importance, contain relevant information for the conservation of the species. To detect new species we are using DNA barcodes. This approach is allowing the discovery of numerous species morphologically cryptic and a more accurate understanding of the real diversity of amphibians in Brazil. For the conservation, the second major issue in this project, we produced, until now, five articles on chytridiomycosis, a disease that has become a major concern for the conservation of amphibians, because it is causing population declines or even extinctions of amphibian species worldwide. Still associated to the conservation, we produced an article on microbiota associated to amphibians and two articles using the environmental DNA as a way to find rare and threatened species in the environment. As an important result, one species that was disappeared for about 35 years was rediscovered (Figure 2). In total, we produced, until now, around 60 scientific publications, like those exemplified below.



Figure 2. *Scinax pinima*, a microendemic species of tree frog from Serra do Cipó, Minas Gerais state, Brazil, was disappeared for about 35 years. Recently it was rediscovered as a result of the project.
Photo by Célio F. B. Haddad.

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SINTESIS – NATURAL RESOURCES MANAGEMENT IN SOCIO-ECOLOGICAL SYSTEMS: INTEGRATING ENVIRONMENTAL CONSERVATION AND LOCAL DEVELOPMENT

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Luciana Gomes de Araújo

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On the left side, the cover of the booklet by Araujo et al. (2017); on the right side, fishers from Paraty (RJ) receiving the booklet. Within Project SinteSIS, we have been working simultaneously on advancing scientific knowledge, and on communicating with non-scientific public, in order to broaden the impact of our results. The booklet synthesizes some of our research and outreach results, written in a comprehensible and involving lay language.

The overall goal of the SinteSIS project is to produce analytical syntheses of the diverse research on social-ecological systems and outreach effort carried out by the Commons Conservation and Management Research Group (CGCommons) over the past decade. We understand that (i) ecosystem sustainability depends, among other things, on ecosystems' adaptive capacity to social and environmental drivers of change, and (ii) system's adaptive capacity results from dynamics involving social actors, institutions, knowledge and resources available to management. In this context, the project seeks to contribute to the management and conservation of ecosystems considering the involvement of people who depend on them, integrating three theoretical approaches: resilience and adaptive capacity of complex socio-ecological systems; participatory management of common-pool resources (the commons); and ecosystem stewardship (integrating conservation and

development). Based on case studies with different levels of analysis and territorial coverage, our main objective is to identify potentialities and obstacles of different institutional arrangements for integrating conservation and development at the local level. The specific objectives are: (i) to analyze the evolution of ecosystem management and conservation arrangements involving local communities; (ii) to investigate how the different social groups (e.g., resource users, government, NGOs, researchers, companies) have worked on ecosystem management and conservation; (iii) to identify how traditional/local ecological knowledge and practices influence the management and resilience of socio-ecological systems; (iv) to investigate monitoring approaches of social and ecological variables in management and conservation of common-pool resources involving communities; (v) to identify feedback mechanisms present in these socio-ecological systems, influencing adaptability and local socio-ecological resilience; and, (vi) to identify contributing factors for community self-organizing processes related to environmental management. Data collection involves multiple research methods based on case studies. The study sites concentrate mostly in the Brazilian Atlantic Forest domain, but there are also cases from other biomes and countries. In exploring a diversity of cases and seeking challenges, opportunities and commonalities among them, we provide insights to advance scientific knowledge and the praxis of environmental management.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Research has been carried out with *Caiçara* and *Caipiras* from the Atlantic Forest, *Cablocos* and *indigenous* groups from the Amazon, *small-scale fishers* along the coast of Brazil, and the *Machangana* people from the coast of Mozambique. The institutional arrangements investigated range from private enterprises, to community and NGO initiatives, to protected areas, including sacred sites. In order to elaborate syntheses of the work developed by our team, we organized four workshops for planning the writing of scientific papers and a textbook. These workshops have shown to be essential for the elaboration of our intended outputs. Building upon different methods for stimulating collaborative work, our workshops have been demonstrating innovative possibilities and perspectives for building knowledge and high-quality science. Some papers are already in advanced stages of development, synthesizing the results of diverse case studies aggregated by theme, for instance (i) stakeholders' participation on the management of common-pool resources; (ii) the role of traditional/local ecological knowledge in coping with change; and (iii) self-organization and feedbacks in collective action related to environmental issues. In addition to scientific articles, the SinteSIS project is preparing a textbook to fill a reference gap for training on conservation and management of common-pool resources in Portuguese. The book, with contributions of more than 20 authors, targets primarily undergraduate students from environmental sciences-related courses, though it may also be useful to managers, practitioners and professionals working on environmental management and conservation. Efforts for communicating academic work to a non-scientific public have been increasingly demanded and valued by society. In this sense, another innovative product by the SinteSIS project has been published: a booklet synthesizing some of our research and outreach initiatives, written in a comprehensible and involving lay language. The booklet *Caiçaras e caipiras: uma prosa sobre natureza, de desenvolvimento e cultura* (*Caiçaras and Caipiras: a prose on nature, development and culture*) (Araujo et al 2017) is intended to communicate to local communities and other social actors from our research/outreach sites the knowledge about environmental conservation and local development that we produced. The SinteSIS project is also contributing to feed comparative analyzes in two international research networks: the Community Conservation Research Network (CCRN) (<http://www.communityconservation.net/>) and Too Big to Ignore (TBTI) (<http://toobigtoignore.net/>) on small-scale fisheries. In Brazil, our team is part of Rede TransForMar (Transdisciplinary Network on Adaptive Co-Management for Ecodevelopment). All these networks are seeking to investigate the contribution of human communities to the management and conservation of ecosystems.

MAIN PUBLICATIONS

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DIMENSIONS US-BIOTA SÃO PAULO: A MULTIDISCIPLINARY FRAMEWORK FOR BIODIVERSITY PREDICTION IN THE BRAZILIAN ATLANTIC FOREST HOTSPOT

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View of Serra do Caraça, MG.

Picture by Claydson Pinto de Assis Bezerra.

We are applying a hypothesis-testing framework to predict spatial patterns of biodiversity in the megadiverse and threatened Atlantic Forest (AF) of Brazil. To describe the current spatial patterns of diversity, we are synthesizing the distribution of producers, consumers, parasites, and symbionts. We are expanding on phylogenetic and phylogeographic analyses and summarizing patterns of endemism and turnover. To advance diversity prediction, we are integrating data on the ecological mechanisms acting on the flora and fauna with climatic models of the last glacial-interglacial cycles. We are using genetic and genomic data to test the fit of the aggregate population histories to the inferred time-calibrated landscape shifts and demographic processes. We are describing the dissimilarity of communities as a function of geographical and environmental turnover in space and time. Based on population histories of co-

distributed taxa, we are inferring community-level macro-ecological processes of community assembly, detecting forces behind regional biodiversity patterns, and understanding how historical changes in environmental features affected species distributions. The remote sensing analyses are providing data relevant to studies of carbon cycling, productivity, and biomass. We are generating a long-term paleorecord to describe the spatial heterogeneity of forest response to climate change over time. This is permitting us to construct a framework for biodiversity prediction and promote cross-fertilization among the fields. Finally, this project has significant impacts in conservation, synergy with existing research, and training. The reconstruction of population histories is revealing regions of high stability and genetic diversity and areas of historical connectivity between the AF and other South American forests. We are determining areas and groups under greater extinction threats and predicting AF responses to future climatic shifts. Numerous postdocs, doctoral, masters and undergraduate students are being trained in a collaborative environment to advance the documentation and conservation of biodiversity.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We compiled phylogenetic, phylogeographic, taxonomic, and locality data for hundreds of vertebrate, butterfly and plant species in a single, comparative analysis of the patterns of geographic distribution of taxonomic and phylogenetic diversities, endemism and turnover along the Atlantic Forest (AF). The preliminary analyses show that taxonomic and phylogenetic diversity have similar distributions and the highest diversity occurs in an area that includes the Serra do Mar.

Two of our multi taxa studies of AF organisms (summarized below) indicate that geological landmarks are not the only drivers of biotic diversification, but climate changes have also been important.

Two climatic domains (northern [above 20oS] lowland and mid-elevation forests vs. southern and southeastern Brazilian cooler and higher elevation forests) with high turnover around the Rio Doce were revealed by an analysis of the genetic diversity of 25 vertebrates (Carnaval et al. 2014). Endemism patterns observed after independent modeling of these domains indicate different climatic drivers. This result is congruent with studies of cave deposits and fossil pollen, suggesting that the northern and the southern AF were differently affected by climatic variability during the last 250 kyr.



Tangara cyanocephala (red-necked tanager), Guarujá, SP.
Picture by Andressa Nuss.

Population genetic structures and models of present and past distributions of 15 birds showed that geographic ranges changed in concert with Pleistocene glacial cycles, but ranges during glacial maxima were slightly larger, equally fragmented and displaced from the interglacial ranges (Cabanne et al. 2016). Population genetic structure was increased by the combined effect of temporal fragmentation variation, small current range size, and range stability. However, genetic diversity was not affected by fragmentation and range stability. In sum, there is a high variance in evolutionary responses of birds to Pleistocene range shifts.

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PARTICIPATIVE ETHNOBOTANY: CONSERVATION AND LOCAL DEVELOPMENT IN SERRA DO MAR STATE PARK - PICINGUABA, UBATUBA, SP, BRAZIL

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FAPESP Process 2015/12046-0 | Term: Jun 2016 to May 2018



Figure 1. Local partner, Ginacil, interviewing one of the specialist and, in the back, one of the researchers taking notes of the interview (Photo: Thamara Sauini, 2016).

Introduction: Studies on ethnobotany that use a participatory approach propose active involvement of local people in its various stages, in order to promote, among other things, local culture strengthening and their empowerment on decision-making about the use of resources available at their environment, aiming local development.

Objective: To develop ethnobotanical surveys between two Quilombos in Serra do Mar State Park - Picinguaba, Ubatuba, Brazil - with participation of its residents called "local partners"; and to produce potential conservation diagnosis of raised plant species.

Methodology: This project will progress in two phases. In phase 1, courses will be offered to "local partners" about plants collection and ethnobotanical data. The partners, together with technical team, and using ethnobotany methods and techniques, will select and

interview experts about various categories of plant use: construction, medicine, food, technology, fuel, among others. Data of each plant used will be noted on specially chips designed for this project, respective plant will be collected and deposited in herbaria: Municipal-SP Herbarium (PMSP) and the Forestry Institute (SPSF). In phase 2, potential conservation diagnosis of plants (the ones collected in Phase 1) will be performed by calculating the Conservation Priority Index (CPI), associated with ecological and phenological data, as well as bibliographic data of each plant conservation status.

Expected Results: The traditional knowledge registered in both quilombos will be systematized, analyzed and compared. Still, data about potential conservation diagnosis of species used by these communities in the Atlantic forest will be obtained; both will generate scientific publications. Moreover, such data will be used for the production of booklet and / or audiovisual documentary, as well as on themed trails construction, contributing to tourism activity. The development of this study will contribute to advancement of ethnobotany research, promoting participation of local inhabitants in the registration of their own knowledge; above all, it will bring progress to ethnobotanic methods that aim conservation and local development, since these have been one of the focus of current studies in this knowledge area. In addition, as a continuation of this project, in medium term, we intend to build a management plan of at least one of those plants, with the local residents participation.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Local collaborators were trained on anthropology methods for interviews and botany, aiming at collecting the plants indicated during the interviews. Until November 2016, during 45 days of fieldwork, 13 specialists have been interviewed by the five "local partners" (Figure 1). Although we have tried to separate the interviews by specialties, we have noted that all of them have indicated plants for different uses.

The 6 interviewed living in Quilombo da Fazenda (QF) have indicated 121 plants for 171 uses (Figure 2A); while 147 plants have been indicated for 177 uses by the seven interviewed from the Quilombo Cambury (QC), Figure 2B. For medication it was cited 81 (47%) plants by the interviewed living in QF and 62 (35%) at QC; 43 for food/ spices (25% - QF) and 50 (28%-QC); 25 for construction (15%-QF) and 21 (12%-QC); 7 indications for handicraft (4%-QF) and 33 (18,6%-QC), among others. Some plants are used for more than one indication, as it is the case of some palms which they eat the fruit and the apical meristem, use the bract for handicraft and the wood and leaves for construction.

Of the 268 plants cited in total, 182 have been collected until the moment (Figure 3) and are being identified and deposited into the Municipal-SP Herbarium.



Figure 2. Local partner, Silvestre, with one of the plants collected (Photo: Priscila B. Yazbek, 2016).

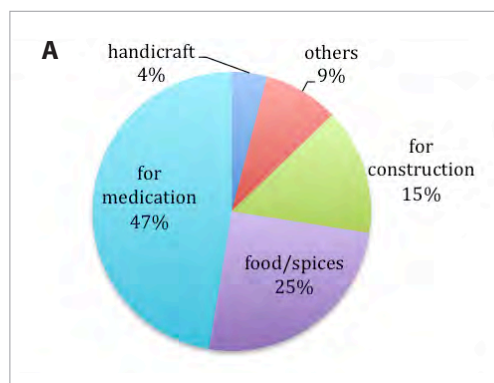


Figure 2A. The main categories of use of the 121 plants cited by the 6 interviewed at Quilombo da Fazenda (QF).

