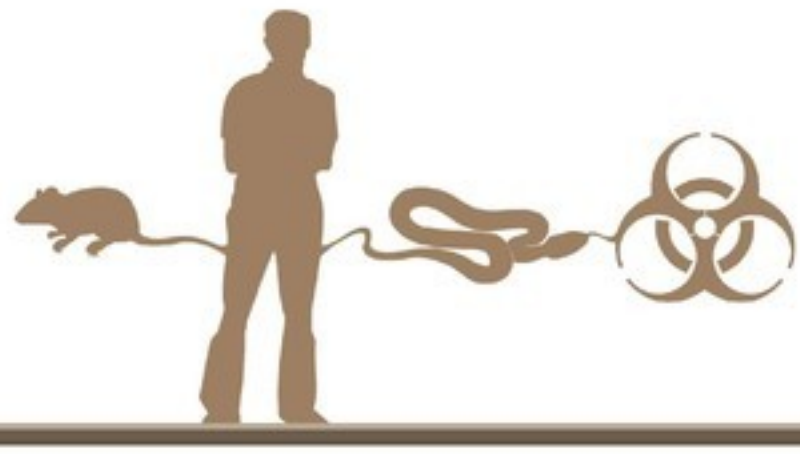
8. Animal Biology



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

8.01 Reproductive alterations caused by *Wolbachia* on its host *Culex quinquefasciatus*

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Introduction: *Wolbachia* are α-proteobacteria that was first reported in *Culex pipiens* mosquitoes early in the twentieth century. Heretofore, these endosymbionts have been detected in dozens of invertebrate species. However the effects of *Wolbachia* on their host reproduction have been increasingly investigated, given the extreme complexity of this interaction, new study cases are welcome to enhance its understanding. The present work addresses the influence of *Wolbachia* on *Cx. quinquefasciatus*, the cosmopolite member of that *Culex* complex that is responsible for diseases transmission. **Objetives:** To evaluate and compare physiological and reproductive differences between *Cx. quinquefasciatus* infected and uninfected by *Wolbachia*. **Methods:** Samples of a *Cx. quinquefasciatus* colony ($wPip^+$) originated from individuals naturally infected by *Wolbachia pipientis* B strain were cured with tetracycline, yielding a *Wolbachia*-free colony ($wPip^-$). Both the presence of bacteria and the efficiency of bacterial removal were checked by PCR of the *wsp* gene and ovary ultrastructure. Some fitness, physiological aspects, shape and size of the wing of mosquitoes from both colonies was measured. **Results and Discussion:** Reproductive aspects were also comparatively evaluated between colonies**.** No bias in the sex ratio was observed in any colony and reproductive fitness was higher among $wPip^-$ than $wPip^+$ females regarding the following parameters. Fertility: rafts/fed females ($p< 0.001$); fecundity: eggs/raft ($p< 0.001$), and viability: larvae/eggs ($p< 0.001$). Conversely, female longevity of $wPip^-$ was lower ($p < 0.001$) and its oviposition time during the first gonotrophic cycle was late ($p< 0.01$). Summarizing, although the infected mosquitoes have advantage on faster oviposition and higher longevity, they have lower reproductive fitness. Forty-eight hours after blood meal we observed, by electron microscopy, an early development of mosquito ovaries in $wPip^+$ females, resulting in increased amount of ovary total protein at the same time. Real-time PCR assays of the vitellogenin (Vg) and vitellogenin receptor (recVg) cDNA showed no difference in the amount of produced Vg and recVg mRNA during the vitellogenic process. After comparison of wing shape and wing size between mosquitoes from both colonies, no differences were detected.

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8.02 Development of an *in vitro* assay to facilitate the study of the bacteria-amphibian skin interaction

Barbosa FF[1], Neves AC[1], Antoniazzi MM[1], Jared C[1], Ferreira CM[2], Elias WP[3], Abe CM[1]
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Introduction: Currently, there are approximately 5,966 described species of anurans. However, factors such as climate change, diseases caused by microorganisms and the introduction of new species, have been increasingly contributing to the decline of global population of these vertebrates. The skin of these animals plays a crucial role in protection against the adversities of the environment. However, this moisturized skin also favors the growth of various microorganisms that are not always associated to the skin in a beneficial association. It is believed that many of these microorganisms associated with skin are opportunistic and potentially pathogenic, and that they contribute to the amphibian population decline. **Objectives:** The aim of this project is the development of an *in vitro* assay to facilitate the study of the bacteria-amphibian skin interaction. **Methods:** In this model, fragments of skin are collected from *Lithobates catesbeianus* immediately after animal's sacrifice. The fragments are maintained *in vitro* in tissue culture medium (DMEM), until being separately infected with different species of bacteria (2 strains each of *Citrobacter freundii*, *Escherichia coli* and *Klebsiella pneumoniae*) diluted in the same medium, and incubated for 6 hours at 37°C. Preparations were fixed according to the analysis method (histology and electron microscopy). The ability of bacteria adhere to the host tissue were also compared with adherence assays performed with HeLa cells (human epithelial cells) cultivated *in vitro*. **Results and Discussion:** Preliminary results showed that in general the ability of bacterial strains to adhere to amphibian tissue fragments was much weaker than the adherence observed on HeLa cells. This difference in adherence intensity in both systems suggests that the adherence assay on HeLa cells may not be a suitable model to study bacterial strains isolated from these animals. However, the comparative study between the two systems can be a very interesting tool to indicate differences in the mechanisms of adhesion of different microorganisms. Histological and electron microscopy analysis to observe possible alterations caused by bacterial infection is currently in progress. The development of this assay using tissue fragments of *Lithobates catesbeianus* (non-native species and commercialized for human consumption) in order to study the interaction of bacteria and amphibian skin may be an alternative for future studies, where interaction with various bacterial species can be studied with skin fragments obtained from a single animal instead of using one animal per every bacteria strain tested.

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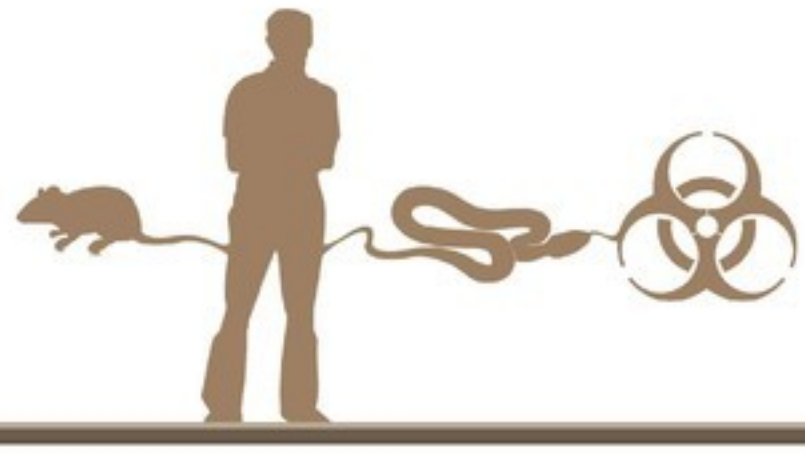

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8.03 Is temperature determinant for timing of spermatogenesis in pitvipers from Northeastern Brazil?

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Introduction: The timing of sperm production is influenced by environmental temperature in most snake species studied to date. Spermatogenesis occurs during the warmer seasons (spring and summer) in pitvipers from the temperate region, where seasons are well defined. On the other hand, climatic conditions are very complex in the neotropical region. A huge variation in temperature and rainfall patterns occurs between Brazilian regions. Temperatures are high throughout the year in Northeastern Brazil (NE) but rainfall is very scarce in some months, characterizing well defined dry and wet seasons. **Objectives:** The aim of this study was to characterize the male reproductive cycle of three species of pitvipers (*Crotalus durissus*, *Bothrops leucurus* and *Bothrops erythromelas*) from Northeastern Brazil,and discuss the influence of the temperature pattern of this area in timing of spermatogenesis in these species. **Methods:** Male reproductive cycles were described based on histological analysis of the testes and ductus deferens. An evaluation of some macroscopic parameters (testes volume and ductus deferens width) has also been made. **Results and Discussion:** Both *C. durissus* from the caatinga region, and *B. leucurus* from the Atlantic Forest exhibited an associated reproductive tactic (i.e. sperm production occurring synchronously to the mating season in autumn). Spermatogenesis may occur at any time of the year in NE as temperatures are maintained high throughout the year. An associated cycle may present some advantages because males do not need to spend energy maintaining the hypertrophy of the sexual segment of the kidney and/or storing sperm for long periods. Despite favorable temperatures to sperm production in any season of the year, *B. erythromelas* from the caatinga region exhibits a dissociated reproductive tatic with spermatogenesis occurring during summer (wet season) and mating in autumn (dry season). This reproductive pattern may be influenced by other environmental variables such as rainfall patterns which affect prey availability or by phylogenetic inertia. Data on reproduction of pitvipers from other Brazilian regions and *Bothrops lutzi* and *Lachesis muta* from NE Brazil may shed some light on this issue.

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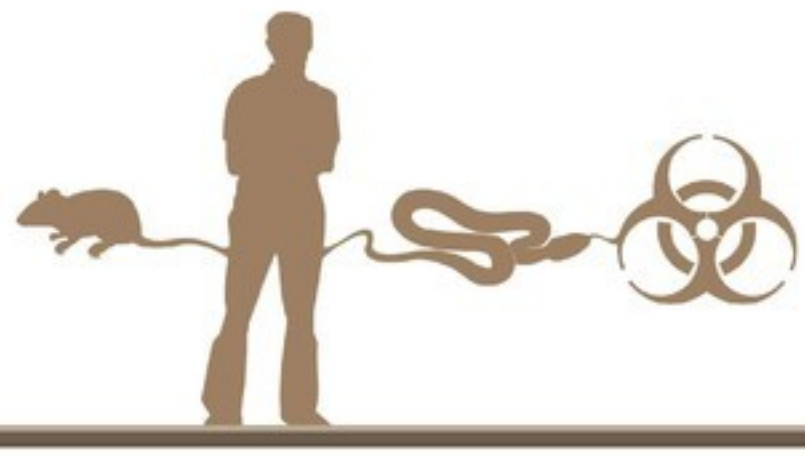

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8.04 Does cold climate hypothesis explain the evolution of viviparity in water snakes of the tribe Hydropsini?

