

8. Animal Biology

8.01 Morphological study of the skin and characterization of the cutaneous secretion in the tree frog *Aparasphenodon brunoi*

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Introduction: One of the most important functions of the amphibian skin is the chemical defence against predators and microorganisms through the secretion of toxins. *Aparasphenodon brunoi*, is a casque-headed tree-frog inhabiting the Atlantic forest from São Paulo up to Espírito Santo. This species is frequently found inside axils of bromeliads, using the head as a lid to close the entrance and protect itself from possible predators, in a behavior known as phragmosis. **Objectives:** The aim of this study was to examine the skin morphology of *Aparasphenodon brunoi* and to present a preliminary biochemical and pharmacological characterization of its cutaneous secretion. **Methods:** Fragments of the dorsal, ventral and inguinal skin were fixed in paraformaldehyde and embedded in glycol methacrylate. The whole head was fixed in Bouin and decalcified in EDTA. Histological sections were stained with toluidine blue-fuchsin or HE. The skin secretion *in toto* was analyzed by SDS-PAGE, and the cytotoxic potential was evaluated by the MTT method using B16F10 murine melanoma cells and L929 fibroblast cells. The antimicrobial potential was tested against two bacterial strains, *Escherichia coli* GFP and *Micrococcus luteus*. **Results and Discussion:** The dorsal skin, including the head, shows a considerable number of granular (or venom) glands while the ventral and inguinal skin, richer in mucous glands, is specialized in water absorption, with prominent *verruca hydrophilica*, a well developed vascularization and the absence of a calcified dermal layer (which is present in the dorsal skin). These skin characteristics are in accordance with the phragmotic behavior of the animal, in which the dorsal skin and the head are much more exposed to water loss and to possible attacks of predators. SDS-PAGE revealed the presence in the skin secretion of at least three different proteins varying between 18.4 and 35 kDa. Different levels of cytotoxicity were observed for both cell lines tested, indicating the potential for further pharmacological studies. This toxicity may be related to the large number of granular glands present in the dorsal skin of *A. brunoi* and is probably used as chemical defense against predators and microorganisms in general. For the two bacteria tested, however, the secretion did not show any effect.

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8.02 Sexual dimorphism in the skin of the tree frog *Aplastodiscus leucopygius* (Amphibia, Anura) correlated to courtship behavior

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Introduction: Amphibian skin is mainly involved in respiration, chemical and mechanical protection, water and ion transportation, sensory reception, temperature control and reproduction. Among amphibians, the anurans possess the greater diversity of reproductive modes. In the family Hylidae (the tree frogs), the genus *Aplastodiscus* shows a sophisticated courtship behavior involving male vocalization, mutual tactile stimuli and, possibly, chemical signaling. Using auditive and tactile stimuli, the male gradually guides the female from the vegetation down to a subterranean nest, where oviposition occurs. **Objectives:** The aim of this study was the comparison of the skin glandular distribution and morphology between males and females of *Aplastodiscus leucopygius* to determine: 1) the occurrence of sexual dimorphism and 2) the possible participation of chemical signaling during courtship behavior, through the correlation of gland distribution with the tactile stimulated regions. **Methods:** Skin fragments from the dorsal, ventral, inguinal, dorso-lateral, mentonian and cephalic regions were fixed in Karnovsky and embedded in historesin. The sections were stained with toluidine blue-fuchsin and submitted to PAS (for mucus substances) and bromophenol blue (for proteins). **Results and Discussion:** The skin of both males and females shows a large number of glands of three different types, mucous, mixed and granular (or venom) glands. In the males, a fourth type of gland is present, with peculiar morphological characteristics. The mucous glands are acinar, formed by a monolayer of cells, which are positive mainly for PAS. The mixed glands are also acinar and show two different types of cells, one positive for PAS and the other positive for bromophenol blue. The granular glands are syncytial and are intensely positive for bromophenol blue. The differentiated glands appearing exclusively in the males are also acinar, but the cells, all of the same type, are columnar and full of tiny spherical glands which are strongly positive for bromophenol blue and PAS. The large number of glands present in the skin of *A. leucopygius*, mainly in the dorsum, is uncommon when compared to the skin of other hylids. This is an indication that these animals have a great dependence on skin secretions for their survival. The presence of a differentiated gland exclusive in males, mainly in the mentonian and dorso-lateral regions of the skin, can be associated with the touching behavior during courtship and is probably related to the secretion of compounds for chemical signaling between the male and female.