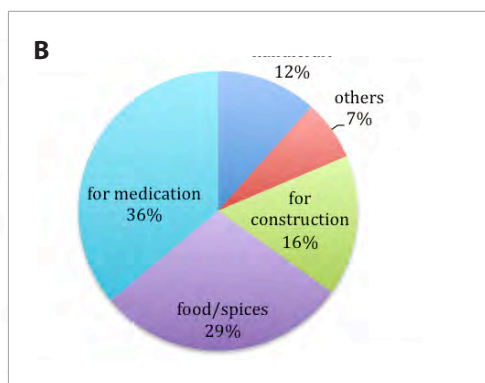


Figure 2B. The main categories of use of the 147 plants cited by the 7 interviewed at Quilombo do Cambury (QC).

Also, about 35 hours were recorded by means of audiovisual by the team, with the purpose of recording the ethnobotanical surveys conducted by the residents themselves, aiming to produce a documentary. Our team also held a workshop among the residents of Quilombo da Fazenda (QF) on how to make soaps, candles and ointments from the Atlantic forest plants.

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INFLUENCE OF ECOLOGICAL AND EVOLUTIONARY PROCESSES IN STRUCTURING AMPHIBIAN COMMUNITIES AT DIFFERENT SPATIAL AND TEMPORAL SCALES

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FAPESP Process 2013/50714-0 | Term: May 2014 to Apr 2018

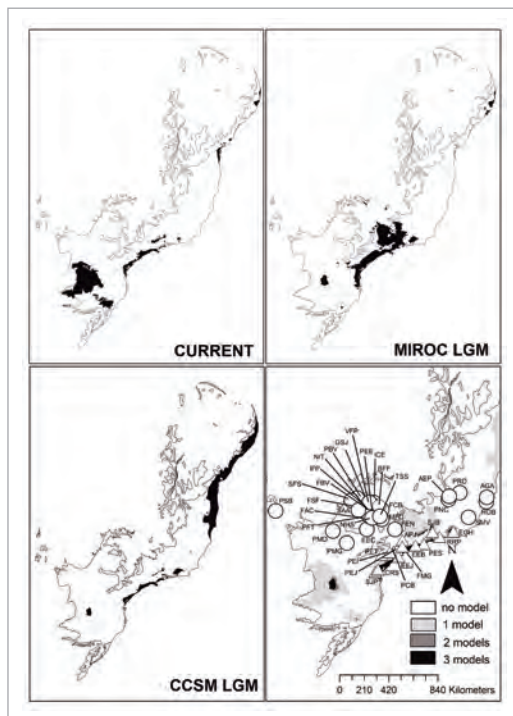


Figure 1. Distribution of the 10% highest values of annual precipitation for the current time, the two last glacial maximum period (LGM), and the intersection of the three maps. Symbols (in the intersection map) indicate the 44 sites in the southern range of Brazilian Atlantic Forest. Dark shading indicates stable regions. White circles represent sites in an unstable region (Quaternary Climatic Oscillations). White triangles represent sites in a stable region (Quaternary Climatic Stability). Reference: da Silva et al. (2014, PLOS ONE).

A key challenge in ecological research is to integrate data from different scales to evaluate the ecological and evolutionary mechanisms that influence current patterns of biological diversity. Previous studies have shown that the climate oscillations of the Quaternary strongly influenced the current species composition of amphibians. However, we do not know how species are distributed in the assemblages in regards to their phylogenetic relatedness and traits (e.g., clustered or even) or how the spatial scale and species pool influence amphibian distribution in this group. Here, I will perform surveys to examine the medium-term effects of ecological and evolutionary processes in the structure of 14 anuran communities considering different spatial scales. Therefore, I will address four main issues: (i) Temporal replacement in anuran composition over three years, considering different spatial scales, local and regional (ii) If reproductive modes in anurans exhibit phylogenetic signal and how they are distributed in the communities, (iii) How different spatial scales and species pool influence phylogenetic patterns of anuran communities, and (iv) If there is an environmental gradient (e.g., rainfall, temperature and topography) that determines the phylogenetic structure of communities of anuran. Thus, I believe that the approach adopted in this project is innovative in that it combines medium-term surveys with different types of data (phylogenetic, phenotypic and environmental space). At the end of this project I hope to contribute with new insights about how evolutionary processes (e.g., convergence or conservation niche), biogeographic (e.g., areas of speciation or extinction of species) and climate contemporaries (e.g., rainfall and temperature) influence the structure of communities amphibians at different spatial and temporal scales. Thus, these results may help in the processes of decision-making on biodiversity conservation of anuran species whereas understanding the different ecological and evolutionary processes that act on different spatial scales provide greater power prediction about which regions or clades will be negatively affected by the possible future climate change.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have carried out two of the three surveys planned for the inventories of amphibian species in the 14 protected areas. So far, we found 102 amphibian species of which 96 were recorded in the adult stage and 64 in the larval stage. We identified 21 reproductive modes (i.e., oviposition site, ovum and clutch characteristics, the rate and duration of development, the stage and size of hatchlings, and the type of parental care, if any) considering the amphibian species recorded in this study. Based on preliminary analyzes, we found that reproductive modes have a phylogenetic signal (i.e, situation in which ecological similarity between species is related to phylogenetic relatedness). Furthermore, the spatial distribution of climatic variables are not random with areas from eastern region presenting mild temperatures and constant precipitation throughout the year while areas from western region presenting high temperatures and marked seasonality in precipitation. Consequently, these patterns of climatic gradients are influencing the lineage distribution of amphibian species. The species that occur in areas with high humidity levels and mild temperatures have specialized reproductive modes in which they deposit their eggs in streams, bromeliads and soil, while species that occurred in the regions with high temperatures and marked seasonality in precipitation have specialized reproductive modes to be more resistant to desiccation. Thus, environmental harshness in the western sites might prevent the colonization of species from specific clades from eastern region that is

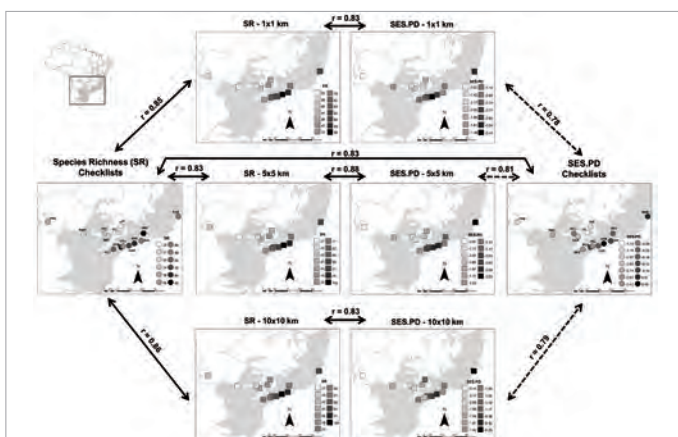


Figure 2. Species richness (SR) and standardized effect size of Faith's phylogenetic diversity (SES.PD) of the 14 areas in the southern range of Brazilian Atlantic Forest and the three grid cell size (1×1 km, 5×5 km, and 10×10 km) used to extract species composition from range maps. Dark shading indicates the Atlantic Forest biome. Continuous arrows indicate 'significant' ($P < 0.05$) Pearson correlations after taking into account spatial autocorrelation in the data, while dashed arrows indicate no statistical significance. For illustrative purposes we increased cell sizes of 1×1 km, 5×5 km, and 10×10 km to enhance visibility. Reference: da Silva et al. (2016, *Natureza & Conservação*).

recognized by its high rates of speciation and endemism. Therefore, our results suggest that niche conservatism (i.e., tendency of lineages to retain their niche-related traits through speciation events) and environmental filters are important processes organizing amphibian communities in the Brazilian Atlantic Forest.

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DYNAMICS OF MALARIA TRANSMISSION UNDER DISTINCT LANDSCAPE FRAGMENTATION THRESHOLDS

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Associated researchers: Eduardo Massad

FAPESP Process 2014/09774-1 | Term: Feb 2015 to Jan 2019

On the road. To Cruzeiro do Sul, Acre state.
Eduardo Bergo. Leonardo Chaves.



On the collections of host-seeking females.
In Machadinho D'Oeste, Rondônia.
Gabriel Laporta.



Malaria is the most prevalent vector-borne parasitic disease in the Americas, and Brazil has the highest regional malaria incidence. The most aggressive and effective Neotropical malaria vector, particularly in the Amazon Basin, is *Anopheles darlingi*. This species' impact is facilitated by its rapid exploitation of the novel environments provided by whole ecosystem alteration via factors associated with deforestation, e.g., so-called frontier malaria outbreaks. There are more than 2,700 recent agricultural settlements in Amazonian Brazil, accounting for over 63,000 malaria cases in Brazil in 2012. The Frontier Malaria Hypothesis postulates that malaria incidence peaks early following settlement and declines with settlement age. The present proposal is linked to a Research Project (n 1R01 AI110112-01A1, Project Title: 'Latitudinal landscape genomics and ecology of *Anopheles darlingi*', Principal Investigators: Jan E. Conn - USA, M. Anice M. Sallum - Brazil) funded by the National Institutes of Health (NIH), USA, 2014-2019. Herein, we will examine understudied aspects of dynamics of malaria transmission in Brazil. We will test the Frontier Malaria Hypothesis (FMH), by explicitly separating the effects of settlement age and landscape fragmentation. We will use a state-of-the-art ecologically based experimental design that compares environmental variables in three levels of landscape fragmentation and two habitat types in both new and old settlements in two regions (east and west) of Amazonian Brazil. Entomological

metrics and the dynamics of malaria transmission will be compared among 12 settlements, by applying mixed effect regression models. Three mathematical models that represent the dynamics of malaria transmission will be employed to understand malaria emergence under distinct landscape fragmentation thresholds. The first model is the Ross-Macdonald (H_0), the second is a biodiversity-oriented model (H_1) and the third is a food-web model (H_2). These models will be calibrated with primary data acquired in the field and their R_0 (Basic Reproductive Number) values will be compared to epidemiological data provided by the Ministry of Health. Simulations using these models in hypothetical scenarios will be performed to identify the main mechanisms responsible for the success in malarial transmission. Expected results are that a modified FMH, that accounts for both age and fragmentation, will provide stronger predictive power for the invasiveness of *An. darlingi* and other vectors causing malaria emergence in a wide range of fragmented landscape types.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Field collections were performed in all the following Amazonian states: Acre, Rondônia, Pará, and Amazonas, Jan. 2015 – Nov. 2016. Municipalities with high malaria transmission were chosen: Cruzeiro do Sul-AC, Mâncio Lima-AC, Acrelândia-AC, Machadinho D'Oeste-RO, Lábrea-AM, Humaitá-AM, Manaus-AM, and Pacajá-PA. Inside each municipality, rural and/or peri-urban human settlements with the highest malaria transmission were selected. In each settlement, a gradient of forest clearing was determined by remote sensing and a 5-km² landscape with replicate was determined in either mature forest (100-65% forest cover), fragmented forest (64-30% forest cover) or cleared forest (29-0% forest cover). In each landscape, a one-night mosquito collection was carried out with Shannon traps, interception nets and small aspirators (Figures 1, 2).

Samples were taken to the laboratory. Species were identified. Specimens of *Anopheles darlingi* species have been tested for the presence of infection by malaria parasites (*P. vivax* and *P. falciparum*). Origin of the ingested blood has been also employed with these specimens.

Landscape analyses have started. There are two aims. The first is to estimate forest cover in each of the 48 landscapes by utilizing Landsat 8 satellite imagery. The second is to estimate time of colonization for each of these landscapes. An undergraduate student with a CNPq fellowship is currently working with the second aim. A second undergraduate student is at the present developing a research proposal to study associations between forest cover and malaria incidence in up to 30 municipalities in the Amazon, in the last 10 years.

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Shannon trap. In the peridomicile.
Machadinho D'Oeste, Rondônia.



Shannon trap. In the forest.
Cururuí settlement, Pacajá, Pará.



Cururuí river.