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Introduction: Viviparity is a derived trait in Squamata. Cold climates (CC) from high latitudes and altitudes are frequently invoked as the selective pressure leading to viviparity. Such hypothesis predicts that (1) recent origins of viviparity are associated with high latitudes and altitudes (cold areas); (2) proportion of viviparous species is larger at higher latitudes and altitudes. **Objectives:** Here, we test these predictions within a phylogenetic context using water snakes of the tribe Hydropsini; a reproductively bimodal taxa widely distributed in South America. **Methods:** Specifically, we (i) reconstructed the evolution of reproductive modes using the available phylogeny for Hydropsini; (ii) analyzed associations between viviparity and high latitudes, altitudes and CC; (iii) compare proportions of viviparous species across latitudinal and altitudinal distribution. **Results and Discussion:** Reconstruction of reproductive modes suggests that oviparity is plesiomorphic in Hydropsini and viviparity evolved four times independently. Only one origin may have occurred in cold climates. Viviparity was not correlated with latitude, altitude and temperatures. Proportion of viviparous species is relatively constant over most of the latitudinal and altitudinal distribution but is larger only at southern limits of distribution. However, this fact may represent only differential survival in these areas and not that CC has played some role in the origin of viviparity. Thus, our results support weakly the CC model and pose the needs to consider other factors as selective agents for viviparity in Hydropsini. We suggest that viviparity may have evolved in Hydropsini as a response to egg mortality in nests due to flooding. Hydropsini snakes inhabit rivers and swampy areas and females lay eggs in crevices in the banks of these environments in the beginning of the rainy season. At this time, the level of the rivers is low but increases as rainy season proceeds. If these eggs require a long incubation period nests could be flooded before hatchings. By retaining eggs for longer periods, females oviposit with embryos at late stages. This would decrease the remaining incubation period and eggs would hatch before the river levels elevate and flood nests. Alternatively, the maternal manipulation hypothesis could also be applied to Hydropsini because gravid females could enhance fitness by maintaining optimal body temperatures for developing embryos than those available in nests.

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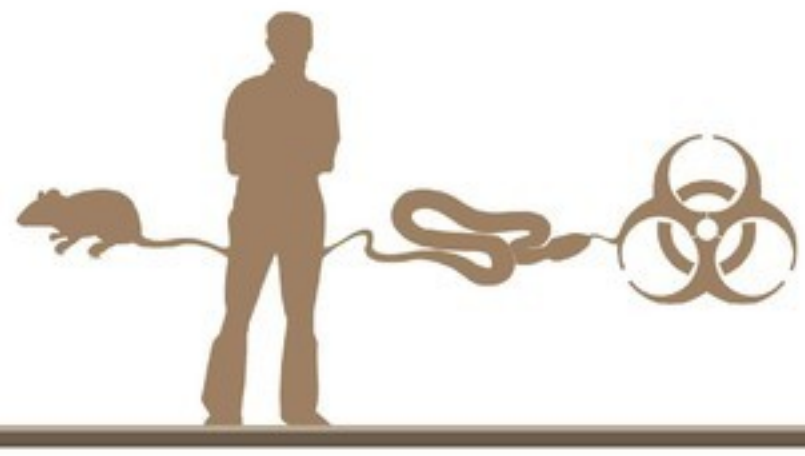

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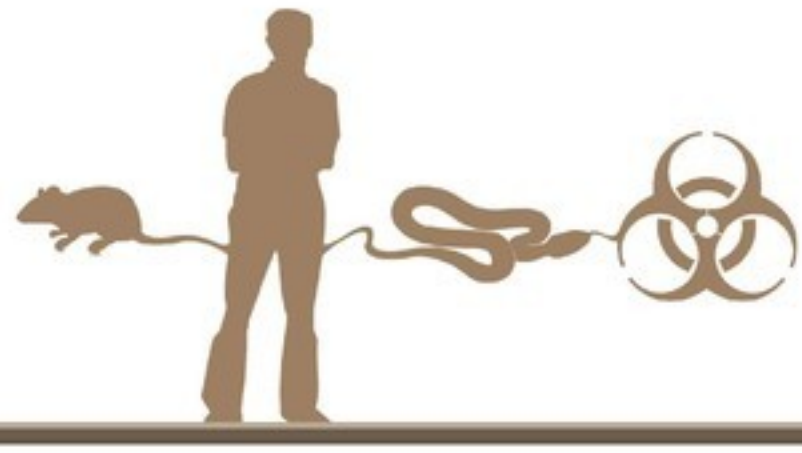
8.05 Observations on the feeding chelonian behavior (Reptilia, Testudines) and strategies in captivity in Outer Court House Vital Brazil, Herpetology Laboratory, Instituto Butantan

Calleffo MEV[1], Barbarini CC[2] Monteiro LPO[1], Giaretta FA[1,3]
[1]Laboratório de Herpetologia/IBu; [2]Núcleo de Difusão do Conhecimento/IBu; [3]Universidade Santo Amaro/UNISA

Introduction: In the Outer Court in Casa Vital Brazil, Laboratório de Herpetologia, are kept in captivity semi-extensive 58 specimens of chelonian obtained by Animals Reception, from donations with unknown origins. During routine handling, specimens are observed as for as strategies and feeding behavior in captivity. **Objectives:** To observe the feeding behavior and strategies of captive wild and exotic specimens. **Methods:** After screening, they were dewormed, micro chipped and sexed before entering the precincts. The precincts were designated by numbers in boxes PVC and terrariums, both with screens caps, plus a swimming pool. Subjects received individual markings designated by the initial letter of the genre over the control number, 2 *Phrynops geoffroanus* (tortoise of goatee), 4 *Mesoclemmys vanderhaegei* (headstrong tortoise), 3 *Hydromedusa tectifera* (long neck turtle), 1 *H. maximiliani* (snake-necked turtle) 2 *Chelidra serpentina* (snapping turtle) and 1 *Apalone ferox* (soft shell turtle). In the pool, 28 *Trachemys* (freshwater turtle) (*T. dorbignyi* and *T. scripta elegans*). Free in the yard, 16 *Chelonoidis carbonaria* (tortoise jabuti-piranga) and 1 *C. denticulata* (tortoise jabuti-tinga). Food is offered three times per week, with varied food items, fruits, vegetables, feed, newborn mice and arthropods (cockroaches, crickets and mealworms) preferably in the hottest period of the day. The temperature ranged from 9 to 28°C during the sample period, except in the pool heater with thermostat which fluctuated between 20 and 30°C. **Results and Discussion:** During feeding we realized that the eating behavior of the species occurs at different stages: foraging, recognition (visual and olfactory) approach, arrest, and disruption of food intake. In aquatic specimens, we observed that first the individual swim to the bottom of the enclosure, with slow movements, neck stretched and head close to the surface or substrate. The second step which is the visual recognition occurs after the location of food. Then, approaching with slow movements, if the food is still (ration, vegetables and fruits) or chasing food when prey is alive agile (arthropods; mice). The approximation can be made in or out of water. Then there is the smell recognition and if interested in the food item will be seized and swallowed, otherwise back to the first step. In the case of tortoises, are conditioned to the keeper and locations of feeders and drinkers, regardless of agile prey or property. Recognize, tear and ingest food. In this space we observed the strategies used by specimens and improvised props for that.

Supported by: Instituto e Fundação Butantan, FAPESP, COBASI



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8.06 Influence of diet on survival and venom extraction of the scorpion Tityus serrulatus (Scorpiones - Buthidae) in captivity

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[1]Laboratório de Artrópodes, [2]Laboratório de Imunopatologia, [3]Programa de Pós Graduação em Toxinologia, Instituto Butantan, Brasil

Introduction: The scorpion envenomation is a major public health problem in Brazil. It was notified around 52,000 accidents in 2010, with 88 deaths. The serumtherapy is recommended by Ministry of health as treatment of moderate and severe cases of scorpion envenomation. Instituto Butantan produces the antivenom serum with *Tityus serrulatus* venom which are kept in captivity. Thus, to maintain scorpions in good health for this purpose, a good diet utilizing insects maintained in captivity is necessary. **Objectives:** This study aims to evaluate the feeding preference of captive scorpions, as well as the mortality and amount of venom obtained from animals submitted to 3 different types of diet. **Methods:** During 1 year, 3 groups of scorpions (104 animals/cage) were fed with *Periplaneta americana* (domestic cockroach), *Phoetalia pallidus* or *Gryllus* sp. Food was provided to scorpions each 15 days in the ratio of 1:2 to cockroaches and 1:1 to crickets. After two days the remaining insects was removed and counted. Three venom extractions were performed during 1 year. **Results and Discussion:** In the group of *T. serrulatus* that received *P. pallidus*, the mortality was 27%, the insect leftover was 29% and the average amount of venom/scorpion was 2.11 $\pm$ 0.013 mg. The scorpions fed with *Gryllus* sp., the mortality was 32%, the insect leftover was 26% and the average amount of venom/scorpion was 1.90 $\pm$ 0.12 mg. The group that received *P. americana*, there was 40% of mortality during a year, the insects leftover was 45% and the average amount of venom/scorpion was 1.69 $\pm$ 0.13 mg. The group that received *P. americana* showed a higher mortality and a lower venom production comparing with the others groups during the study period. In the hot months (December to March) the diet was more accepted, ranging from 6 to 10% of leftover per month, according to the type of diet. On the other hand, in the winter there was 50% of leftover. Although the more common diet for scorpions in urban areas is the *P. americana*, the present study revealed a higher preference for *P. pallidus* and *Gryllus* sp. In conclusion that it is more productive to keep wild insects for captivity feeding, since the scorpions survive and produce more venom with this diet.