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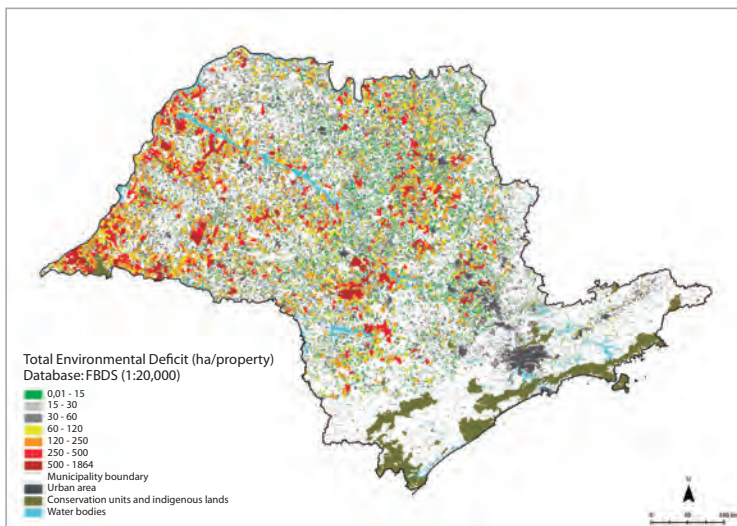
PRIORITY AREAS FOR LEGAL RESERVE OFFSETTING: DEVELOPING A DECISION-MAKING TOOL TO GUIDE THE ENVIRONMENTAL ADJUSTMENT PROGRAM IMPLEMENTATION IN SÃO PAULO STATE, BRAZIL

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FAPESP Process 2016/17680-2 | Term: Nov 2016 to Oct 2019



The implementation of the "New Forest Code" law, due to its complexity and its several sector disputes involved, needs to be supported by technical analysis, strongly based on scientific research. Scientific knowledge organized specifically for supporting the implementation of this law can assist the decision makers in the public consultation and in the necessary dialogues between various stakeholders (environmentalists, farmers, and legislators), strengthening the accuracy of technical discussions and providing a more credible material. The major focus of dispute in the implementation of the "New Forest Code" in the State of São Paulo (Brazil) is the offsetting of Legal Reserves (LR), which

involves the mapping of farmland, the restoration potential in cultivate areas with low agricultural suitability, the offsetting possibility on currently cultivated, and the possibility of buying additional properties covered with natural vegetation. This project intends to accomplish: (i) the preparation of priority areas for offsetting of LR maps and agricultural land use admission in LR (according to Art. 68 of Federal Law # 12.651/2012), (ii) the development of an automatic tool prototype for query georeferenced information, that assists public managers and allow access and query information by other stakeholders (landowners, NGOs, etc.), in order to guarantee the transparency on implementation of the Environmental Adjustment Program (PRA) in São Paulo State. This research comes from a demand of governmental sectors both to identify and to prioritize areas for LR compensation because of federal and state legislation and should contribute to the state role in the development and application of methodology for ensuring the compliance of the New Forest Code.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Until now the project had carried out three meetings with stakeholders from different sectors engaged in the discussion of the New Forest Code (NFC) implementation in São Paulo State (e.g. environmental NGOs, governmental organizations, rural producers). The goal of these meetings is to create a neutral and safe space for an open dialog among stakeholders and the research team. Through these meetings it is possible to identify socially relevant and scientifically challenging queries to guide the research and, consequently, to generate information to support decision-making for the establishment of the NFC in São Paulo State. The project requests along with the stakeholders' claims had already generated three main outcomes for São Paulo State: (i) the assessment of its Rural Environmental Registry (Portuguese acronym: CAR); (ii) the analysis of the available databases of the Areas of Permanent Preservation (APP) (iii) and the new numbers of the NFC to the State.

The assessment of São Paulo CAR shows that, contrarily to the believes of many stakeholders, about 63% of the registers do not show significant problems of overlapping areas. Thus, the CAR database for São Paulo presents an adequate accuracy. The assessment of the databases available to generate a map of the APPs associated to water bodies (IBGE 1:50,000; FBDS 1:20,000) showed that each one has its own advantages and drawbacks. Therefore, it is necessary to create an effort to improve these data and to consolidate a database for the NFC implementation. Finally, the NFC model was updated using a geographically more accurate database (FBDS) and including the article 61a from the Federal Law (Brazil 12,651/2012). Using this model, we created a map of the environmental deficit (LR and APP) in terms of property size for São Paulo State (Figures 1, 2). The outcomes showed that large rural properties (i.e. > 15 FMs) on their own concentrate more than 75% of the State environmental deficit. Furthermore, we accessed the environmental deficit in terms of primary land use using two different databases (IBGE and FBDS). The results showed that the majority of the State environmental deficit is found in lands used for sugarcane production (approximately 40%) and livestock (around 28%). This information will help stakeholders in the process of decision making about the NFC implementation.

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DIMENSIONS US-BIOTA-SÃO PAULO: SCALES OF BIODIVERSITY – INTEGRATED STUDIES OF SNAKE VENOM EVOLUTION AND FUNCTION ACROSS MULTIPLE LEVELS OF DIVERSITY

Inácio de Loiola Meirelles Junqueira de Azevedo & Lisle Gibbs

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Co-PIs: Erika Zaher, Ana Moura-da-Silva, Hussam Zaher, Darin Rokyta, Christopher Parkinson

FAPESP Process 2016/50127-5 | Term: Sep 2016 to Aug 2021

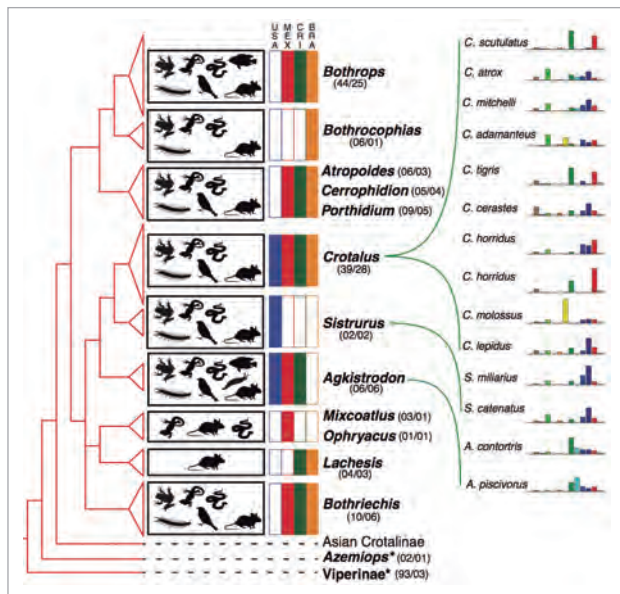


Figure 1. Phylogeny illustrating one of the target clades (Viperidae family). Colored boxes indicate occurrence in the U.S. (blue), Mexico (red), Costa Rica (green), and Brazil (orange). Taxa in bold will be sampled, if available, and taxa with an asterisk (*) indicate outgroups for comparative analyses. Numbers in parentheses (S/C) describe number of species within each clade (S) and the number of species with a high probability of being captured (C) based on museum records and personal experience. Animal silhouettes indicate documented prey in each clade (amphibians, squamates, birds, arthropods, small mammals, fish, and soft-bodied invertebrates). Histograms are preliminary transcriptomic data for different toxin-gene families, measured as percentage of reads mapped. (Source: draw provided by Felipe Graziotin based on unpublished preliminary data).

This recently started project supported by the partnership between FAPESP and NSF (National Science Foundation) involves researchers from Brazil (Instituto Butantan and Museu de Zoologia da USP) and US (Ohio State University, Florida State University and Florida Central University). As part of the Dimensions of Biodiversity Program, it aims to investigate the interface between genetic, phylogenetic and functional diversities, focusing on the evolution of advanced (Caenophidia) snakes.

The first step to understand the rapid diversification within an evolutionary lineage requires the determination of the factors that promote diversification. Traits that are key innovations certainly have a role in adaptive radiations, but few systems exist with one trait that is readily identifiable and genetically tractable such that the precise mutational pathways to species diversification can be ascertained. In snakes, venom has been hypothesized to be an innovation that initiated the radiation of species by expanding trophic opportunities, with subsequent toxin recruitment and toxin-gene neofunctionalization, promoting further diversification.

The relationships between function and properties of venoms, including complexity, composition, and enzymatic activities, and cladogenic patterns across the advanced snakes will be addressed, as shown in the Viperidae family phylogeny in figure 1. The genetic pathways underlying specific cases of rapid functional evolution will be characterized to test for generalities in the processes leading to the observed patterns of diversification. Based on that, it will be possible to determine how secondary key innovations within the venom system contributed to diversification patterns in the advanced snakes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The first part of the project will be dedicated to test for relationships between venom diversity and function and diversification rates using data from more than 100 species belonging to three families of venomous snakes. The specimens used here will be collected in areas of exceptionally high biodiversity in North and Central America and in different domains of Brazil. Using data from venom-gland transcriptomics, quantitative mass spectrometry, and functional assays we will estimate phylogeny, quantify venom function and complexity and test for a link between clade diversification and venom composition. The second part will focus on the evaluation of the genetics of phenotypic divergence and mutational biases in the generation of venom as an adaptive trait. This will be achieved by comprehensively investigating species pairs that show recent divergence and significant differences in venom function. For these species, we will assess the importance of pre-transcriptional and post-transcriptional mechanisms as determinants of venom protein diversity.

The molecular details of species divergence and any inherent mutational biases will be determined since they could be as important in generating macroevolutionary patterns as the ecological context and the nature of the selective pressures driving species divergence. This will provide unprecedented detail about microevolutionary

processes that underlie a key trait that influences macroevolutionary patterns, providing an integrated perspective from the molecular to organismal level on the fundamental processes generating biodiversity. The results will also substantially improve our knowledge about the toxins produced by medically important species or by less studied ones, as exemplified by the recent discovery of a new family of toxins in Dipsadidae snakes (Figure 2).

As broad impacts, the project aims to promote undergraduate research through the partnership between the institutions involved, and by offering a series of technical symposia and lectures for distinct audiences. Moreover, an interactive traveling display and educational pamphlets about snake diversity and evolution are planned to be used in public outreach to local schools in São Paulo. All results and events such as field trips and courses will also be available in a website designed to engage the public and promote public understanding of science.

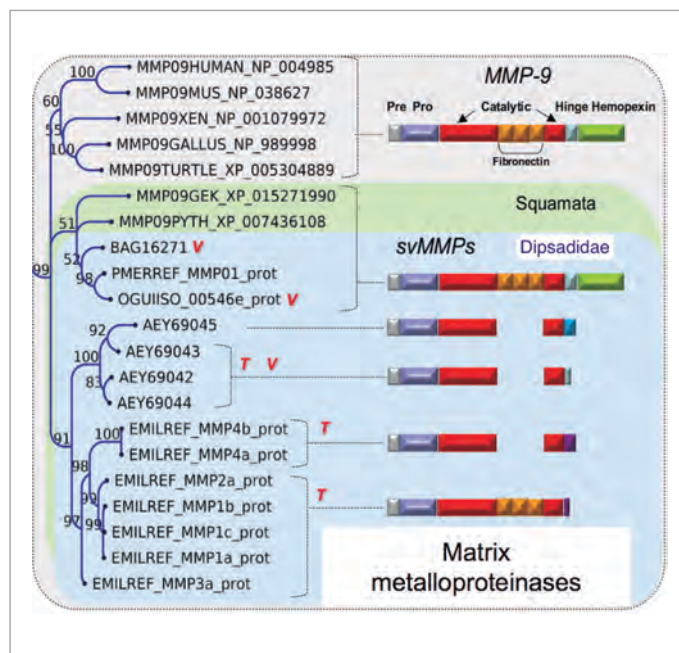


Figure 2. Relationship among sequences of snake venom Matrix Metalloproteinases (svMMPs), a class of toxin identified only in Dipsadidae snakes. On the right, schemes representing the structural organization of protein domains indicate processes of domain loss in venom proteins in comparison to non-venom orthologous protein (MMP 9). Evidences for the presence in venoms are indicated by "T" (transcribed) and "V" (detected in venom proteome).

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INTERFACE PROJECT: RELATIONSHIPS AMONG LANDSCAPE STRUCTURE, ECOLOGICAL PROCESSES, BIODIVERSITY, AND ECOSYSTEM SERVICES

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FAPESP Process 2013/23457-6 | Term: May 2014 to Apr 2019



Figure 1. Pollinator exclusion experiment in coffee bushes in Poços de Caldas (MG). Photo: Adrian David Gonzáles Chaves.

Faced with the challenge of planning multifunctional landscapes to ensure the retention of both original biological diversity and ecosystem functions, as well as agricultural production, this project aims to investigate how parameters of landscape structure directly or indirectly regulates key ecosystem services through influencing a series of distinct ecological processes and specifically investigate the likelihood of both thresholds and trade-offs in service provision. We will do this by relating rates and stocks measurements of key ecosystem services, including regulatory (e.g. pollination, pest and disease control), provisioning (i.e. water storage) and supportive services (i.e. carbon stocks) with parameters associated with landscape structure, including the proportion of native habitat, the proximity and number of edges between native vegetation and agricultural areas, and landscape composition. By considering these relationships for 40 landscapes located in distinct agricultural matrices (i.e. coffee, extensive cattle pasture, and eucalyptus), within a highly biodiverse and threatened biome (Brazil's Atlantic Rainforest), we can assess the generality of these relationships, compare landscape-service relationships for multiple services across within the same matrix type, as well as single service across different matrices. It is expected that: (i) regulating and supporting ecosystem services demonstrate non-

linear threshold dynamics along gradients of habitat loss, (ii) these thresholds are governed by a series of ecological processes related to both movement patterns of the biodiversity associated with service provision, and changes (usually non-linear) in landscape configuration associated with habitat loss, and (iii) that thresholds in service decline occur at lower levels of forest cover for those landscapes with more forested matrix habitat. Taken together, these related datasets will provide a critical scientific subsidy to ongoing political land-use planning processes, and the maintenance of ecosystem services in the working agricultural landscapes of the Atlantic Rainforest.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Pollination: The presence of bee pollinators in coffee plantations can increase coffee productivity in 28%. Therefore, fruit set is especially enhanced in farms where coffee cover is low and nearby forest remnants. Moreover, coffee management (organic vs conventional) and the diversity of flower resources interspersed among coffee lines are determinants of flower visitors in coffee plantations.