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8.07 Diversity of anurans in matrix of cane sugar in adjacent areas of Parque Estadual do Aguapeí, São Paulo, southeastern Brazil

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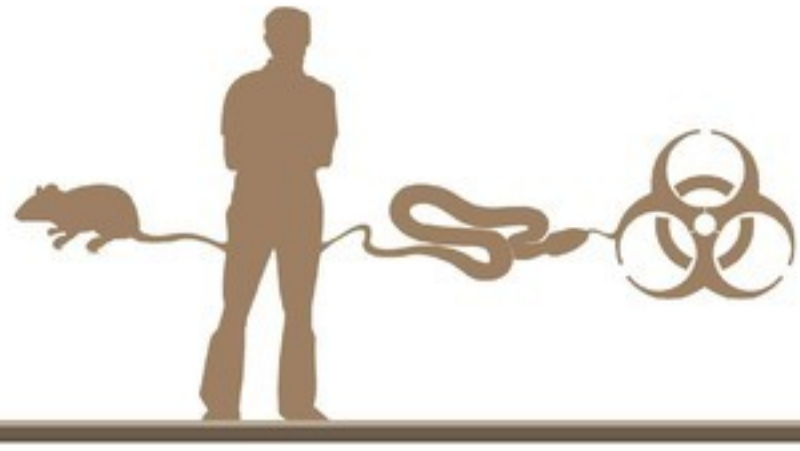
Introduction: São Paulo State has 28% of the Brazilian richness but this number can be overestimated. **Objectives:** This study aimed to inventory the anuran species of the around of "Parque Estadual do Aguapeí". **Methods:** The Parque Estadual do Aguapeí was established in 1998, has an area of 9.043,97 hectares and located between geographic coordinates S 21° 07' and 21° 17' and W 51°20' and 51°39'. The vegetation is characterized by tropical semideciduous forest, Brazilian Atlantic rainforest domain. Due the seasonal flooding in these regions, this environment is unique in the State, commonly called "Pantaninho Paulista". This study was realized in the Castilho, Junqueirópolis and Nova Independência municipalities, all studied areas are embedded in a matrix of cane sugar. Active methods, active searching and vocalization, were employed. **Results and Discussion:** Was recorded 27 anuran species belonging to 5 families: Bufonidae (*Rhinella schneideri*), Hylidae (*Dendropsophus minutus; D. nanus; Hypsiboas albopunctatus; H. lundii; H. raniceps; Phyllomedusa azurea; Pseudis platensis; Scinax fuscomarginatus; S. fuscovarius; S. similis; Trachycephalus venulosus*), Leiuperidae (*Eupemphix nattereri; Physalaemus centralis; P. cuvieri*; *P. marmoratus*; *Pseudopaludicola falcipes; P. sp.* (*aff falcipes I*); *P. mystacalis*), Leptodactylidae (*Leptodactylus chaquensis; L. furnarius; L. fuscus; L. latrans; L. mystaceus; L. podicipinus*), Microhylidae (*Dermatonotus muelleri; Elachistocleis bicolor*). The specie *P. azurea* was record in the Castilho municipality, and is the second record of this specie in the São Paulo State, the first occurred in the União Paulista municipality. The species *H. lundii* and *L. mustaceaus* was recorded just inside forest areas in a good condition of conservation, indicating, in this study, the dependence of these species in preserved environments. The high richness of species, when compared with others studies in the west paulista, and the presence of species habitat-dependent indicate a good state of conservation area, and highlight the importance of recent regional forest remnants in the region. The agricultural activities predominant in the studied cities are cattle raising and the cultivation of sugar cane. According to environmental public policies imposed on alcohol sector, the rescue of forest fragments, Legal Reserves and Permanent Preservation Areas enabled significant improvements in the landscape. Acknowledgments to: Rio Vermelho Açúcar and Álcool SA; Viralcool Açúcar and Álcool LTDA; Pedra Agroindustrial SA; Project-Projetos e Consultoria LTDA.



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8.08 Use of an acupuncture technique (moxibustion) as a supplementary therapy in the veterinary treatment of the snake *Bothrops jararaca*

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Introduction: Moxibustion is a technique included in the acupuncture methodology. It consists in burning herbs (usually *Artemisia vulgaris*) next to the skin, in acupuncture points. The claimed physiological effects of this treatment are: anti-inflammatory, immunologic stimulant, cicatrization and anti-oxidant. That therapy is believed to be a good complementary treatment in wild animals, as long it could be performed without touching the animal, which is safer considering venomous reptiles. **Objectives:** Investigating the results of the use of moxibustion as a supplementary therapy in reptiles. **Methods:** A snake *Bothrops jararaca* gave birth in February 2012. After giving birth, the animal has shown apathy, anorexia, lack of tongue flicking and dehydration for four months. During the three first months the animal was treated with fluid therapy, vitamins and forced feeding without showing signs of recovery. From May 2012 on, moxibustion was applied once a week, during 5 to 10 minutes, for two months. **Results and Discussion:** After the first week the animal showed an increase in tongue flicking and started moving inside its cage. After 6 weeks the animal captured its food without help and after 8 weeks it showed normorexia and returned to its normal condition. Moxibustion technique could be easily performed on the animal without stress or the need of contention. Although the results are not conclusive and more experiments are necessary to prove the benefits of the technique, these findings can suggest that moxibustion might be a good complementary therapy, somehow contributing to the traditional veterinary treatments.

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8.09 Evaluation of clinical parameters of a litter of *Boa constrictor constrictor* (Red tail boa), from the collection of the Museu Biológico of the Instituto Butantan

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[1]Anhembi Morumbi University, Veterinary Medicine, São Paulo, Brazil; [2]Museu Biológico, Instituto Butantan, São Paulo, Brazil

Introduction: Evaluation of the clinical parameters of an animal is important to determine his health state. The knowledge of which are the normal parameters is an essential tool to recognize the physiological changes undergone by an animal. **Objectives**: To evaluate the clinical parameters of a litter of *Boa constrictor constrictor* born and kept in the Museu Biológico of Instituto Butantan. **Methods:** Twelve *Boa constrictor constrictor* belonging to the same litter and with four years of age were kept in plastic boxes with corrugated cardboard, receiving water *ad libitum* and in an environment with controlled temperature between 24 -27°C and 60-70% of relative air humidity. The clinical exam followed a protocol: the animals were inspected visually and then restrained manually. During the visual inspection was conducted and determination of respiratory rate. In the manual inspection, the following parameters were verified: heart rate, muscle tone, condition of the mouth and vent, tongue flicking, presence of secretion in the nostrils, abnormalities in the eye membrane and appearance of the skin. External palpation was performed along the animal's body, together with a neurological examination for alertness, posture, poise and tongue flicking. Data collected was analyzed descriptively. **Results and Discussion:** Respiratory rate ranged from 48 to 68 movements per minute (mpm). Heart rate ranged from 40 to 48 beats per minute (bpm). In snakes, heart rate can vary from 22 to 136 bpm and these values are changed during the restraint. Good muscle tone was observed, with the snakes showing strength and mobility, without any abnormality. The coloration of the mouth and cloacal was pale pink, within the normal range. There weren't any abnormalities in the oral cavity, nostrils and eye membranes, nor in the skin, without presence of ectoparasites. The animals flicked the tongue continuously during the evaluation and were alert. On palpation, it was possible to notice the presence of normal fecal content. The neurological exam evidenced that the snakes were alert, darting and with the positive righting reflex. The results showed that the animals were in good health and with the clinical parameters within normal range.

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8.10 Behavior and feeding preference of the species *Trachemys scripta elegans* and *Trachemys dorbignyi* in the Outer Court House Vital Brazil, Herpetology Laboratory, Butantan Institute - São Paulo

Giaretta FA[1,3], Barbarini CC[2], Calleffo MEV[3]
[1]Universidade Santo Amaro/UNISA; [2]Núcleo de Difusão do Conhecimento/IBu; [3]Laboratório de Herpetologia/IBu

Introduction: Reptiles are the first group of vertebrates that have adapted to terrestrial environments. The dry skin, scales and shells resist moisture loss from the body and make life easier on dry surfaces. The order Testudines, which includes the turtles, tortoises and freshwater tortoises, can be found in different habitats and the impact has been suffering with human disturbance and environmental degradation. The behavior and diet are important factors in animal life, and has a key role in the adaptation of biological functions in captivity. **Objectives:** This study addressed changes in relation to behavior and feeding preference of the species *Trachemys scripta elegans*, *Trachemys dorbignyi* and in twenty-eight individuals of various ages and sex. One of the common characteristics of the genus *Trachemys* sp. is the adaptation of life in captivity these animals by imposing conditions very different from those found in their natural environments. **Methods:** The study was conducted in the Outer Court House Vital Brazil annexed the reception Snakes, Herpetology Laboratory, Butantan Institute. During the months of August 2011 and January 2012, the specimens underwent several procedures, biometrics (size and weight); individual external markings (to distinguish the specimens) and were separated into groups. From the screening and identification of specimens, food was offered individually three times a week with a varied diet consisting of small vertebrates (mice and neonatal mice of strain *Mus musculus*) and invertebrates (*Gryllus assimilis* (crickets), *Phoetalia pallida* (cheap) and *Tenebrio molitor* (larvae)), beyond the commercial type diets (for reptiles and dog). **Results and Discussion:** We realized that under the influence of captive management, specimens accepted and the diet offered, indicating that they are opportunistic and their diet varies with the condition, there was no preference for food items between sexes and ages, ie, all items offered were readily ingested. Regarding behavior, there were differences and inter-specific variation in physical activity (aggression, disputes, submission) and biological (weight in grams and growth - measures of the plastron and carapace) between pups, juveniles and adults. From the results, this study indicates that management techniques and changes in diet promoted better quality of life of captive specimens and it is necessary to continue the schedule of food supply for their effects in the long term.

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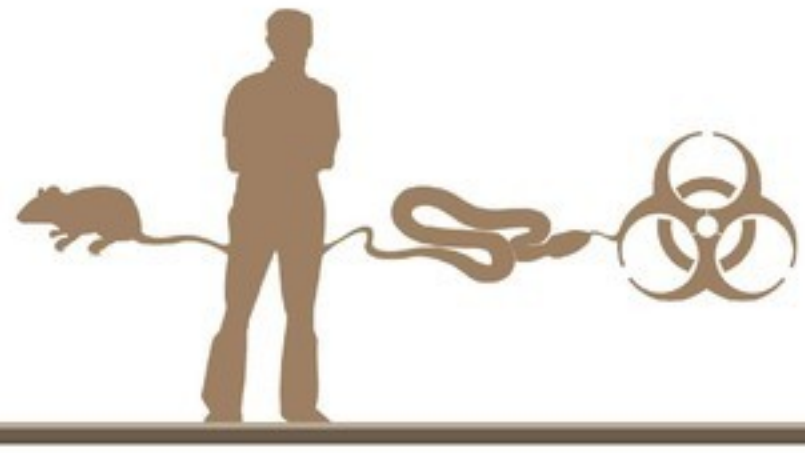

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8.11 Development of an efficient spray bath box for snakes

Grego KF, Stuginski DR, Monteiro LPO, Vidueiros JP, Fernandes W, Sant'Anna SS
Laboratório de Herpetologia - Instituto Butantan

Introduction: Snakes kept in captivity sometimes are prone to inadequate environment moisture which causes dehydration or fungal and bacterial infections of the skin. One symptom of dehydration is abnormal shedding (dysecdysis), while moisture excess can predispose the animal to a wide variety of fungi and bacteria that have been recorded as the etiologic agents found to cause superficial cutaneous infections in snakes. In some cases, the microorganisms invade tissue that has already sustained some physical or chemical injury. The symptomatic treatment to dysecdysis and the curative treatment to skin infections is to immerse the snake in tepid water with an appropriate diluted medicine. The disadvantages of using immersion bath are: 1) As the snake floats, its back does not come into contact with the water and 2) weak animals can drown and should not be left unattended while soaking. The development of a more efficient method to bath snake was urgent. **Objectives:** improve the method of bathing snakes, making it more efficient and secure for the animals. **Methods:** A spraying bath was developed using a transparent plastic box with 564 x 385 x 371 mm. On the lid of the box was attached a hose of 2" with several micro holes along its length. The end of the hose attached to the lid was sealed and the other end attached to an aquarium powerhead pump (mod. PH 2000). A stainless steel support with the height of 17 cm was put inside the box to support a perforated plastic tray where the snake remained during the bath. The warm water together with the diluted medication was placed in the box in an amount sufficient to completely submerge the pump but not exceed the stainless steel support. **Results and Discussion**: Ten Viperidae (*Bothrops* and *Crotalus*) snakes with mild fungal skin lesions were put in the spray bath box with antifungal medication during four different times (30 minutes, 60 minutes, 120 minutes and 240 minutes). All snakes recovered from skin lesions and showed no discomfort during the bath. The new system allowed direct contact of the whole snake's body (ventral and dorsal part) with the drug diluted in the water and there was no danger of drowning the snake. The pump maintained the water warm until the end of the spray bath. Even weaker snakes could benefit from this method, since there was no accumulation of water in the tray where the snake remained.