Pest control: Agricultural matrices containing coffee plantations might facilitate biological movement among forest patches postponing species loss within patches. A substantial proportion of the species pool of forest dependent birds (25%) can spillover into coffee plantations promoting pest control services. Exclusion experiments in coffee plantations (birds and bats) demonstrate that herbivory was lower in landscapes with higher amounts of forest cover, and new evidences are indicating ants as important predators of the coffee borer beetle (*Hypothenemus hampei*).

Disease control: Landscape structure and both social and climatic factors are associated to hantavirus infection risk. Future scenarios are indicating that sugar cane expansion and expected increase in temperature can increase up to 34% the population at risk for infection in São Paulo state. Contrary to our expectations, parasite regulation services in cattle pastures was increased in landscapes with larger pasture expansion. Moreover, disease transmission may be a strong threat to native biodiversity as well due domestic dog invasion, especially in less forested landscapes.

Water supply: More forested areas are associated with increased rainwater interception, increased groundwater recharge, and a longer time of rainwater residence in watersheds. These results highlight the importance of vegetation cover maintaining hydrological resources quality and regulating the basin streamflow.

Carbon storage: Both deforestation and fragmentation processes have led to important carbon stock's losses in our study system. The above-ground carbon stored is lower at patch edges compared to patch inners. By its turn, the carbon stored below-ground responds to land use intensity, where more intensive land uses (e.g. pastures) are carbon poor.

Public Policies: The enrollment on Payment for Environmental Services programs (PES) influences the on-farm extent of native forest cover. PES engaged rural properties had a higher rate of forest regeneration after PES implementation, when compared to non-engaged similar properties.

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THE METAZOO PROJECT: STUDIES OF THE MICROBIAL DIVERSITY IN THE SÃO PAULO ZOO

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Co-PI: Aline Maria da Silva

FAPESE Process 2011/50870-6 | Term: Aug 2012 to Jul 2017



Figure 1. The São Paulo Zoo composting facility.
Credit: Fundação Parque Zoológico de São Paulo.

The São Paulo Zoo Park is located within the urban area of the São Paulo city (Brazil) and includes a remnant Atlantic rain forest patch. This project has as general objectives the collection, analysis, and interpretation of molecular data from three microbiomes found in this Zoo. These microbiomes are: compost, water from a reservoir, and stool samples from *Alouatta* monkeys. We are using an array of methodologies, with the main ones being metagenomic and metatranscriptomic sequencing, microbial isolate identification by mass spectrometry, and bioinformatics. The project emphasizes the biodiversity characterization of microbes and specific biomes; the establishment of microbial strain collections; development of strategies to identify strains with industrial and biotechnological potential; and mining of microbial gene products for enzymes of technological interest.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Composting: we have collected data from four distinct composting cells, and for two of them our samples included time-series data. The composting process exhibits a sustained thermophilic profile (50°C to 75°C), which seems to preclude fungal activity. The time-series data showed that the turning procedure has a strong impact on the compost microbiota, restoring to a certain extent the population profile seen at the beginning of the process; and that lignocellulosic biomass deconstruction occurs synergistically and sequentially, with hemicellulose being degraded preferentially to cellulose and lignin. Key players in the biomass degradation process are members of the orders Bacillales, Clostridiales, and Actinomycetales. From our metagenomic sequencing data we have obtained near-complete genomes of six bacterial species, one of which is a novel biodegrading species, likely a new genus in the order Bacillales. Our compost isolate collection has more than 800 strains.

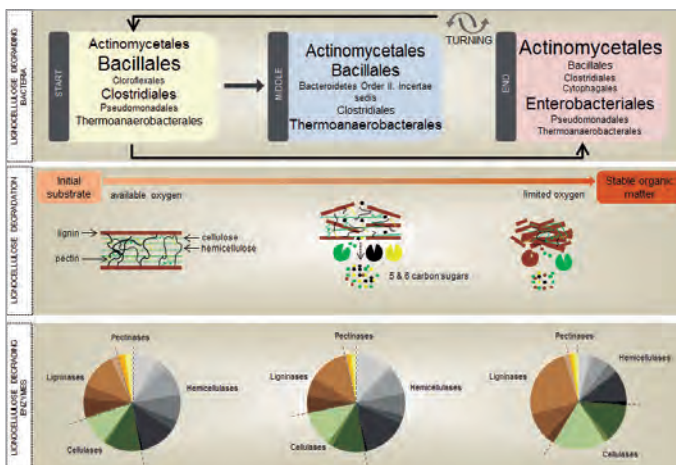


Figure 2. The composting process at the microbial-molecular level. Credit: Antunes et al., *Scientific Reports*, 6:38915, 2016.

Allouatta feces: In the Zoo there are two populations of *Allouatta* monkeys: one held in captivity and another free-living. We have collected stool samples from several individuals from both populations. We have obtained ribosomal RNA 16S amplicon sequences from all samples. These show that the microbiota of these two populations are markedly different, with the most likely cause being differing diets.

Reservoir water: This reservoir is called the São Francisco Lake. We have collected monthly samples during a whole year, at the reservoir's outflow point. For these samples we have both 16S and total DNA shotgun data. Preliminary results indicate seasonal variations in the microbiota, which roughly matches variation in ambient temperature.

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MOLECULAR DIVERSIFICATION IN AVIAN MALARIA IN THE ATLANTIC FOREST

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Figure 1. Male individual of *Pyriglena leucoptera* (Thamnophilidae), one of our focal host species. Photo credit Sidnei Sampaio dos Santos. Not published.

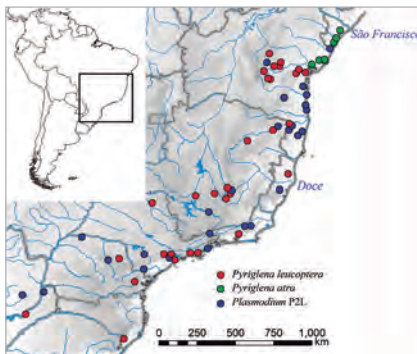


Figure 2. Sampling localities of *Pyriglena* antbirds in the Atlantic Forest. Localities where *Plasmodium P2L* was recovered are shown in blue, localities where *P. leucoptera* was sampled but P2L not recovered are shown in red, and localities where *P. atra* was sampled but P2L not recovered are shown in green. Figure not published.

A major challenge for evolutionary biologists is to illuminate the various factors influencing species diversification. In broad terms, genetic drift and selection enhance evolution and gene flow mediated by migration inhibits it. Selection operates on traits that exhibit heritable variation within a population, and only when some variant(s) increases the fitness of the individuals that possess it. Factors that normally influence the dynamics of diversification in free-living organisms may also operate on parasites. For example, (1) environmental variation can influence population sizes and transmission potential, (2) history may limit parasite distributions, and genetic drift may result in differentiation in the absence of selection. Unlike free-living organisms, however, parasites are intimately connected with their hosts, which provide both a habitat and nutrients. By definition, parasites impose a cost to their hosts, which defend themselves against parasites through their immune system. This reciprocity of the interaction results in coevolution. Thus, in addition to the abiotic environment, historical events, and drift, parasite diversification may also be affected by host evolution. A framework in which one can simultaneously examine multiple factors would be a powerful means towards a deeper understanding of the influence of these different factors in parasite diversification. The field of phylogeography provides this. In this project, we investigate the evolutionary history of a *Plasmodium sp.* in the Brazilian Atlantic Forest (AF) in a phylogeographical framework.

Avian *Plasmodium* (malaria) and the closely related *Haemoproteus* parasites have served as a model study system in several scientific disciplines. Yet, we know little about the genetic variation below the species level in this system. In this project, we will determine whether a *Plasmodium* species is structured genetically across the AF and if so identify factors that may explain this structure. Obtaining this information will provide new insights to how changing environments affect the interaction between host and parasite at the molecular level.

A major limitation in the study of bird-*Plasmodium* interactions is that genetic variation and divergence in the parasite mtDNA cytochrome b (cyt b) gene, the standard marker for diversity studies on avian malaria, is low. We therefore apply whole genome sequencing and sequence capture methods to analyze more rapidly evolving markers. Specifically, focusing on one *Plasmodium* species infecting a non-migratory bird genus in the Brazilian AF (*Pyriglena leucoptera* and *P. atra*, Thamnophilidae), we (1) describe neutral genetic variation (2) describe adaptive genetic variation, and (3) relate recovered patterns to history, ecology, geography, and host evolution.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have screened 574 *Pyriglena* individuals from 63 localities throughout the AF for malaria infection using standard PCR techniques, and we recovered over 30 malaria lineages in the AF. One *Plasmodium*, P2L, was by far the most abundant, and in addition, appears endemic to the AF.

The *Plasmodium* species investigated here does not currently have genomic data. Our approach, therefore, is to sequence the whole genome for P2L, and then perform sequence capture on the majority of isolates. Because we have obtained parasites from host tissue, we reduce the host contamination of the parasite DNA using the NEBNext® Microbiome DNA Enrichment Kit (New England Biolabs Inc., Ipswich, Massachusetts). We tested the efficiency of the kit on seven samples and found the ideal starting amount to be half of that recommended by the manufacturer. DNA is expected to be low for all samples; thus, we use the Nextera XT library preparation kit for small genomes with low starting material.

We have so far found 77 P2L isolates from 26 localities (Figure 2), but only 52 are being processed for this project. Based on *cyt b* sequencing, 16 isolates exhibited multiple infections. Because it would be impossible to distinguish two parasites found in the same host individual, we exclude all multiple infections. To identify markers that vary geographically, we sequence the whole genomes of four isolates from distant sites. The samples are being subjected to paired-end sequencing on an Illumina platform through RAPiD Genomics in Florida, USA. Up to 5000 probes are being designed, targeting both neutral markers and genes under selection (for example, those genes encoding surface proteins, involved both in host cell invasion and immune evasion).

Early on in molecular studies of avian malaria, it became clear that the gene (*cyt b*) used to identify parasite haplotypes and infer evolutionary lineages evolves more slowly than the homologous gene in the bird and thus provides low resolution for intraspecific studies, preventing researchers from analyzing the effects of historical events, climate, habitat, and host evolution on the evolution on the parasites. Our current project will hopefully shed light on what molecular markers are appropriate for phylogeographic inference of avian malaria parasite species and what factors are relevant to parasite evolution.

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ASSEMBLY AND EVOLUTION OF THE AMAZONIAN BIOTA AND ITS ENVIRONMENT: AN INTEGRATED APPROACH

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FAPESP Process 2012/50260-6 | Term: Sep 2012 to Aug 2017

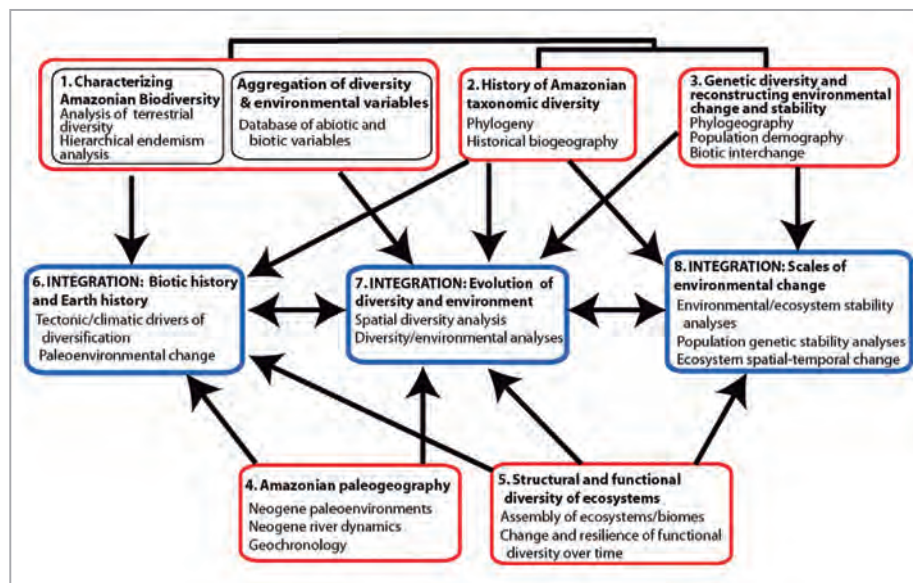


Figure 1. Summary of activities being developed within the project.

The Amazon is one of the most biodiverse areas on Earth; however, little is still known about the processes that led to such great diversity. Indeed, many uncertainties remain about its geologic history, age of formation, and extension of its terrestrial and aquatic systems. For instance, while some models claim that the Amazon was established during mid-Miocene, others established its origin in the Pleistocene. The resolution of these historical uncertainties and a better understanding of how the Amazonian biota has responded to past

paleogeographic/climatic events are of extreme importance for a better understanding of the processes associated with the generation and maintenance of its biodiversity. This knowledge is also vital for predicting the future of this extremely important biome.

This project aims to achieve a new evolutionary and environmental synthesis of Amazonia biodiversity, integrating findings from phylogenetics, historical biogeography, phylogeography, remote sensing, geology and biogeochemical cycles in a new model about the origin and evolution of the Amazonian biota, from the Neogene to present. New geological data and biological studies with plants, butterflies, birds and primates will help solve several uncertainties about the mechanisms responsible for the diversification, spatial organization and dynamics of Amazonia over the last 20 million years.

This project aims to answer biogeographical, evolutionary and geological questions such as: (1) How species diversity is distributed and organized at varying spatial scales into common distribution patterns? (2) What has been the phylogenetic history and pattern of diversification of Amazonian taxa? (3) What has been the paleogeographical history of the Amazonian drainage system and terrestrial tropical Amazonia, particularly in the west, and since the latest Neogene when the World became cooler and drier and the effects of climate forcing more pronounced? (4) To what extent do large-changes in ecosystem structure relate to the distribution of species and ecosystem diversity? (5) How did the history of Amazonia influence global-scale changes in biogeochemical cycling?

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

During the first years of this project, we compiled the most complete georeferenced database for Amazonian vascular plants and terrestrial vertebrates to date. These data are now being analyzed using macroecological approaches to characterize patterns of Amazonian diversity and endemism and address fundamental questions about how that biodiversity has evolved. DNA sequences of hundreds of species of our target taxa (e.g., birds, butterflies, primates, and plants) from across Amazonia were obtained and used to generate a series of time-calibrated phylogenies and phylogeographic networks for biogeographic studies.

Geological studies to date have focused on the sedimentary geology and palynology of the Amazon Basin, as well as on the study of climatic changes in the Amazon during the last 20.000 million years. These studies aimed at describing the evolution of the Amazon river basin and Amazonian forests based on geological sedimentary sequences and their pollen content. In addition, ongoing studies using oxygen isotope records in cave speleothems have indicated an antiphased pattern of precipitation between Eastern and Western Amazonia. The new data obtained covers the last 250.000 years and represents the longest absolute paleoclimatic record for the Amazon thus far and a unique record of climate change during the last glacial/interglacial periods in the Amazon. As genetic data are overlaid on this new paleoclimatic data, new insights about are gained on the complex biogeographic history of Amazonia.

Great efforts were also invested towards planning and integrating international research teams. Four meetings, one at FAPESP (São Paulo, 2013), one at INPA (Manaus, 2014) and two at USP (São Paulo, 2015, 2016) provided multiple venues for collaborative research. These meetings included extensive discussions among project members and open symposia to the whole scientific community. Symposia included talks on the biogeographic history of Amazonian organisms, as well as overviews of the Amazonian past geological history, among others.

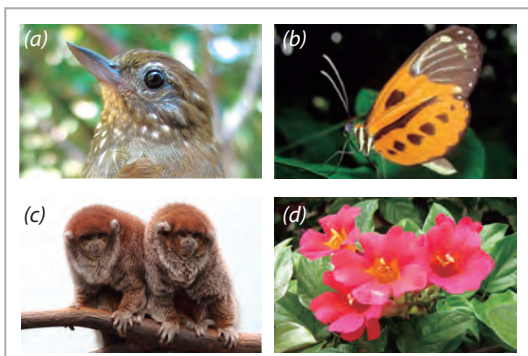


Figure 2.
Project's focal
organisms:
(a) birds,
(b) butterflies,
(c) primates,
and (d) plants.

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CONSERVATION GENOMICS OF COPAIBA IN DIFFERENT VEGETATION FORMATIONS OF THE CENTRAL WEST REGION OF THE SÃO PAULO STATE

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FAPESP Process 2014/18306-1 | Term: May 2015 to Apr 2017



Collecting leaves from *Copaíba* (*Copaifera langsdorffii*) tree.
Credits: Marcos Siqueira.

The ecosystems destruction in Brazil is among the most alarming national and international conservation issues. Both the conservation of natural remnants, through the establishment of Conservation Units, and the possibility of optimizing management strategies in these sites are necessary for the conservation of these biomes and their native species. Genetic diversity is one of the main bases for biological conservation, and the genetics of populations has evolved in this sense for better understanding the biodiversity. This project aims to conduct a detailed study on the diversity and genetic structure of the copaíba (*Copaifera langsdorffii* Desf.) in different vegetation formations. Samples of the Brazilian Savannah, Rain Forest, and ecotones will be collected, including samples from highly anthropized areas in the Central West region of the São Paulo state. This native tree, which is used in restoration projects and known for its herbal importance and inexhaustible ecological value, is one of the symbols of this study area that is composed of all these vegetation complexes. Part of the plant material collected will be used for morpho-anatomical studies, and the other part will be used for extracting genetic material. In this project, we will seek the target area of the species genome studied, locus with adaptive genetic variation, which hypothetically responds to the varied selection in different vegetation formations. Thus, SNP markers will be used by means of the Next- Generation Sequencing. Data obtained will be used to establish management units and priority conservation areas for the copaíba populations. During its execution, an environmental component will be developed with junior high and high school students aiming to show the importance, the objectives, and outcomes of this study. Based on the scientific data obtained, this study intends to contribute with decisions and management strategies of forest fragments in the region, as well as create a higher environmental awareness in basic education through teaching methodologies and from the conservation genetics.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We evaluated the feasibility of the RADseq in 95 individuals distributed in fragmented forests in the Central West of the São Paulo state (Jardim Botânico Municipal de Bauru (JBMB), Forest reserve of the campus in Bauru of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Horto Florestal Aimorés, Floresta Estadual de Pederneiras (FEP), Reserva Natural Olavo Setúbal, and Estação Ecológica dos Caetetus) to identify and genotype single nucleotide polymorphisms (SNPs). We identified and genotyped 2797 high-confidence SNPs across six sites distributed along different vegetation structures, demonstrating the value of RADseq for generating polymorphic loci for population genetic analyses in these trees. Genetic diversity measures revealed slight differences for all populations; however, the population of the JBMB showed moderate high values. H_o values were lower than H_e values for all populations, indicating an excess of homozygotes. Differentiation of F_{ST} was low (0.023), but significant (0.007 - 0.044, 95% CI) among populations. A clear correlation was observed between the geographic distances versus the genetic distance. The analysis of the spatial genetic structure (SGS) in populations of *C. langsdorffii* suggested isolation by distance. Similar results were found in previous studies, suggesting that the spatial isolation of populations by habitat fragmentation may reduce the genetic diversity and the effective population size, restrict pollen and seed gene flow, and increase the SGS of new generations. Future analysis will search outliers loci associated with the adaptations of these species in different biomes. An additional study was developed with the leaf anatomy to analyze its adaptation characteristics to environmental conditions and compare the numbers and density of the stomata. In the samples of the populations of *C. langsdorffii*, we found paracytic type stomata only on the abaxial face of the leaflets, which characterize them as hypoestomatic. The population of the FEP had the highest stomata density (509.48 stomata/mm²), and the JBMB had the smallest value (381.31 stomata/mm²). It is important to highlight the JBMB concentrates the largest Brazilian Savannah area among the samples of vegetation formations. In parallel with this, we used the popularization of sciences to promote this project, evaluating the biological knowledge of 193 High school seniors. We could verify there is no significant difference between the private and public schools analyzed in the city of Bauru. Genetic / morphological data may indicate the human pressure on these areas, in special, the ones not protected by the State are accelerating the loss of the *C. langsdorffii* diversity, and the Young people play an important role in the dissemination of knowledge and sciences in this region.

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COMPARATIVE PHYLOGEOGRAPHY, PHYLOGENY, PALEOCLIMATE MODELING, AND TAXONOMY OF NEOTROPICAL REPTILES AND AMPHIBIANS

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FAPESP Process 2011/50146-6 | Term: Jan 2012 to Dec 2017

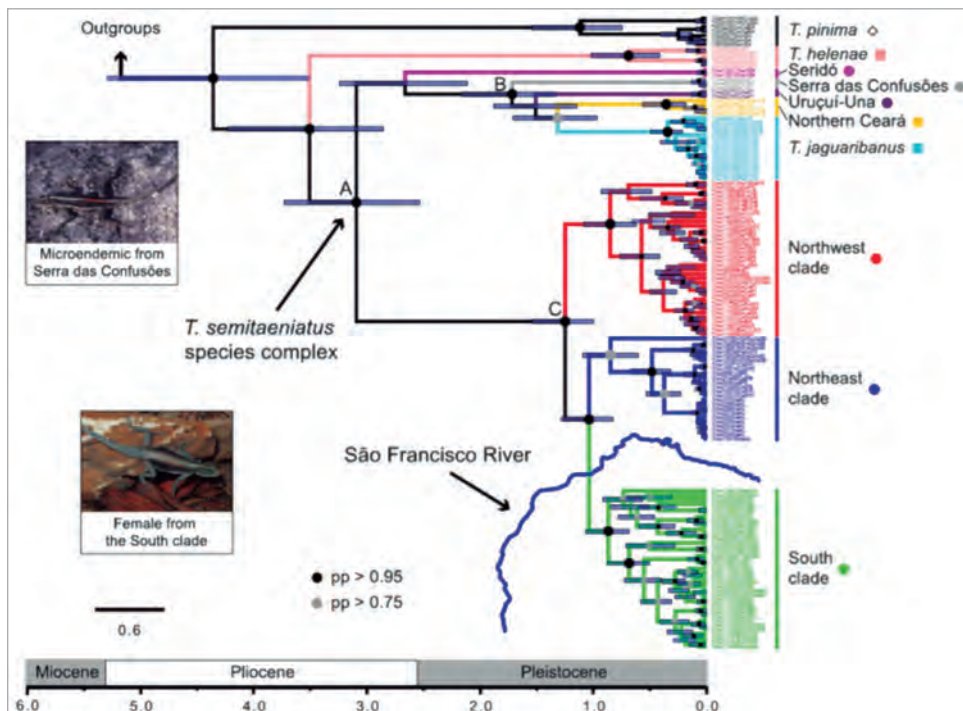


Figure 1. *Tropidurus semitaeniatus* species groups mtDNA maximum clade credibility Bayesian tree and divergence dates. Posterior probability (pp) values are indicated by node colors and nodes with no indicative of support have pp < 0.75. (Photos by MTR).

Recent analyses of endemism and levels of anthropic threat identified the so-called hotspots targets for conservation. However, our knowledge about the origin and distribution of the biodiversity in those areas remains scarce. Such a deficiency limits our power of conservation with respect to the environmental changes caused by a man. One of the aims of this project is addressed to study the influence of Pleistocene climatic fluctuations of the herpetofauna and the levels of genetic diversity of frogs and lizards living in elevated and lowland regions of the Atlantic

Forest. By combining climatic modeling coupled to phylogeography, the project will be recording diversity patterns in an insufficiently studied, highly threatened and megadiverse region, bringing important contributions for the conservation of the remaining fragments of this hotspot. We will be also focusing on the role of mountains as areas favoring adaptive diversification of the herpetofauna, either as refuges to species adapted to cold climates during hot phases, or as evolutionary scenarios for ecogeographic speciation. We also proceed further our ongoing research with reptiles and amphibians from several Brazilian ecosystems under morphological, karyological, taxonomic, phylogenetic and phylogeographic approaches, contributing to a better understanding of their evolution and the biogeographic history of the continent. We will be also conducting a comparative phylogeographic study of a South American clade of lizards and its ecological equivalent in Africa in order to establish intercontinental correlations on their history and origin. The collections obtained in unexplored or little known areas in State of São Paulo and in other Brazilian ecosystems will improve the knowledge on our biodiversity, describing new taxa which, associated to the phylogenetic and phylogeographic data obtained, will contribute to reevaluate conservation strategies to target species.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We sampled reptiles and amphibians in about 120 localities in all Brazilian biomes totalizing 8609 specimens (4873 reptiles and 3589 amphibians); about 600 additional specimens were obtained in Moçambique for comparative molecular studies, now in course. The specimens and tissues obtained allowed the discovery of three new genera and 35 new species that are described in the about 160 publications of the project. Interdisciplinarity was our permanent guideline resulting in integrative papers focusing one or more of the following areas: taxonomy and systematics, phylogeny, phylogeography, physiological ecology, developmental biology and cytogenetics. Using molecular and phenotypic data of selected taxonomic groups we reconstruct their biogeographic history and its bearing to understand the landscape evolution of our biomes. We detect an unsuspected amount of cryptic diversity in the majority of groups studied, rendering mandatory a review of the presently adopted conservation policies. Comparative studies addressing molecular and phenotypic evolution of selected lizards in Brazil and Africa are underway.