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05 a 07 de Dezembro 2012, São Paulo, SP

8.12 Life-history variation in island populations of the *Bothrops jararaca* complex (Serpentes, Viperidae)

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Introduction: Island species often show different life-history traits compared to their mainland relatives. Body size variation is certainly one of the most commonly documented. In snakes, body size influences important reproductive parameters as sexual maturity and fecundity. Hence, variation in body size also implies variation in several aspects of life history. **Objectives:** Here, we investigate body size variation and its influence on sexual maturity and litter size in island populations of the *Bothrops jararaca* complex (*B. jararaca*, *B. alcatraz* and *B. insularis*). **Methods:** Data for adult body size, litter size and body size at maturity were obtained from 321 preserved specimens from four island populations: *B. jararaca* from Búzios Island (BI) and Ilhabela (II), *B. alcatraz* from Alcatrazes Island (AI) and *B. insularis* from Queimada Grande Island (QI) and compared with a mainland population of their closest relative: *B. jararaca* from São Paulo city (SP). **Results and Discussion:** Female body size differed among populations. *B. jararaca* females (SP, BI and II) did not differ in mean body size, however they were larger than *B. insularis* (QI) and *B. alcatraz* (AI). Adult *B. insularis* were larger than *B. alcatraz*. Litter size was influenced by maternal body size and differed among populations. Litter size did not differ among SP, II and BI populations but it was higher than the island populations from QI (mean = 7.9) and AI (mean = 2.7). Litter size in *B. alcatraz* was smaller than other populations. This interpopulational variation in litter size is likely a result of the variation in the maternal body size. After removing body size effects litter size did not vary among populations. Mean adult body also size influenced body size at maturity. Minimum body size at maturity increases as mean adult body size increases: mean adult body size explains 80% of the variation of body size at maturity. In general, females attain sexual maturity with body size between 69% (II) and 95% (BI) of the mean adult body size. Body size at sexual maturity varied among populations. *B. jararaca* females from SP and II and *B. insularis* females (QI) attain sexual maturity with similar body sizes (relative to mean body sizes) (mean = 74%). *B. alcatraz* female mature at 81% of the mean body sizes and *B. jararaca* (Búzios) at 95%. Thus, *B. alcatraz* and *B. jararaca* (BI) seem adopt the strategy of delaying maturity in relation to *B. insularis* and *B. jararaca* from SP and II. Such late maturity provides attain relatively large body sizes at maturity.

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8.13 The skin of amphibians and the habit of exposing in the sun: the case of the tree frog *Bokermannohyla alvarengai*, from Serra do Cipó (MG)

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Introduction: *Bokermannohyla alvarengai* Bokermann (1956) is an anuran species with a geographical distribution restricted to the Cadeia do Espinhaço, from Serra do Caraça (MG) to the south of Bahia. It is an endemic species inhabiting stony fields, a phytogeographic landscape typical of the Serra do Cipó. *B. alvarengai* occurs in altitudes above 1000 m, and is commonly found on the surface of rocks. The tree frog is characterized by a large and robust body, a short and truncated snout, a thick forearm and a developed spine in the prepolex. The most striking aspect of its biology is the behavior of basking: the animal stays immobilized exposed on a rock throughout the day, receiving the sunlight, with rock temperatures around 40°C. Basking is a quite common behavior among several species of lizards but very rare among amphibians. Draws attention the bright whitish color that the skin acquires during basking, which is immediately lost when the animal is removed from the sunlight. **Objectives**: This work aims at the integrative study of the skin morphology and of basking behavior in *B. alvarengai*, in light of the natural history of the species. **Methods**: six specimens were collected, three sacrificed during basking with bright color, and three sacrificed with dark skin, with the bodies not exposed to the sunlight. Samples were taken from dorsal, mental, pectoral and inguinal skin. The skin samples were fixed in Karnowsky fixative and processed for histology and ultrastructure. **Results and Discussion**: Preliminary data indicate that the skin morphology of *B. alvarengai* shows peculiar characteristics, both qualitative and quantitative, that were related to the basking behavior and the stony and hilly environment were the tree frog lives. Draws attention the large amount of mucous glands and iridocytes, pigment cells that are probably associated with the reflection of sunlight during basking. The number of poison glands is very low, indicating that the animal must have low toxicity. Another conspicuous fact is the great blood vascular net in the skin including many projections into the epidermis that reach very close to the skin surface. This extensive blood capillary net may promote efficient body warming during basking, keeping sufficient heat inside the organism to be used in nocturnal activities. During the night, up in the mountains, temperature drops abruptly, reaching values around 8°C. In addition, the calcified dermal layer, related to protection against water loss, is thick and occurs only in the dorsal region directly exposed to sunlight.

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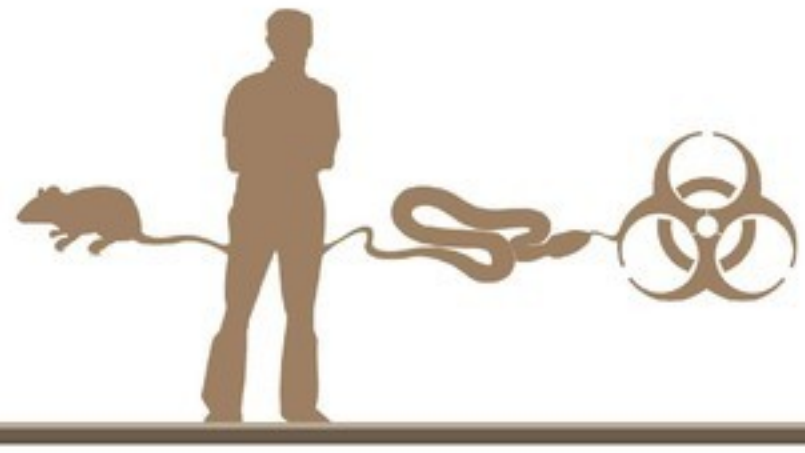

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8.14 Wing geometry of five Neotropical species of *Culex*

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Introduction: Species of the *Culex* genus have high epidemiological importance because of their ability to transmit to man and animals etiological agents. In spite of their epidemiological relevance, species of this genus has been little studied. Many species of *Culex* are morphologically similar in the adult form, which causes problems regarding its identification and thus the control efficacy of these vectors. **Objectives:** Owing the taxonomical similarity among females of *Culex* species, the aim of this study was to comparatively analyze female individuals of five species of *Culex* using geometric morphometrics. **Methods:** Five species: *Culex corniger* (n=27), *Culex quinquefasciatus* (n=16), *Culex nigripalpus* (n=15), *Culex coronator* (n=25) and *Culex sacchetae* (n=16) was analyzed. Eighteen wing landmarks (vein crosses) were digitized and coordinate data assembles into matrices. Data were then submitted to Procrustes superimposition and other standard Geometric morphometric procedures. **Results and Discussion:** Discriminant analysis of principal components of shape showed wing differentiation among the five species. Morphospace of the first two cannonical variables originated from the pairwise comparison among the five taxa showed that *Cx. quinquefasciatus, Cx. nigripalpus* and *Cx. coronator* slightly overlapped. The neighbor-joining phenogram of Procrustes distances resulted in a tree with three main branches as follows: (*Cx. sacchettae* ((*Cx. corniger*, *Cx. nigripalpus*)(*Cx. quinquefasciatus*, *Cx. coronator*))). This result was not consistent with the current phylogenetic hypothesis of *Culex* genus based on mouthparts. This apparent contradiction between phylogeny and wing phenetics might be due to natural selection acting on wing shape. Although geometry failed to reproduce phylogenetic history, discriminant power of geometric data was strong and allowed to differentiate among the species. The highest Mahalanobis distance was observed between *Cx. sacchettae* and *Cx. quinquefasciatus* (9.51) whereas the lowest was between *Cx. quinquefasciatus* and *Cx. nigripalpus* (5.45). Scores of validated reclassification of individuals ranged from 100% between *Cx. quinquefasciatus* and *Cx. sacchettae* to 62.5% between and *Cx. coronator* and *Cx. sacchettae*. Metric disparities of shape were statistically significant in all pairwise comparisons, according to nonparametrical permutation tests. Thus, wing geometry has proven to be a useful tool to diagnose some *Culex* species from wing characters of females and should be applicable to other vector species of family Culicidae.