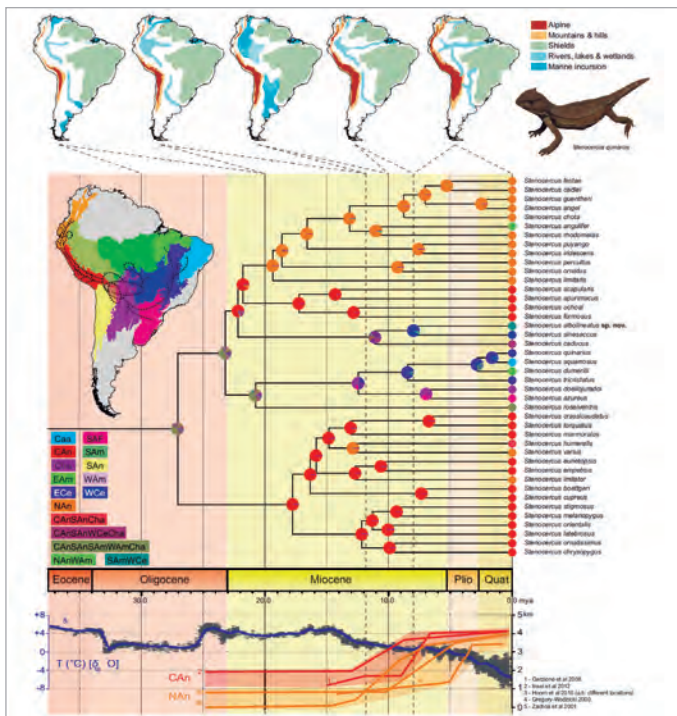


Figure 2. Biogeographical history of *Stenocercus*; top panel: landscape changes (adapted from Cook et al., 2012; Hoom et al., 2010; Lundberg et al., 1998); middle panel: amcestra; area reconstruction (Caa = Caatinga; CAn = Central Andes; Cha = Chaco; EAm = Eastern Amazonia; ECe = Eastern Cerrado; NAn = Northern Andes; SAF = South Atlantic Forest; SAM = Southern Amazonia; SAN = Southern Andes; WAM = Western Amazonia; WCe = Western Cerrado); lower panel: temperature changes (adapted from Zachos et al., 2001) and Andean uplift (adapted from Garzzone et al., 2008; Gregory-Wodzicki, 2000; Hoom et al., 2010; Insel et al., 2012).

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AN INVENTORY OF THE CHARACIFORM FISH FAUNA (TELEOSTEI, OSTARIOPHYSI) FROM SOUTH AMERICA

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Co-PI: Osvaldo Takeshi Oyakawa

FAPESP Process 2011/50282-7 | Term: Aug 2011 to Jul 2017



Figure 1. *Hyphessobrycon procyon* Pastana & Ohara, a new characid species from rio Aripuanã, rio Madeira basin (Source: Pastana ML, Ohara WM. 2015. Zootaxa. 4162 (3): 386-398). (Photo by William Ohara).

The project SACI (South American Characiformes Inventory) focus on the taxonomic study of the Characiformes and aims at the production of identification guides, atlases, catalogues and checklists of species, and phylogenetic studies of higher-level relationships among characiforms based on material already available in fish collections of participant institutions as well as representative samples from poorly collected regions to be added to permanent collections. In phylogenetic studies both morphological and molecular data will be used.

A project website and a system of electronic mail was created and will continue after the project end for dissemination of data and products and will enable the continuous communication among the participant taxonomists.

The project made possible and stimulated the continuous inventory of South American freshwater fishes through contact of the participants with other Brazilian and foreign taxonomists.

Master, doctoral and postdoctoral scholarships have provided training for students, a most important issue within the project. Students and researchers engaged in graduate and postdoctoral programs have been working with senior taxonomists in the field and museum laboratories thus largely increasing their knowledge in taxonomy and systematics.

To know the species and higher-level relationships of a large and widely distributed clade as Characiformes, will open research opportunities in evolution, ecology, and organismal biology. The research will encompass studies in systematics, phylogeny, historical biogeography and comparative biology.

Degradation of aquatic ecosystems in South America is enormous and aquatic species are among the most threatened. Conservation biologists and fishery managers depend on accurate taxonomic work and up dated information on specimens deposited in collections for determination of priority areas for protection and establishment of management programs. In addition, the project has provided a considerable progress in taxonomic knowledge of the group and the opportunity to improve museum collection data through the work of characiform experts.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The website of the project has been created (<http://www.usp.br/peixes/>) and is already available. Photographs and all the pertinent information about type specimens have been introduced and will continue until completed. In taxonomic studies type specimens of species contain important data needed for precise definitions and when available provide information for systematists worldwide. Original descriptions of the types are also included.

The first expedition was undertaken to the headwaters of the Xingu river, in the region of Altamira, and surroundings by a team including research members of the project. The expedition was extremely successful since not only specimens of rare species formerly described from the area, but specimens representing new species were collected. Since then 6 more expeditions were undertaken during which almost 10,000 specimens and about 280 species were collected. Nearly 50 new species and 2 new genera were described.

The collecting efforts have been primarily concentrated on headwaters of the rivers especially those of major tributaries of the Amazon basin such as the Xingu, Tapajós, Tocantins and others that are targets for construction of new hydroelectric power plants. Damming of headwater streams and rivers for hydroelectric purposes are among the most severe threats to aquatic ecosystems in South America and knowing the aquatic fauna, especially fishes, at the species level through accurate taxonomic work, is of utmost importance for determination of priority areas for conservation, recovery, and establishment of management programs.



Figure 2. *Moenkhausia uirapuru* Ohara, W.M. and Lima, F.T.C., a new characid species from Rio Guaporé, Chapada dos Parecis, Mato Grosso, Brasil (Source: Ohara WM, Lima FTC. 2015. *Ichthyological exploration of freshwaters*. **26**(2): 150-170). (Photo by Willian Ohara).

MAIN PUBLICATIONS

In order to speed up the process of publication of the results, a special issue of the official journal of the Brazilian Society of Ichthyology (*Neotropical Ichthyology*, volume 12, number 2, 2014) was published including 22 articles exclusively on characiform fishes by members of the project. In addition 244 individual papers have been published to date in indexed journals by participants of the project.

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DISTRIBUTION AND METABOLISM OF NATURAL AND SYNTHETIC XENOBIOTICS: FROM THE COMPREHENSION OF REACTIONAL PROCESS TO TISSUE IMAGING GENERATION

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FAPESP Process 2014/50265-3 | Term: Jul 2015 to Jun 2019

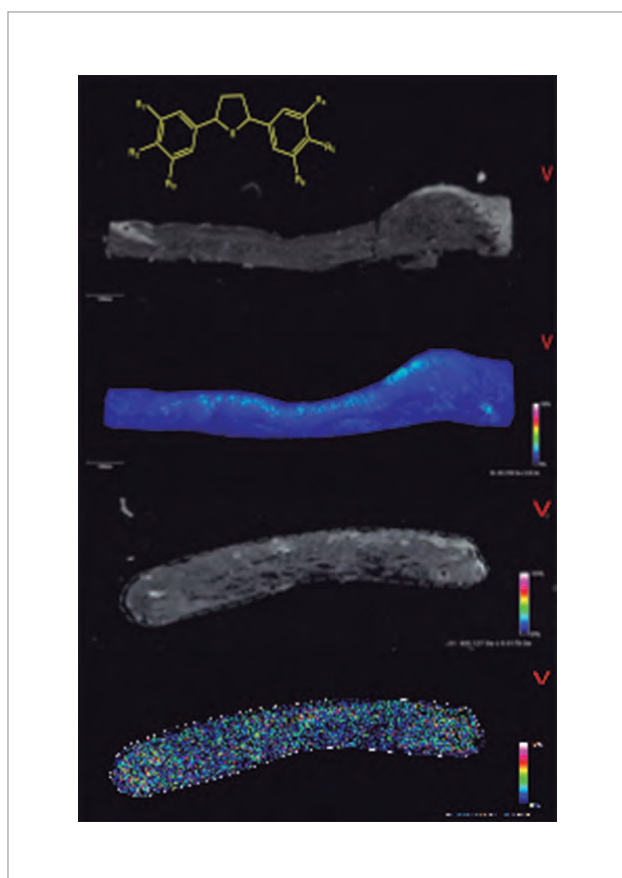


Figure 1. MALDI-MS/MS, image reconstructed from the protonated ions of a new anti-leishmaniasis agent (patent under analysis). Optical image (obtained by scanner), used to create the MALDI-MS/MS image illustrated in details of pig skin anatomy.

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, but also compounds with ecological relevance. 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 flow that envisions supporting pre-clinical studies or for understand ecological interactions. Since the platform model still is somewhat uncommon the team size may oscillate during project execution, having involved in this first two fifteen 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.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The selected active natural products were submitted to biomimetic studies, in which the organometallic 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 more than 10 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

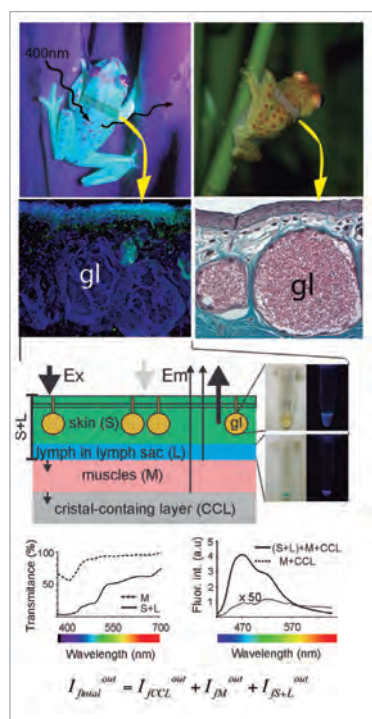


Figure 2. Fluorescence in *Hypsiboas punctatus*. **Top.** Adult males under UV-blue light (left), and under white light (right). **Middle.** Transverse sections of dorsal skin. Confocal images of fresh dorsal skin samples using a 405 nm laser line (left), and stained histological section (right). Fluorescence emission is observed from epidermis, dermis and glands (gl). **Bottom.** Schematic representation of the skin and subcutaneous structures. Incident excitation light and fluorescence emission from each tissue layer are attenuated by the structures above and depend on the transmittance of each layer. Fluorescence from subcutaneous structures is almost completely filtered by skin with lymph. The total observed fluorescence take into account the contribution of each tissue of the frog. We considered that the photon flux reaching CCL may be estimated as the incident photon flux (I_0) attenuated by the absorption of S+L and M.

in gas phase allowed the definition of three complete pathways from three classes of natural products. Additional collaborative work open the possibility to introduce strategies for the identification of plant secondary metabolites on the GNPS platform. The initial pharmacokinetic study has clarified the elimination mechanism and half life time of the several alkaloids, and terpenoids. Initial results has shown the viability of the proposal and has generated the expectation of better

understanding the absorption, distribution and metabolism mechanism for selected natural products. The MALDI-imaging development allowed us to verify in details the distribution of specific compounds and its metabolites in several organs. The same methodology was also applied to understand ecological function of Natural Products.

MAIN PUBLICATIONS

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ECOLOGY OF INTERACTIONS, BEHAVIORAL ECOLOGY, AND GENETICS OF NEOTROPICAL ANT POPULATIONS

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FAPESP Process 2014/23141-1 | Term: Sep 2016 to Aug 2021



Figure 1. Workers of (A) *Camponotus renggeri* and (B) *C. rufipes*. These two ant species are often difficult to differentiate in the Cerrado. An integrative approach using morphology, natural history, and molecular data confirmed the valid status of these species. (Source: Ronque et al. 2016. *Zool. J. Linn. Soc.* **176**:170-181; photographs by L. Mota). Reproduced with permission from John Wiley and Sons.

Ants outnumber all other terrestrial animals and individual colonies may contain several million workers. The numerical dominance of ants in terrestrial habitats is combined with a broad taxonomic diversity and a widespread distribution, especially in the tropics. Among other things, the ecological success of ants is attributed to their eusocial mode of life, local abundance, and diversity of adaptations. Such traits result in a wide variety of feeding habits and foraging strategies, including the use of plant foliage as a foraging substrate. Intense foraging on vegetation appears to have set the scenario for a multitude of interactions with many plant species worldwide, ranging from facultative to obligate ant-plant associations. By frequently foraging on the plant surface, ants often affect (positively or negatively) the life of a particular trophic group – the herbivores. The abundance of ants on the ground of tropical habitats is also remarkable. Recent studies have shown that ground-dwelling ants actively interact with a wide range of fleshy fruits, and can markedly affect seed fate, as well as seedling growth and survival. Ants thus perform a crucial role in tropical ecosystems, acting as detritivores, predators, mutualists, granivores, and herbivores. Through this diversity of functions in the trophic web of terrestrial communities, ants generate a range of interspecific interactions – from antagonism to mutualism – with animals, plants, fungi, and a wide variety of microorganisms. The biodiversity inherent to this multitude of interactions is essential for community structure and should form an integral part of conservation programs. This project encompasses the following research avenues: multitrophic interactions at the ant-plant-herbivore interface, secondary seed dispersal by ants, natural history and behavior of fungus-growing ants, integrative taxonomy, and landscape genetics of ants. Our investigation involves field work in two representative Brazilian ecosystems, the Cerrado savanna and the Atlantic rainforest.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our research on multitrophic interactions is focused on plants bearing ant attractants such as extrafloral floral nectaries and honeydew-producing hemipterans, and on how intense ant foraging on such exudates can affect the associated community and host plants in Cerrado. Recent results have shown that slow-moving caterpillars (Lepidoptera) employ a variety of defense strategies to avoid or to survive attack by ants on leaves. Negative effects of predatory ants on caterpillars can affect host plant use not only by single species, but are also detectable at the community level, affecting patterns of abundance and host plant use by lepidopterans in general.