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8.15 *Anopheles* (*Kerteszia*) *cruzii*: rapid microevolution and altitudinal preference

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Introduction: In Brazil, the autochthonous transmission of extra-Amazonian malaria occurs mainly in areas of the Southeastern coastal Atlantic Forest, where *An. cruzii* is a primary vector. Although it inhabits a wide altitudinal range (between 5 and 263 meters over the sea level), these anophelines have differential ecological affinities to the microenvironments of the Atlantic Forest. We hypothesised that gene flow is not evenly distributed across that altitudinal transect containing diverse microenvironments. Considering that humans are sympatric with this species and may also exhibit altitudinal preferences, estimating the dispersion of mosquitoes across the altitudinal axis may help to understand the spreading of malaria. **Objectives:** This study aimed to evaluate populational similarity of *An. cruzii* between two altitudinal microenvironments in Atlantic Forest: the "plain" (between 5 - 20 meters) and the "hill" (between 81 -263 meters). The two samples were compared regarding the genetic and morphological markers. **Methods:** Mosquito larvae were sampled from hill and plain at municipality of Cananeia (State of São Paulo, Brazil) in two moments: years 2009 and 2012. For morphological characterization, right wings of males and females were photographed and submitted to standard geometric morphometrics. As the genetic marker, a 400bp DNA-fragment of the mitochondrial cytochrome oxidase gene was PCR-amplified and sequenced. **Results and Discussion:** Wing shapes were significantly distinct between populations from hill and plain. Validated reclassification tests classified males from hill with 82% and from plain with 94% accuracies. Among females, accuracy scores were 92% (hill) and 100% (plain). Mitochondrial gene was highly polymorphic (haplotypic diversity = 0.98) and altitudinally-stratified. Only one haplotypes (out of 33) was shared between hill and plain populations. Taken together, results points to a low gene flow between plain and hill, which indicates subpopulations with distinct altitudinal preferences. Morphological and genetic data were consistent, which reinforces our interpretation. Additionally, morphological comparisons involving the two chronological samples showed clear wing differentiation occurred between 2009 and 2012. Wing geometric morphometrics is enough sensitive to detect microevolutionary changes in a period as short as three years. Whether those morphological changes across the years are due to genetic drift, migration or selective pressure derived from environmental changes is yet to be investigated.

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8.16 Wing morphological variation in *Aedes aegypti* in one year

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Introduction: *Aedes aegypti* (Diptera: Culicidae) is the most important vector of dengue viruses in Brazil. As the vaccine against dengue is still in development, the only way of preventing the disease is to control the vector. Currently, the methods of control have different efficiencies and vector microevolution is a limiting factor. **Objectives:** In this study, we evaluated during one year, the morphological variation in wing size and shape of *Ae. aegypti*, in the Butantã neighborhood of São Paulo city. **Methods:** The mosquitoes were collected in five periods: fall/2011, winter/2011, spring/2011, summer/2011-12 and fall/2012. Wing size and shape were assessed through geometric morphometrics. Female wings were mounted between a slide and a coverslip and scanned under stereomicroscope. Eighteen wing landmarks (vein crosses) were digitized and Cartesian coordinates data assembled into matrices. **Results and Discussion:** We verified conspicuous morphological wing variability whithin the examined period. Mahalanobis distances among the five seasonal samples were significant ($p<0.0001$) when compared pairwise. Therefore, there was differentiation in the wing shape in periods as short as three months. These variations in wing shape occurred possibly by quantitative allelic variation. On the other hand, wing size was significantly different only between winter and summer and between spring and summer. Data from the literature showed that at lower temperatures than the wing size tends to increase. However, there was no difference in size between winter and spring, possibly due to the influence of other environmental factors. Hitherto, similar surveys concerning Culicidae only had been conducted with individuals kept in captivity. Altogether, our results indicate that genetic variations may produce morphological differentiation in a short period of time and can be detected in wing geometry. In our interpretation, wing shape was confirmed as a powerful indicator of microevolution in *Aedes aegypti*.

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8.17 Frequency of non-venomous snakes (Anomalepididae, Colubridae and Dipsadidae) received at Instituto Butantan from the state of São Paulo and their use in research

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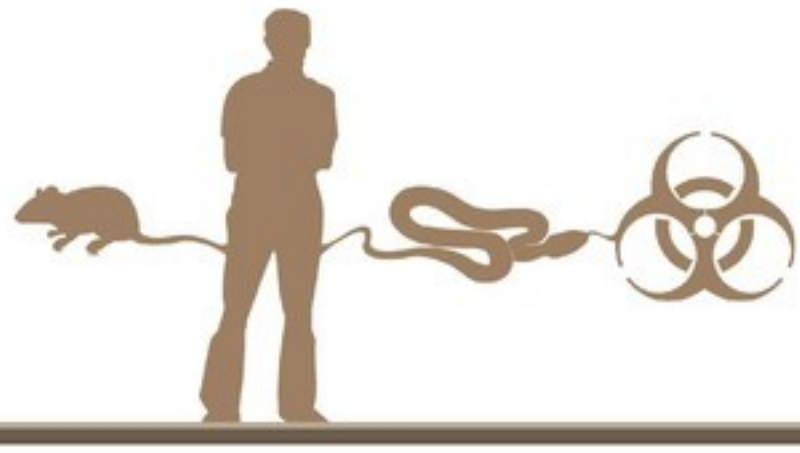
Introduction: Instituto Butantan (IB) receives daily many snakes captured and donated by the community. These snakes are referred to different Laboratories of IB, with different purposes. All research projects using snakes submit the Animal Ethics Committee Certificate to the Laboratório de Herpetologia before withdrawing the animals. **Objectives:** Present biological and ecological data about the species inhabiting São Paulo, their biodiversity, abundance and the influence of seasonality in the encounter of these animals. **Methods:** From September/2012 to June/2012, 2849 specimens of Anomalepididae, Colubridae and Dipsadidae snakes from the state of São Paulo were classified as soon as they arrived at IB and the records analyzed according to frequency of arrival and origin. **Results and Discussion:** In the period of study 2849 non venomous snakes from the state of Sao Paulo were received at IB, representing 28 genus and 61 species. The five most frequent species were *Oxyrhopus guibei, Sibynomorphus mikani, Tomodon dorsatus, Liophis miliaris* and *Philodryas patagoniensis*, which together represented 66.4% (N = 1893) from the total of snakes received. Eighteen species were considered less frequent, representing 1.1% of the snakes received. Anthropization is a factor that may affect the diversity and abundance of species. The seasonal activity of snakes can be influenced by abiotic factors such as temperature and rainfall and biotic factors such as reproductive season and prey availability. Considering the season in which the animals were captured in 2011, summer had the highest number of encounters, 35.8% (568 individuals), followed by fall with 25.2% of the total meetings (399 individuals). The number of genus and species was also higher during summer and autumn months. In respect to the period and the families analyzed, IB received snakes coming from 184 counties. Most snakes came from São Paulo, capital (N = 665), followed by Cotia (N = 233), Itu (N = 158), Barueri (N = 131) and São Bernardo do Campo (N = 129). Snakes from other states were not analyzed in the actual study (138 individuals). Besides the important data obtained, the snakes received were used in research projects at IB different laboratories (N = 96 snakes) and also at USP (N = 67), were used to feed ophiophagous snakes at the Laboratory of Herpetology (N = 134) and at the Biological Museum of IB (N = 1201), were submitted for public display at the MIB (N = 56) and at São Paulo Zoological Park Foundation (N = 27), and were also deposited in IB Herpetological Collection (N = 1211).



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8.18 Chemical defense in amphibians: the eccentric case of the Amazon toad *Rhaebo guttatus*, which is able to voluntarily squirt poison from parotoid macroglands

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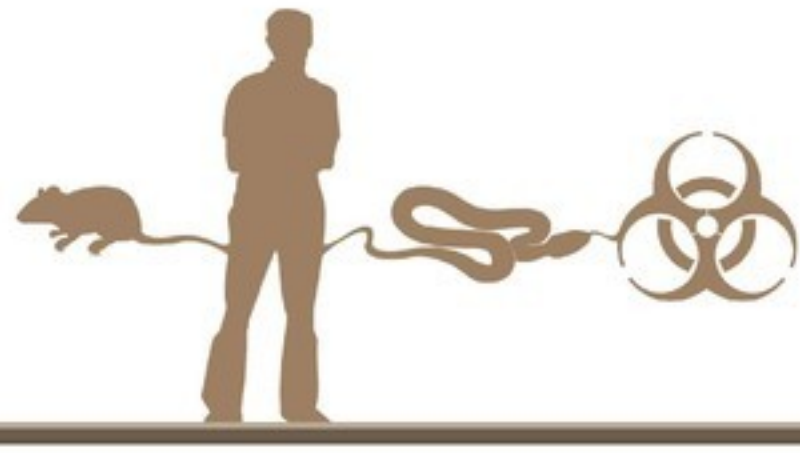
Introduction: Animals have two basic types of chemical defense: active defense, in which toxins are injected into the aggressor, and passive defense, in which the aggressor causes its own poisoning when biting a prey. Amphibians have typical passive defense, possessing skin poison glands scattered throughout the body. In some groups these glands accumulate forming macroglands, such as the parotoids of toads. Surprisingly, the toad *Rhaebo guttatus* (*Rg*) contradicts amphibian defense, and is able to squirt jets of poison towards the aggressor. **Objectives:** To study the morphology and toxinology of the *Rg* chemical defense system, comparing it with *Rhinella marina* (*Rm*) a sympatric species showing typical toad passive defense. **Methods:** *Rg* and *Rm* were observed and collected in Pará and Amazonas. The parotoids of *Rg* and *Rm* were processed for histology. Their venom was characterized by SDS-PAGE, HPLC and mass spectrometry. Tests of lethality, edema and nociception were conducted using a murine model. **Results and Discussion:** When in danger, *Rg* presents stereotyped behaviors: it lowers one of the shoulders, inflates the lungs, and with eyes and mouth closed, directs the parotoids to the aggressor, shooting jets of poison towards its face. Anatomical analysis showed that only in *Rg*, the scapula is adhered to the parotoid and may play a key role in poison ejection, taking active part in parotoid compression and poison ejection. In the two species each parotoid secretory unit is connected to the outside through a duct obstructed by an epithelial plug. In *Rg*, the plugs emerge from wide pores on the parotoid surface, contrasting with the pores of Rm, which show the form of a narrow slit. The ability of *Rg* to squirt poison must be directly related to the series of stereotyped defensive behaviors, together with the morphological characteristics of the parotoid. The poisons of *Rg* and *Rm* are basically composed of proteins, biogenic amines and steroids, but with characteristic compounds in each species. Compared to *Rm*, the poison of *Rg* is less lethal, induces edema and provokes a four-time more intense nociception. The poison of *Rg* must act "positively" on the learning of predators that feel the effects of poisoning but have little chance of dying. The analysis by the side of folklore, indicates that the eccentric defense of *Rg* can mistakenly justify the popular myth that all toads are dangerous and squirt poison into people's eyes.

Supported by: CAPES, CNPq, Fundação Butantan, Secretaria da Saúde (PAP), FAPESP and INCTTox.