Most plants producing fleshy fruits are dispersed by vertebrate frugivores in tropical habitats. Large amounts of nutritious fruits, however, fall to the ground and are exploited by litter-foraging ants. Recent results from Cerrado and Atlantic forest have shown that ants can provide a range of benefits to seeds and seedlings, including protection from pathogens and predators, increased germination success, and dispersal to nutrient-rich ant nests where seedling establishment is enhanced. Even leaf-cutter ants, traditionally viewed as pests, have recently been shown to positively affect the biology of primarily vertebrate-dispersed seeds. Recent experiments in fragmented areas of Cerrado and Atlantic forest have shown that anthropogenic disturbance can negatively affect such ant-mediated dispersal systems.

Species identification in difficult groups requires many types of data in addition to traditional morphology. We used an integrative approach to distinguish sympatric *Camponotus* ant species (Formicinae). Natural history (habitat preference, nesting biology) and molecular tools (nuclear and mitochondrial markers) performed well in delimiting two common Cerrado ants, *C. renggeri* and *C. rufipes*, as valid species (Figure 1). Given that Cerrado physiognomies range from open grassland to dense woodland, we are currently investigating multiscale responses by ant species to landscape variation using recent approaches of landscape genetics.

Our multidisciplinary approach at ant ecology and behavior, encompassing multiple levels of interactions and incorporating genetic data into basic information of ant species, should help management and conservation programs of Cerrado and Atlantic forest reserves.

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BIODIVERSITY AND CONNECTIVITY OF BENTHIC COMMUNITIES IN ORGANIC-RICH HABITATS IN THE DEEP SW ATLANTIC – BIOSUOR

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FAPESP Process 2011/50185-1 | Term: Aug 2011 to Jul 2017

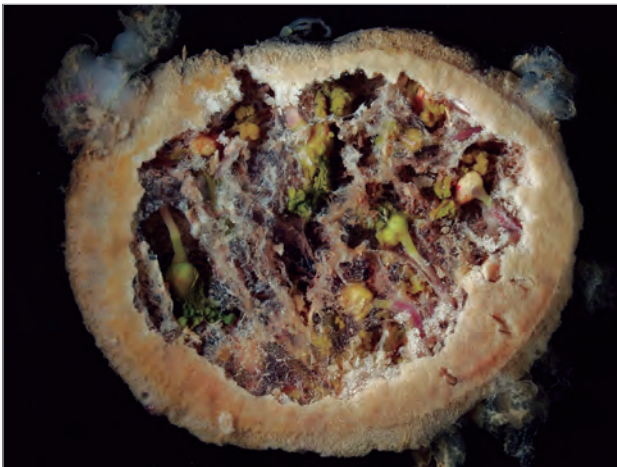


Figure 1. The bone specialist *Osedax sp. nov.* (green) infesting a minke whale intervertebral disk. This is the deepest living species of *Osedax* found to date (ca. 4204 m) and the first to be found in the deep Atlantic Ocean. *Osedax* produces acid to dissolve the bone matrix in order to absorb collagen and lipids. Photo: Yoshihiro Fujiwara (JAMSTEC).

The BIOTA/FAPESP program is an effort that has been mapping the biological diversity of terrestrial and aquatic ecosystems in the State of São Paulo with extreme success. In marine areas, this effort has historically been concentrated in coastal ecosystems, leaving the deep ocean biota habitat - one of the largest global repositories of species - largely unknown. Evidence shows that organic islands contribute significantly to deep benthic biodiversity, with highly specialized life histories. However, there is a large gap on the large-scale patterns of biodiversity, biogeography and connectivity of the populations that exploit these islands, as well as on the relationships between diversity and ecosystem function. The present proposal aims to study the biota associated with organic-rich islands in the deep-sea (whale bones and wood parcels) in the SE Brazilian margin, in areas under whale migratory routes and historical proximity to large continental forests. In order to address these questions, we will use an advanced experimental design through the implantation of whale bones and wood

parcels using low-cost autonomous vehicles (landers) at depths of 1500 and 3000 m along the Brazilian continental margin for 15 months, allowing the colonization of substrates by specialized micro- and macroorganisms. Ecological, molecular and genetic analyses of whale bone and wood associated macro- and microfauna will be used to address regional and bathymetric patterns of benthic biota, the connectivity between populations of specialists in the Southwest Atlantic and also their trophic and ecosystem function in the degradation of organic substrates in the deep SW Atlantic. Interbasin comparison of biodiversity and functioning of these islands (SW Atlantic vs. NE Pacific) will be achieved through an international collaboration project, which will be jointly submitted to the National Science Foundation by Dr. Craig R. Smith of the University of Hawaii. This is a highly innovative scientific proposal in Brazil, with national and international partners of high scientific level, and will use a pioneer low-cost methodology in deep sea ecosystems, which are areas of difficult access with the resources previously available for institutions in São Paulo and Brazil, but that should be facilitated by investments made by FAPESP and IOUSP, with the recent purchase of a new oceanographic vessel and a research boat.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The deep-sea is a highly oligotrophic environment which, over the evolutionary time, has selected organisms that take advantage of any organic material they might find. This is seen in the extremely toxic hydrothermal vents, where organisms live symbiotically with chemoautotrophic bacteria in nasty, sulfide rich environments. Large food falls may also produce such reduced habitats and attract a suite of opportunistic organisms. Even more, they have also produced evolutionary novelties that specialize in taking advantage of such large food feast. However, food falls like large whale carcasses and wood parcels are very localized islands in the huge deep-ocean. How do organisms find such islands and how are the populations connected? We proposed that connections would be higher along places with similar depths but not across isobaths, regardless the distance (i.e., hundreds of kilometers) (Figure 1). Our data, still under analysis, show surprisingly these islands may be highly connected even across depths. While some species may be isolated by depth, many others occur in very different conditions (1500 and 3300 m depth). What is more astonishing is that many of our species are shared with those from the NE Pacific Basin. These data are supported by morphological and molecular analyses.

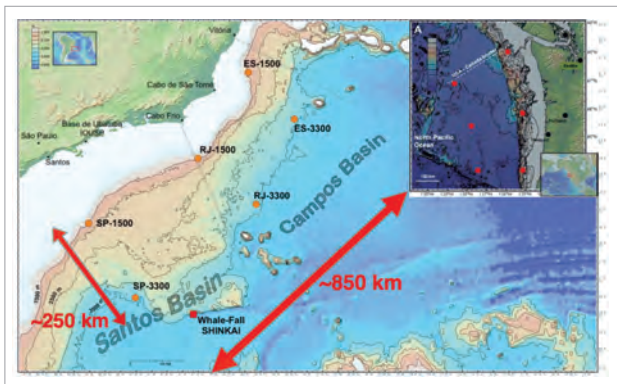


Figure 1. The large map shows the stations where the organic parcels were implanted along the SE Brazilian continental margin. The inset shows the same experiment carried out by our collaborators of the University of Hawaii and Auburn University on the continental margin off the coast of Oregon and Washington States, NE Pacific, USA.

Therefore, our data suggest that populations are highly connected by the probably common food falls. In addition, results found for the vent/seep endemic shrimp *Alvinocaris muricola* indicate that whale falls may serve as ecological stepping stones for the dispersal in reducing environments. *A. muricola* was found for the first time in whale bones in the present study, supporting the hypothesis that organic falls may have had an important role in the colonization and maintenance of hydrothermal vent and cold seep faunas.

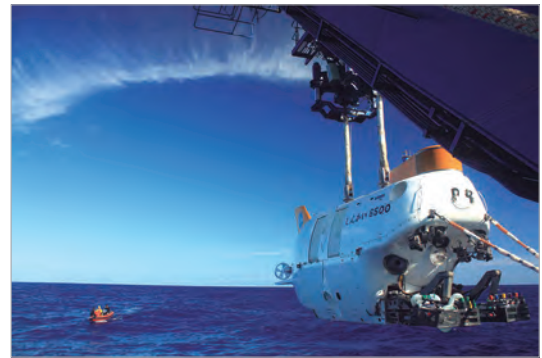


Figure 2. The deep diving research submersible Shinkai 6500 (JAMSTEC) prepares to dive in the deep SW Atlantic Ocean off the coast of Brazil. During one of the dives, a natural whale carcass was found lying at the bottom of the ocean and it has been used for comparison with the bone fauna implanted during the BioSuOr Project. (Photo: Paulo Sumida).

MAIN PUBLICATIONS

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USING ECOLOGICAL RESTORATION OF RIPARIAN FORESTS, DEGRADED FOREST FRAGMENTS AND ECONOMICALLY EXPLOITED NATURAL FORESTS TO SCIENTIFICALLY TEST THE PREMISES OF THE NEW BRAZILIAN FOREST CODE, BASED ON THE ECOLOGY OF REFERENCE ECOSYSTEMS

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Assistant researcher: Pedro Henrique Santin Brancalion

FAPESP Process 2013/50718-5 | Term: Feb 2014 to Jan 2019

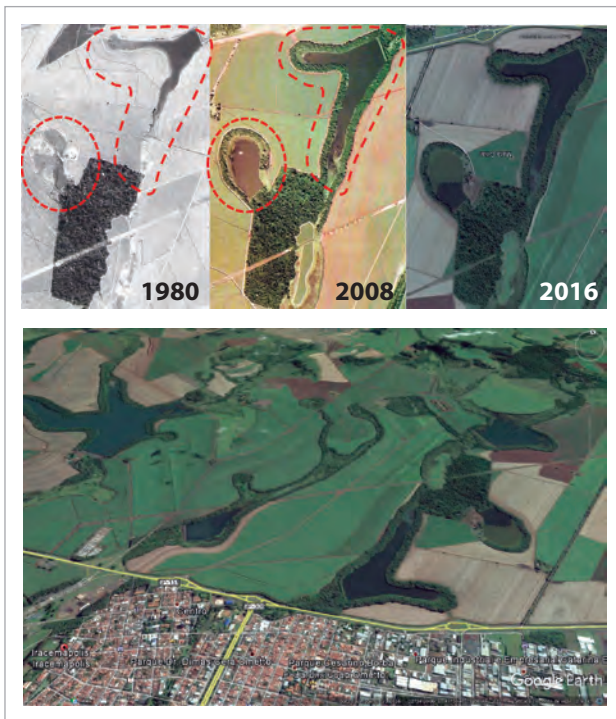


Figure 1. 2016 – Iracemápolis, SP: Forest restoration seen in the Regional context, including a water supply dam.

Ecological restoration has been considered one of the main strategies for combining biodiversity conservation and ecosystem services with agricultural production in Brazil. However, there are still many bottlenecks to be overcome. This research project is designed to investigate the challenges to restore forests in disturbed landscapes. The objectives are to test management practices on forest fragments in order to enhance their role in biodiversity conservation and to provide forest products and ecosystem services; to test methods for restoring riparian forests, aiming to meet the current demand for scientific support related to the recent changes in the Brazilian Forest Code; to test methods for restoring the Legal Reserve allied with the production of native timber and non-timber forest products; to conduct long-term monitoring of the vegetation in permanent plots deployed in reference ecosystems to different threatened biomes: the Atlantic Forest, the Cerrado and the Amazon, in order to understand forest dynamics and support restoration initiatives; and to test methods for evaluating and monitoring natural and restored areas to define efficient indicators of ecological sustainability and economic viability in order to produce consistent protocols. Altogether, our results may provide scientific support for economically viable tropical forest restoration initiatives. Furthermore, we expect to influence new and current public policies related to this issue.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We achieved important scientific advances related to the structure and diversity of forest fragments in agricultural landscapes and also to case-by-case management needs. We found that frequent disturbances and area history can lead fragments to have a different vegetation, which can result in higher levels of community divergence in forests and landscapes. As a result of the differentiation, secondary forests can reach a high level of beta diversity. Local management actions were implemented to enrich forests with threatened species, and landscape management actions were implemented in order to understand how forest cover, proximity to other fragments, size and shape can be related to the floristic data. Among the enrichment methods tested, seedling plantation resulted in higher survival rates compared to direct seeding. Through this type of approach, we aim to enhance the role of fragments in conserving regional flora diversity and in creating more diverse habitats for local fauna. Studies on forest structure, ecological processes and soil attributes in restored riparian forests in agricultural matrix are in progress. Restored riparian forests are growing well, except for some areas that had to be replanted due to a severe drought. Several forest inventories evaluated stem and wood quality, measured eco-physiological traits, assessed natural regeneration, harvested Eucalyptus and calculated economic returns. Now, the restoration areas move into the next stage, after the first rotation of Eucalyptus. We are testing these different methods to combine nature conservation and forest restoration with the production of native timber and non-timber products. Sampling is in progress in the Atlantic Forest, Cerrado and Amazonian domains using permanent plots in continuous and fragmented areas. Partial results suggest that adaptive management efforts are needed to assist long-term biodiversity persistence in highly fragmented agricultural landscapes. Frequent fires were the main factor affecting negatively biodiversity and our results also support the importance of soil gradient for maintaining species composition. Restoration monitoring covered several different ecological aspects, including fauna and vegetation structure and diversity and how they are connected; landscape analysis, as well as changes in soil and water during secondary succession. We collaborate with the "Pact for the Restoration of the Atlantic Forest" and our results have contributed to the design of an online system for monitoring. We generated practical knowledge on how young restoration forests are structured, which can already be applied on the ground. Restored forests play an important role together with forest remnants for nature conservation, even increasing biodiversity at the landscape level.