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8.19 Male reproductive cycle of the false-coral snake *Oxyrhopus guibei*

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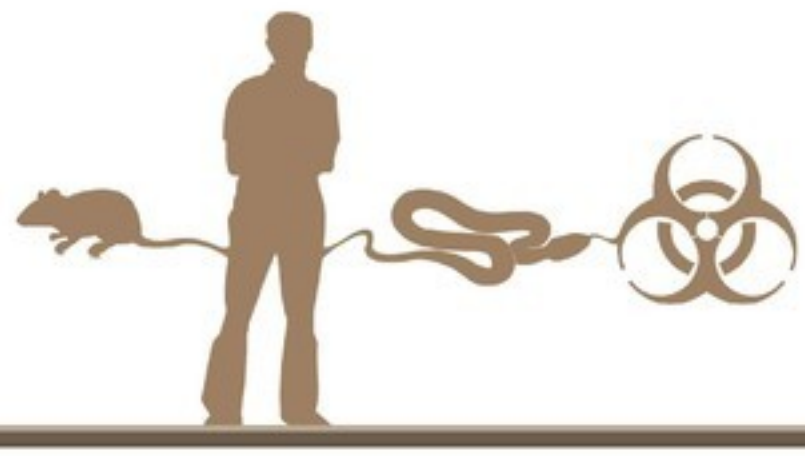
Introduction: Studies on reproductive cycles of neotropical snakes are still insufficient and the available data in the literature do not represent the majority of data existing specimens. Furthermore, these data are inadequate since they only address reproductive biology simply based on morphological measurements. Recent studies have shown that the reproductive morphology alone does not represent the actual reproductive status of the studied animal, it is necessary to join other techniques such as histology to really know what is happening seasonally with the animal. The false coral *Oxyrhopus guibei*, is a non-venomous snake belonging to the tribe Pseudoboinii. It is recognized as one of the most common snake species in the city and the state of São Paulo. Studies on reproductive biology based solely on morphological measurements showed that males of this species have a continuous reproductive cycle. **Objectives:** This study aims to evaluate the reproductive cycle of *Oxyrhopus guibei* males by gonadal histological analysis. **Methods:** 27 specimens of adult males *Oxyrhopus guibei* (> than 388 mm) of São Paulo deposited in the Herpetological Collection of the Instituto Butantan (IBSP) and the Museum of Zoology at Unicamp (ZUEC) were analyzed. The following data were taken: snout vent length (SVL), tail length, length, width and thickness of the right and left testes, width of the ductus deferens. In addition, samples were collected from the testes, ductus deferens and sexual segment of the kidney for histological analysis. For statistical and histological analysis, the individuals were evaluated according to the seasons (spring, summer, autumn and winter). Analysis of Covariance (ANCOVA) test was used in the program Statistic 7 to verify possible differences between the seasons and the testicular volume and diameter of the ductus deferens. Result were considered significant when $p > 0,5$. The tissue fragments were analyzed by light microscopy and stained with hematoxylin and eosin. **Results and Discussion:** Testes volume and ductus deferens diameter did not show any significant differences among the seasons. Analysis of preliminary data indicates that recrudescence may occur (increased testicular activity) at different periods. Neither of the specimens examined showed complete regression of testicular activity. We observed a spermiogenesis peak in specimens during the summer and autumn, but it not was possible to determine a seasonal pattern, because spermiogenic activity was observed in other seasons. Detailed analyzes integrating the cycle of the sexual segment of the kidney will be held for the final characterization of the male reproductive cycle.



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8.20 First record of the occurrence of *Lonomia descimoni* Walker (Lepidoptera, Insecta) in Belterra, Pará, Brazil

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Introduction: The genus *Lonomia* Walker (Saturniidae, Hemileucinae) includes eleven species that inhabit the Neotropical region, two of which are recorded from the Brazilian Amazon: *Lonomia achelous diabolus* Draudt and *Lonomia descimoni* Lemiare. Nevertheless, the record for the latter species has no definite locality. Among the eleven species, two of them (*Lonomia obliqua* Walker and *Lonomia achelous* Cramer) are responsible for causing Hemorrhagic Syndrome in humans after contact with the bristles of the caterpillars. Little is known about the other nine species, especially concerning their biology, morphology and biochemical properties of the poisons. Most published studies deal with systematics and are based mainly on morphological features of adults (moths). **Objectives:** Record for the first time the presence of *Lonomia descimoni* in Belterra, Pará. **Methods:** In April 2010, entomological samplings were carried out in the Mata do Butantan (S02 ° 37'21 .9" W054 ° 56'09 .5") in Belterra, during the activities of the project Butantan Amazônia. Light traps of the "Luiz de Queiroz" model were used during the night for one week, from 22h to 5am the next morning. After sorting the collected material the presence of a male of the family Saturniidae was detected. At the Laboratório Especial de Coleções Zoológicas of the Instituto Butantan, it was identified, by means of the morphological study of the genitalia, as *Lonomia descimoni*, one of the nine poorly known species. The specimen was included in the Entomological Collection of the Instituto Butantan under n°. 975. **Results and Discussion:** The importance of this capture lies in the fact that this is the first record with a defined locality for the occurrence of *L. descimoni* in the Brazilian Amazon. This species differs from other congeners, especially in the lack of sharp harpes in the uncus of the male genitalia. New samplings are scheduled in order to capture specimens of both sexes, especially inseminated females, to obtain caterpillars.

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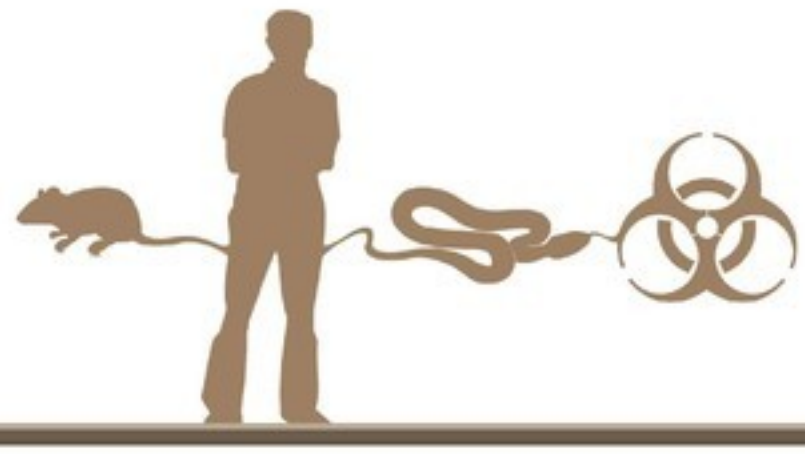

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8.21 Characterization of three populations of *Aedes scapularis*, using morphological and genetic markers

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Introduction: *Aedes scapularis* is a mosquito of family Culicidae (Diptera) with vectorial capacity for "rocio", "melão", "ilhéus", "Venezuelan encephalitis" viruses and filarias as well. Despite their importance to public health this species is poorly studied regarding variability and population structure. There is suspicion that the nominal species actually constitutes a species complex. Considering that the pathogen / vector / host interaction is generally specific, it is urgent to elucidate this taxonomic-microevolutionary point. **Objectives:** Our main objective was to investigate the genetic and morphological differentiation among three populations of *Ae. scapularis* and if the difference is indicative of possible species of complexes. **Methods:** Population samples of *Ae. scapularis* were collected at the municipalities of Tremembé (TRE, State of Sao Paulo), São Paulo (SP, São Paulo State) and Itaboraí (ITA, State of Rio de Janeiro). As biological markers, we used mitochondrial gene cytochrome oxidase subunit-I (COI) and wing geometry. These markers are traditionally recognized by the discriminating power in similar studies. **Results and Discussion:** Populations ITA, TRE and SP showed form distinct wing, suggesting low gene flow between them. It was also observed sexual dimorphism in the isometric size, wing shape and in the degree of population differentiation. The population of ITA presented smaller centroids than other populations studied. It was found an extensive genetic polymorphism since we detected 69 COI haplotypes. Among the few haplotypes observed, only 8 are shared by populations. Taken together, genetic and morphological data suggest low gene flow between the studied populations *Ae. scapularis*.

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05 a 07 de Dezembro 2012, São Paulo, SP

8.22 New records of the ocurrence of *Ornithodoros fonsecai* and *Ornithodoros mimon* (Acari: Argasidae) in Brazil

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Introduction: Brazilian studies of argaside ticks were intensified in the past 10 years, with 6 new species described, being 3 of the genus Antricola, 2 the genus Ornithodoros and 1 of the genus Nothoaspis. These researches demonstrates that the Brazilian fauna of ticks from that family is underestimated, and can be much larger than what known, currently 19 species. The genus *Ornithodoros* is represented in Brazil by 15 species, being 4 of them endemic. **Objectives:** To report new records of two species of Ornithodoros recently collected in new areas, *O. fonsecai* Labruna & Venzal, 2009 and *O. mimon* Kohls, Jones & Clifford, 1969. **Methods:** The first specie was found in the Blue Lagoon cave, in the municipality of Nobres, MT, in December of 2011. The specimens were sent to IBU (3 females and 5 nymphs) and, after identification, they were listed in the IBSP collection. The other one, *O. mimon*, was identified in two different locations, infesting the lining of the houses from Natal, RN, (1 male and 1 female) and Jaboticabal, SP (3 males, 2 females and 1 nymph). Considering that the nymphs and the adults are stages that do not permit a secure identification based only on morphology, the species reported here were prepared for DNA extraction and the sequences obtained were compared with those deposited in Genbank. **Results and Discussion:** Both species are associated with bats. The species *O. fonsecai*, was known only from specimens collected on the cave walls of São Miguel, municipality of Bonito, MS. The species *O. mimon*, in turn, has wider distribution, occurring in Bolivia, Argentina, Uruguay and Brazil. The previous records of this species are the Brazilian municipalities of Araraquara and Rio Claro, both in the State of São Paulo. The specimens of the *O. fonsecai* from Nobres were listed in the Instituto Butantan (IBSP 10571) collection. So was the male of *O. mimon* from Natal (IBSP 10569) and the male and female from Jaboticabal (IBSP 10570).