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BIODIVERSITY COMPONENTS, AND ITS METABOLIC CHARACTERS, OF BRAZILIAN ISLANDS – AN INTEGRATED APPROACH

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FAPESP Process 2013/40228-8 | Term: Nov 2014 to Oct 2019

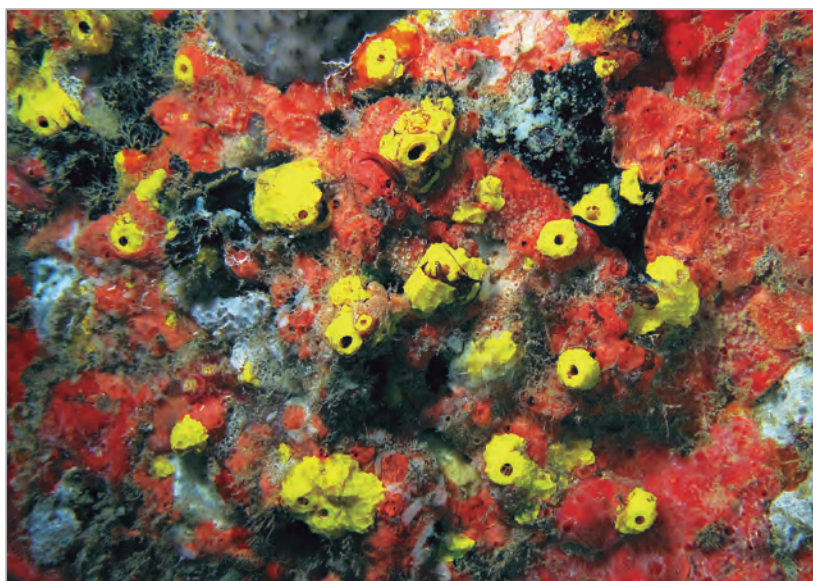


Figure 1 Sponges from Fernando de Noronha

Yellow sponge: *Aiolochoira crassa*

White sponge: *Dysidea sp*

Orange/pink sponge with irregular pores: *Hemimycale sp*

Orange/pink sponge with white channels: *Monanchora arbuscula*

(Photo by Dr. Eduardo Hajdu – Museu Nacional, UFRJ).

Islands represent about 5% of the Earth's land cover. The natural environment of islands very often present a variety of endemic species, many of which are particularly vulnerable. Therefore, the importance of biodiversity conservation of biological species present on islands is extreme. The marine biodiversity of the South Atlantic west coast is still essentially unknown. Anthropogenic impacts observed in the Brazilian coastal region are of increasing intensity. Coastal and oceanic Brazilian islands have submerged marine environments somewhat better preserved than the coastline ones. Taking into account

the diversity of species estimated for such islands, the study of its biodiversity is most necessary and should be conducted jointly with bioprospecting activities. This project aims to develop fauna surveys associated with studies of biodiversity, taxonomy, phylogeography, bioprospecting, microbiology and metagenomic analyses. Particular emphasis are directed to the survey and description of invertebrate species to be found in sites still poorly explored. Such strategy aims the isolation of "exotic" microbial strains using different approaches for growth in culture media, the isolation of novel bioactive metabolites using dereplication tools and refined analyses of chemical profiles (by UPLC-UV-MS/MS), as well as the investigation of specific cases of micro-organism/macro-organism interactions that may be related to metabolism expression of one or both organisms, using metagenomic techniques associated with metabolomics analyses of specific biological groups.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

During the two first years of the project, collections of invertebrates at Alcatrazes Archipelago, Fernando de Noronha Island, Abrolhos Archipelago and at the Amazon river delta revealed a very unique distribution of marine species, many of which are endemic. Invertebrates include mainly marine sponges, but also ascidians, bryozoans and nudibranchs. Local plants have also been collected and are under taxonomical investigation. Marine invertebrates and associated microorganisms are subjected to a survey towards the identification of secondary metabolites active against parasitic and cancer diseases. Several compounds, mainly alkaloids, have been isolated and identified, which presented potent anti-parasitic activity. Promising lead compounds are under development using total synthesis and pharmacological assays. Biological species description, biogeography and phylogeny analysis using molecular tools are also being extensively developed, aiming to understand the natural history of several invertebrate groups within Brazilian islands. Finally, a series of microbial-holobiont investigations using “omics” tools aim at to unveil ecological and functional relationship between these organisms, very often as a result of metabolic interchange and expression. Partial results of fish-microbial, sponge-microorganisms and plant-endophytes are very promising to reveal the uniqueness of symbiotic relationships between holobionts and their respective microbiota.



Figure 2. *Geomyces sp* fungus
Dra. Lara D. Sette Collection (UNESP Rio Claro)
(Photo by Dra. Raquel Peres Moraes-Urano).

MAIN PUBLICATIONS

Santos MFC et al. 2015. Anti-parasitic guanidine and pyrimidine alkaloids from the marine sponge *Monanchora arbuscula*. *Journal of Natural Products*. **78**: 1101-1112.

Newmister SA et al. 2016. OxaD: a versatile indolic nitrone synthase from the marine-derived fungus *Penicillium oxalicum* F30. *Journal of the American Chemical Society*. **138**: 11176-11184.

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NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY IN BIODIVERSITY AND NATURAL PRODUCTS – INCTBIONAT

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Chemistry of Department / Ceará Federal University (UFC)

FAPESP Process 2014/50926-0

Term: Jul 2017 to June 2023

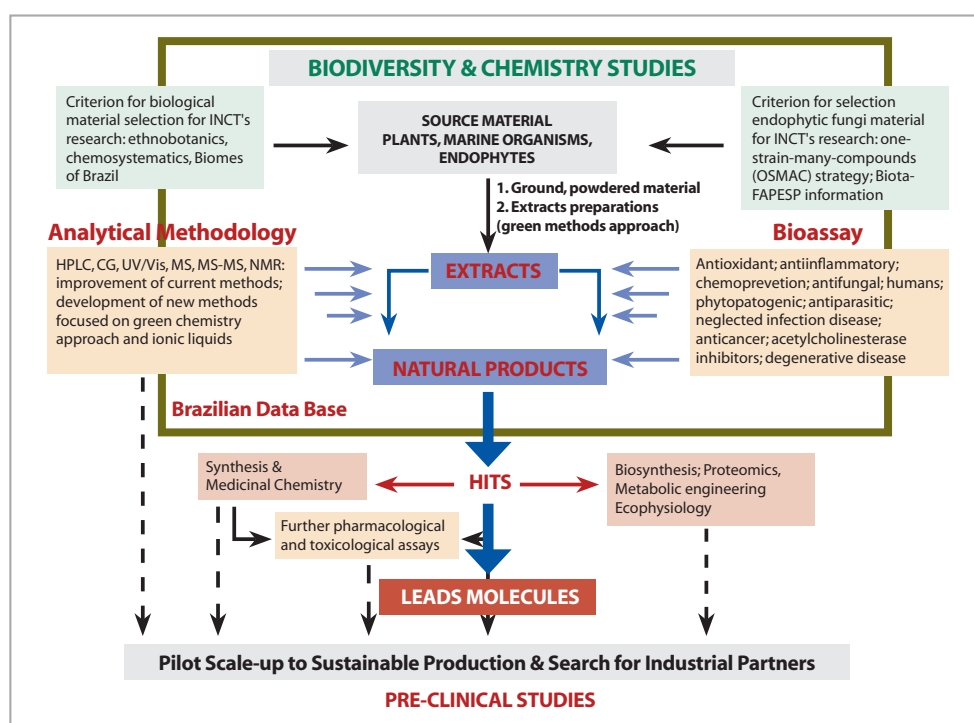


Figure 1. Experimental design of INCTBioNat

The INCT-BioNat is dedicated to the search of bioactive natural products from the fantastic chemical diversity synthesized by Brazilian biological resources. These compounds are important, as they communicate with their ecosystems, the physical environment and the living community, thus resulting in indispensable for the survival of the species in land and sea. Also, secondary metabolites are important supplies for the production of drugs, foods, cosmetics, fragrances, colorants, and agrochemicals, which support the vigorous bioeconomy in developed countries. The success of this initiative depends on most comprehensive collections of materials from different biomes, on the amount of identified natural products in plants, marine organisms and microorganisms, and on the optimization of bioassays and structural derivatization of the prototype compounds.

Taking into account the results we have achieved over nearly 15 years of research on biodiversity supported by BIOTA-FAPESP and CEPID-FAPESP CIBFar, our perspective in this national project is to optimize the searching methods to maximize the natural products-based leads. It is expected the compilation of a huge number of bioactive compounds with new structures for following pharmacological and toxicological assays, and further medicinal chemistry optimization. The state-of-art on natural products chemistry associated with modern analytical methodologies (HPLC-LC / MS, GC / MS, LC / NMR) and chemometric strategies are used by the research groups to map extracts and secondary metabolites from complex mixtures. Furthermore, metabolomic and dereplication tools will be applied for extracts, fractions and pure compounds screening in preliminary and intermediaries' bioassays.

The first database of natural products in Brazil was created on Nuclei of Bioassays, Ecophysiology and Biosynthesis of Natural Products Database (NuBBEDB webpage <http://nubbe.iq.unesp.br/portal/nubbedb.html>) with detailed information on all compounds already isolated by members of the NUBBE, IQAr-UNESP (For more information see Pilon et al., 2017. NuBBEDB: An updated database to uncover chemical and biological information from Brazilian biodiversity, Scientific Reports 7, 7215). The expansion this database is also a goal of this Institute. The organized information on biological, chemical and pharmacological data are vital for the complete understanding of our biodiversity, and so far, to contribute to public policies and to attract the interest of industrial partners.

This collaborative research institute embraces a team of national and international investigators aimed at to the development of scientific and technological research within the principles set out in public notice INCTs CNPq/FAPESP, and BIOTA-FAPESP, as an audacious national program. Facing this challenge and goal to expand the integration of national staff working on natural products chemistry, the INCT BioNat was designed to identify value-added bioproducts and to promote the dissemination of generated knowledge for the high schools and the public.

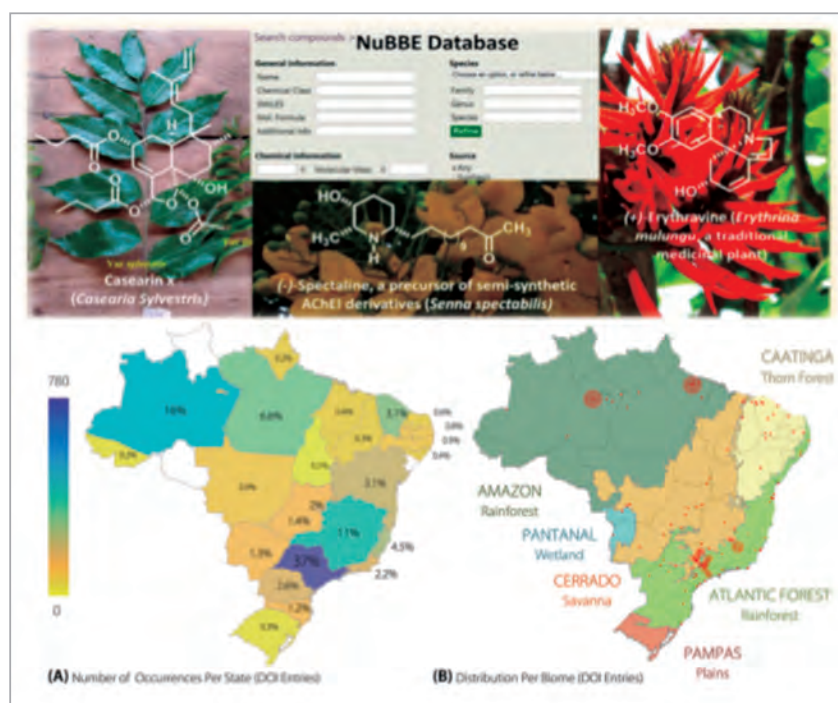


Figure 2. Representation of NuBBEDB and the distribution of compounds in Brazilian territory and its biomes (Pilon et al., 2017)

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