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8.23 Armed spiders *Phoneutria nigriventer* (Araneae: Ctenidae): description of anti-predator behavior

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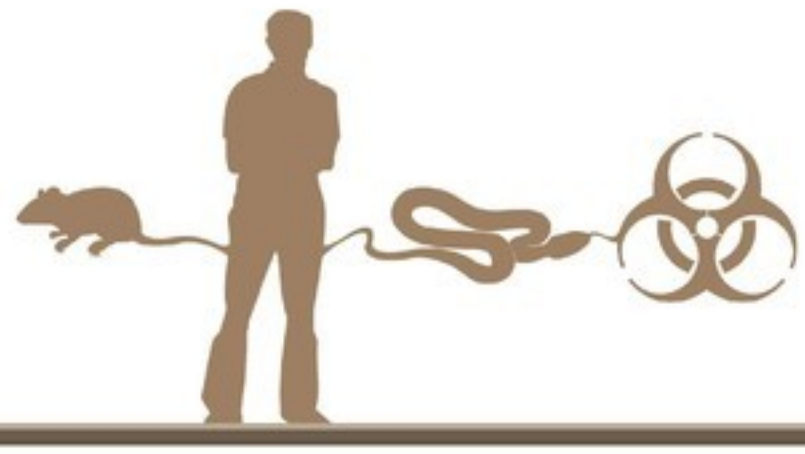
Introduction: *Phoneutria nigriventer* (Keyserling, 1891) are aggressive spiders and causes serious accidents. They have active venom in human being so they are considered spiders of health interesting in Brazil. These spiders are called armed spiders because when feel endangered they raise theirs anterior appendages off substratum, elevating the cephalothorax. This behavior becomes more aggressive when these spiders jump on the stimulus. Normally the accidents happen when the people manipulate firewood, construct or refuse materials around or into their houses. In spite of this behavior has been known and quoted, its topology has not been described yet in literature. **Objectives:** The aim of this work was described the anti-predator behavior of *P. nigriventer* under two aversive stimulus. For this, we follow up the methodology proposed for Blatchford, Walker e Marshal (2011) to study Mygalomorphae spiders. **Methods:** The first stimulus (n=20), considered less invasive was a puff test when the air was blown onto the dorsal surface of the spider. The second (n=18), considered more invasive was a prod test that consisted in touches to the dorsal body with a pipette. **Results and Discussion:** Spiders raise their anterior appendages more often in second stimulus than the first and they take longer armed in this treatment too. Furthermore, spiders run away from the puff test than the prod. These results suggest that when the spider is touched on it fells more disturbed as if it were encounter the "predator". This didn't happen when it receive the puff of air because probable it could have the chance to run out of this situation. The armed behavior was very similar between the treatments but the others behavior before or after that showed be different, like jumps and bites. This work was the first to describe this behavior as well known in the literature.



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8.24 Diversity representativeness of Brazilian snake fauna in the collection Alphonse Richard Hoge

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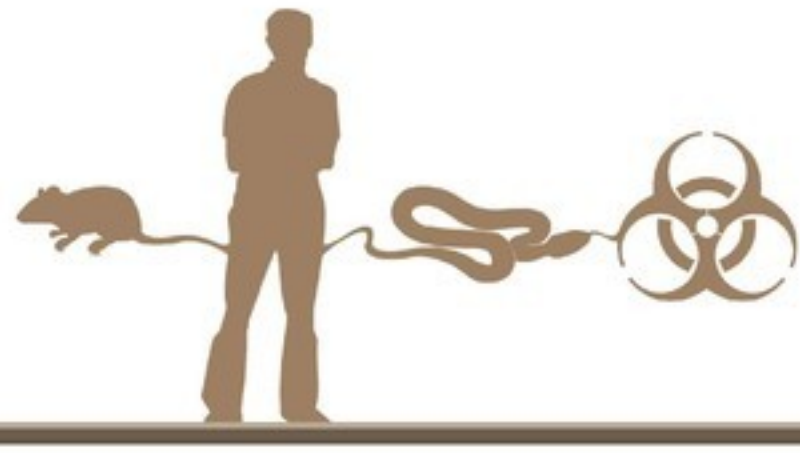
Introduction: Museum collections are very useful for evaluating occurrence and distribution of taxa, as a tool in comparative biology and more recently to infer species richness. The herpetological collection Alphonse Richard Hoge (ARH), Instituto Butantan (IBUT), Brazil, has been a reference on Neotropical snake fauna studies, mainly in public health. **Objectives:** In this study we analyzed the material recorded in the ARH collection collected in Brazil over the last hundred years in order to check temporal diversity of snakes acquired comparatively to capture effort employed. **Methods:** Register books from Lab. Herpetologia were surveyed, collectors of specimens were consulted, since fire May 2010 destroyed the asset. **Results and Discussion:** We listed about 71000 specimens recorded from all over the world. From that approximately 60000 captured in the Brazilian territory. We accounted 21088 venomous representatives (34.2%) namely 13208 *Bothrops* within 24 recognized species, 3314 *Crotalus* within 5 recognized subspecies, 300 *Lachesis muta*, 4 *Porthidium hyoprora*, 4262 Elapidae and 40584 non venomous snakes (65.8%). Among *Bothrops* the species *B. jararaca* (n=2803), *B. leucurus* (n=1584), *B. moojeni* (n=1533) and *B. neuwiedi* (n=1337) were the commonest whereas *B. alcatraz* and *B. pirajai* were the rarest. Regarding non venomous snakes the collection ARH had specimens from the families Boidae, Aniliidae, Tropidophiidae, Leptotyphlopidae, Typhlopidae, Anomalepididae and Colubridae. Among them the most abundant genera recorded were *Boa* (n=1540), *Chironius* (n=3184), *Liophis* (n=6612), *Oxyrhopus* (n=2533), *Philodryas* (n=3609), *Sibynomorphus* (n= 2271) and *Thamnodynastes* (n=2201) whereas the rarest (n<10) were *Typhlophis*, *Boiruna*, *Calamodontophis*, *Dendrophidion*, *Drepanoides*, *Lioheterophis*, *Rhinobothrium*, *Taeniophallus* and *Xenopholis*. Field work, scientific expeditions and donations represented the main source of specimens during decades of first half of XX century concerning venomous and non venomous snakes (about 25%). During the second half, however, captures resulting from snake fauna rescues increased significantly the number and representativeness of the collection, since 75% of remaining snakes were then incorporated, especially in 1980s. These data are corroborated by observations of total recruitment of snakes at Instituto Butantan over the last hundred years in which individual donations have diminished including the number of snakes per donation. Snake fauna rescues carried out in Tucurui, Pará and Porto Primavera, SP were the most contributory for the collection ARH.

Supported by: CNPq



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8.25 Cross-species transfer of microsatellite markers in the Neotropical pitvipers *Bothrops moojeni* and *B. marajoensis* (Viperidae, Crotalinae): Insights on snake reproduction

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Introduction: The reproductive events recorded within *Bothrops* (sensu lato) have been observed in several lineages of Central and Northern American representatives, such as *B. asper* and *Porthidium yucatanicum* respectively. This widespread occurrence of such characters indicates the existence of a pattern for the entire group similar to the situation observed in some temperate vipers and pitvipers. Autumnal uncoupled mating and delayed fertilization with LTSS (Long Term Sperm Storage) through UMT (Uterine Muscular Twisting), polyandry and even parthenogenesis seem to be very conservative traits, probably retained from the ancestor. Lately, the discussion and differentiation between litters resulting from LTSS and parthenogenesis have become more frequent and complex, mainly after several records of births, including multiple ones, of surely virgin mothers. Genetic investigation is fundamental to test for such novelties, however, molecular markers potentially useful to address these questions in these species, such as microsatellite, are scarce in literature. **Objectives:** Thus, In this study we tested some primers developed for *Bothrops insularis* in an attempt to perform cross amplification in *Bothrops atrox* snake complex, particularly *B. moojeni* and individual from Marajó island, Pará, Brazil, assigned as *Bothrops marajoensis*. **Methods:** From about 30 *loci* identified, we selected seven of those assigned as: 52.17; 52.22; 52.7; 52.8; 60.3; 60.6; 60.9, to test in three individuals from Rinópolis, State of São Paulo, Brazil and other three from Marajó Island. Total DNA was obtained from liver tissue kept in 80% ethanol and blood samples collected in EDTA and kept in SDS/Tris solution using DNeasy Blood & Tissue - Kiagen. Once PCR was optimized using Taq polymerase Platinum, those primers that successful amplified were used to genetic characterization in 23 *B. moojeni* from northwestern São Paulo and six *B. marajoensis*. **Results and Discussion:** All seven loci tested produced amplification product in both taxa investigated. In *B. moojeni* the Hardy–Weinberg expectation and gametic disequilibrium were tested using Genepop 4.1. The same way, genetic identity and paternity exclusion probabilities were estimated for each polymorphic locus and overall loci using Identity 1.0. From seven loci tested, one (60.6) was monomorphic, whereas the remaining ones showed moderate polymorphism levels (from 2 to 17 alleles per locus). One of these loci (52.8) was in Hardy-Weinberg disequilibrium and no of them was in genotypic linkage disequilibrium. This battery of loci also showed high paternity exclusion probability (0.968) and low probability of genetic identity ($8.16x10^{-5}$), proving to be helpful for multiple paternity and parthenogenesis tests in these species.

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8.26 Malformations in recently caught pregnant *Crotalus durissus* newborns (Serpentes,Viperidae)

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Introduction: Organogenesis, the most complex stage in embryonic development, is particularly sensitive to exogenous agents, being therefore an important period for the occurrence of malformations, which may lead to newborn or premature death due to predation and/or diseases. Malformations have been described in humans, domestical and laboratory animals, but congenital defects are less reported in free ranging wildlife. **Objectives:** This study describes the frequency and type of newborn malformations occurring in rattlesnakes at the Butantan Institute (IB), Brazil. **Methods:** Free-ranging pregnant specimens of *Crotalus durissus* delivered to IB were kept in captivity until the birth of their offspring. Newborns were weighed, measured and anatomically evaluated for the presence of malformations. After natural death or euthanasia, RX images were done and complete necropsy was performed. **Results and Discussion:** From 2008 to 2012, 36 rattlesnakes gave birth to 341 snakes. Congenital anomaly was observed in 43 (12.6%) newborns. Spine anomalies (kyphosis, lordosis and scoliosis) were the most common (65.1%) followed by fusion of the ventral scales (11.6%) and tail tip wound (11.6%). Fissure in the ventral midline was found in four snakes (9.3%), as well as eye malformations (microphtalmia or buphothamos). Prognathism were observed in 4.7% of the animals. Bicephaly, a common anomaly found in literature, were not seen in this sample. Internal organs were little affected and most alterations were related to liver (7%), heart (2.3%) and kidney (2.3%). Several of these malformations are incompatible with wildlife, reducing the fitness of the rattlesnakes. Complementary studies are necessary to properly understand the etiology and significance of these malformations.

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8.27 Structure-function relationship of the spines of *Echinometra lucunter* sea urchin: a toxinology approach

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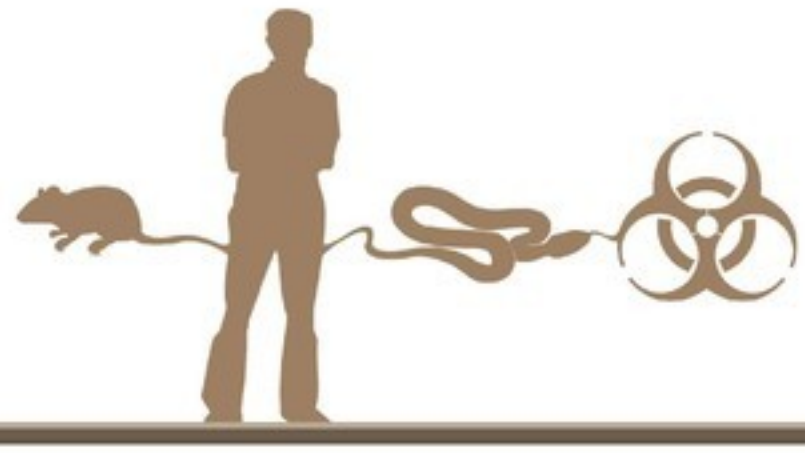
Introduction: *Echinometra lucunter* is a common Brazilian sea urchin, responsible for 50% of marine animal accidents; however, little is known on its spines' structures or toxins. **Objectives:** Prepare a spine aqueous extract from *E. lucunter* and evaluate its pro-inflammatory and enzymatic activities, and correlate them to the spine morphology and ultrastructure. **Methods:** The aqueous extracts were obtained by submerging the spines in 100 mM NH_4COOH (pH 7.4), 4°C. This extract was fractionated and assessed for inflammatory activity, on mice microcirculation leucocytes, as well as for proteolytic activity, over synthetic substrates. Moreover, the spines were analyzed by light and scanning electron microscopy. **Results and Discussion:** The spine extract was able to induce inflammation, being one purified molecule (600.34 Da) positively related to this activity. Moreover, a peptidase present in the extract was able to cleave Z-R-MCA and Abz-GIVRAK(Dnp)-OH following pre-incubation with DTT, which was inhibited by E-64. The pH-dependence of these hydrolyses and the cleavage proportion indicate the presence of both mono and dicarboxypeptidase activities. Immunochemistry and Western-blot assays were positive for the anti-cathepsin B antibody. Spines histological analyses indicate the presence of granulous cells, which stained positively with bromophenol blue, throughout the spine, but more concentrated at the tip. These cells are located within the calcified matrix, which is longitudinally disposed and radiates outwards, as observed by SEM. This observation clearly indicates the presence of a secretory structure, probably the source of bioactive molecules and toxins in this animal. The complex structure contains several cells, including dense granular cells with developed Golgi apparatus, as well as other cellular populations in which developing spiculae could be observed. Once this structure seems to reach the exterior of the spine through regularly disposed longitudinal fenestrations, it was classified as a secretory structure. The presence of, at least, one inflammatory molecule in the spine certainly leads to complications in the accident that, associated to a secreted proteolytic enzyme, surpass the purely traumatic response raised by the mechanical spines punctuation of the skin. The analyses of the kinetic parameters for the cleavage of the FRET substrates, combined with the enzymatic and immunochemical results, point out to the presence one Cathepsin B/X in the spine that, besides its toxic effect, would aid in remodeling the calcified matrix at the fragile spine tip.

Supported by: FAPESP, CNPq, INCTTox



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8.28 Seminal evaluation of in the golden lancehead (*Bothrops insularis*) under captive conditions

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Introduction: The assisted reproduction plays a very important role in conservation of endangered species since the most captive populations are not self-sustaining, particularly in the case of snakes. Analysis of semen quality constitutes a powerful tool to evaluate the potential fertility of males in threatened species and could provide solutions to wildlife conservation and contribute with useful data for research on economically important or endangered species, providing remarkable contributions from the areas of assisted reproduction. The golden lancehead (*Bothrops insularis*), a critically endangered species, is a pitviper endemic to Queimada Grande Island in southeastern Brazil. **Objectives:** This study aimed to characterize semen parameters of the golden lancehead (*Bothrops insularis*), including appearance, volume, concentration and sperm motility; and determine if these parameters varied with body size, condition or age. **Methods:** Single semen samples were collected from 12 adult golden lanceheads, including 5 males born in captive, by the same investigator during the mating season of this species, in autumn (June). After antiseptic cleansing of the skin near the cloaca, the snakes were injected subcutaneously with a dose of 15 mg/kg of 1% solution of lidocaine around the cloaca. Semen was collected with ventral massages directly from genital papilla inside the cloaca, after relaxation of this region, using to graduated micropipette. Seminal volume, motility, and appearance were assessed immediately after collection. Motility was expressed as the percentage of motile sperm. Sperm concentration was assessed after dilution of 1 μL of semen to 499 μL of 10% buffered formalin solution. The concentration was counted using a hematocytometer chamber (improved Neubauer, 0.10mm depth). **Results and Discussion:** The appeareance of the semen of the golden lancehead was generally cloudy and viscous, and the color was white. Spermatozoa exhibited a median motility of 50% and the seminal volume was too small (median 2μL), lower than in other studies on the snakes *Elaphe guttata* and *Crotallus durissus terrificus*. However, the concentration of spermatozoa in the ejaculate was high, with an average of 0.9×10^9 spermatozoa/mL, compared to *Elaphe guttata.*

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8.29 Reproduction of captive Brazilian insular snakes

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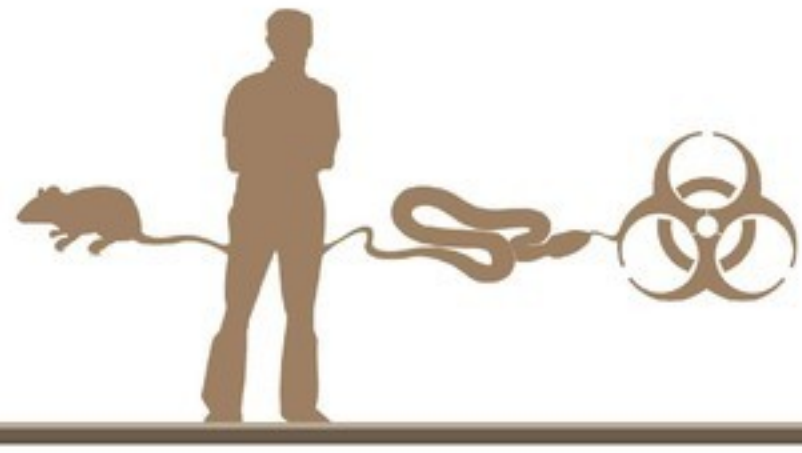
Introduction: Insular habitats are important for evolutionary studies, and the geographical reclusion favors endemic species. Two islands off the coast of SP are known and important players in the Brazilian ecological scene: Queimada Grande, where we find *Bothrops insularis* and Alcatrazes, where *Bothrops alcatraz* is found. Due to the ever increasing habitat destruction both venomous species are threatened with extinction. Also a new insular species, *B. jararaca aff.*, which is still in the description phase, and inhabits the Moela Island in the coast of SP, is being studied. **Objectives:** To study the reproduction (courtship, mating and litter size) of these snakes in order to develop methods to contribute in *"ex situ"* conservation projects and venom extraction for research. **Methods:** *B. alcatraz*, *B. insularis* and *B. jararaca aff.* kept in captivity. **Results and Discussion:** One male and five females of *B. alcatraz* have been kept for about 10 years with zero mortality rates. In captivity these snakes accepted rodents as food. Four snakes were born (in March) from a female already pregnant when collected. In captivity, four mating have been observed, however no female has completed conception. But one new mating was observed in 2012, and the female can be pregnant. In captivity *B. insularis* also feed on rodents, gerbils, hamsters and newly hatched chicks. One female and one male of *B. insularis*, born in the Herpetology are now 14 and 12 years old respectively, produced three litter in captivity. From a mating registered in 2008, four snakes were born in March 2009. From the matings recordeds in 2009 and 2010, eight snakes were born in February 2010, and nine snakes were born in March 2011, respectively. In 2012 other mating was observed. Of the new species, *B. jararaca aff.*, two males and three females are kept and they have already begun to accept small rodents as food. Four snakes were born (in March) from a female already pregnant when collected. Four mating in captivity was also observed. In two occasion (2011) the females no completed conception, but two new mating was registered in 2012. In the three insular species, the courtship and mating was observed in autumn and winter. The courtship behavior was similar in between species however the duration and the time copulating varied greatly. The success of the reproduction in captivity is very important for the conservation, furthering the studies related to the behavior, courtship and reproduction. Researchers are seeking new raw materials in order to conduct pharmacological studies and the search for venom is ever increasing. Endemic species, threatened by extinction that live inside protected areas cannot be removed, therefore the success of these endeavors depend on the reproduction of these species in captivity.



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8.30 Microevolution in *Aedes albopictus*

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Introduction: Although native to the tropical and subtropical areas of Southeast Asia, *Aedes albopictus* is now found on five continents, primarily due to its great capacity to adapt to different environments. This species is an epidemiologically important vector for the transmission of many viral pathogens, such as dengue fever, yellow fever virus, West Nile virus and St. Louis encephalitis virus. The efficacy of control procedures for *Aedes* spp. depends on many contextual aspects, with microevolution being one relevant limiting factor. Currently, there is an increasing tendency to use phenotypic characters, primarily wing shape, to describe microevolutionary patterns. **Objectives:** In this work, we investigated the metric differentiation of wings of *Ae. albopictus* samples collected over a four-year period (2007–2010) in a park in São Paulo city, Brazil (23.566° S, 46.719° W). **Methods:** Left wings of each individual (males and females separately) were mounted between a slide and a coverslip and digitally photographed. Coordinates of 18 landmarks represented by vein intersection were obtained using TpsDig version 1.4. **Results and Discussion:** Wing size significantly decreased during that period for both sexes. Additionally, wing shape also changed with time, with males being more different after the year 2008 and females after 2009. Given that wings play some sex-specific roles, males and females could be affected by differential evolutionary pressures. Consistent with this hypothesis, sexual dimorphism was detected and quantified: females were larger than males (with respect to the mean) and had a distinct wing shape, regardless of allometric effects. To our knowledge, this is the first description of the phenotypic variation in a natural population of *Ae. albopictus* over time. The results showed that wing morphology in *Ae. albopictus* may change in periods as short as one year and that this process is continuous. In conclusion, wing changes, especially concerning shape, are a sensitive indicator of microevolutionary processes in this species. As a result of this investigation, some new complex questions have arisen, confirming that this is a promising field for further research.

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