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8.03 Diet and feeding behavior of turtles and lizards kept in the Anexo da Recepção, Pátio Externo da Casa Vital Brazil, Herpetologia, Instituto Butantan

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Introduction: Reptiles are ectothermic; they depend on external heat to regulate temperature, which is important for their metabolism and behavior. They require favorable conditions for their maintenance. Their feeding is related to energy flow and temperature. With species kept in captivity, suitability of enclosures to ensure efficiency in thermoregulation is recommended. **Objectives:** The aim of this study was to analyze the diet and feeding behavior of different species of turtles and lizards kept in captivity for three years. **Methods:** This work was carried out in the Anexo da Recepção, Pátio Externo da Casa Vital Brazil, Herpetologia, Instituto Butantan, since 2007, with those reptiles brought to the Reception. Every selected species received a record in the admission in order to detail the procedures, mass (in grams), vermifuge, microchip, quarantine, daily observations about the diet and feeding behavior. The *Trachemys* are placed weekly in a swimming pool to eat newborn animals, various arthropods, and fodder when it is available. *Phrynosoma*, *Hydromedusa* – wild and *Chelidra* and *Apalone* – exotics, are conditioned in plastic boxes with water and substratum and are given feed, neonates and fishes. Land turtles (*Geochelone*) stay free in an open place with a shelter and water available. They feed three times a week with a varied diet. *Iguanas* are kept in a heated yard, and feed on various items and eat *hibiscus* flowers daily, and occasionally eat *tenebrios*. The adult, omnivorous teius (*Tupinambis*) that settle down in the same place where turtles stay, except the young kept in the terrarium, receive diverse food; the offer and the quantity of food vary depending on the size and weight. Food is provided as donations from the employees and the local restaurant. **Results and Discussions:** It is notorious and proved in the different seasons of the year, that these reptiles make use of feeding for energy control and thermoregulation. The species consume the food items according to their biology, especially in the warm seasons because they need additional energy value to maintain metabolism and spend on their activities. In the coldest months, the acceptance of food is lower, just because they are not active. Land turtles when not heated, do not eat and easily become sick. Despite that teius hibernate in the winter; we can observe a dispute of food and territory among them. We have also observed that the animals are adaptable to the different kinds of food, due to a variety of food offered. This work was hampered since the register of entry and records of feeding were lost during the fire at Butantan Institute last May. Despite that, we have compiled the available data for the conclusion of this compendium.

8.04 Seasonal changes of the renal sexual segment in the male reproductive cycle of *Sibynomorphus neuwiedii* (sleep snake)

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Introduction: In neotropical regions, reproductive aspects of snakes are poorly known, especially for species of the family Dipsadidae (non-venomous snakes). The male reproductive cycle of the Squamata (snakes, lizards and amphisbaenians) is inferred by changes in length/ weight of the testes and vas deferens and mainly seasonal changes of the renal sexual segment (RSS) during the reproductive season. The RSS is an androgen-dependent organ present in the distal urinary tubules of some reptiles, such as the Squamata, but absent in turtles and crocodilians. The hypertrophy of these tubules is caused by increased production of sexual granules, which indicates the species' breeding season. This hypertrophy of the renal sexual segment, is also related to sexual behaviors such as combat ritual, court and copulatory guard. **Objectives:** The aim of this work was to determine the influence of the RSS at the time of mating *Sibynomorphus neuwiedii* (sleep snake). **Methods:** The cranial portion of the right kidney of 27 males *Sibynomorphus neuwiedii* was processed for light microscopy by the methods of hystoresin (glycol metacrylate, Leica) and paraffin. The material was sectioned (2-5 mm) and stained with toluidine blue-fuchsin and hematoxylin-eosin. After preparation of the slides seasonal variations in the diameter of the tubules, epithelial height, density of granules and staining intensity of granules of the RSS were recorded. Variations in tubule diameter and epithelial height were tested by analysis of variance (ANOVA). **Results and Discussion:** Structural changes were noted in different seasons, with significant increase in tubular diameter ($F = 20.6$, $n = 270$, $df = 3$, $p = 0.0002$) and epithelial height ($F = 23.9$, $n = 270$, $df = 3$, $p = 0.0001$) of the RSS in the spring-summer. The presence of many eosinophilic granules intensely stained and scattered throughout the cytoplasm of the cell in the spring- summer indicated a higher activity when compared to autumn-winter. The RSS of the *Sibynomorphus neuwiedii* shows a seasonal cycle characterized by a hypertrophy of the distal convoluted tubules in the warmer seasons of the year. The hypertrophy of the RSS in the spring-summer coincided with increased Leydig cell nucleus (mating), spermatogenic activity and presence of sperm in the female, previously described in the species. The data presented in this paper indicate that it is possible to determine the mating season in *Sibynomorphus neuwiedii*, through the study of the RSS.

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8.05 Early learning in prey preference of the lynx spider *Peucetia rubrolineata* (Oxyopidae)

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Introduction: Spiders are known as generalist predators, although many species show preferences for certain types of prey. For some species, it is not clear which mechanisms are involved in the establishment of this kind of behavior. However, studies indicate that prey preferences in spiders of the family Oxyopidae could be related to imprinting processes, which occur early in the development of these animals. **Objectives:** This work focused on the study of prey choice in young spiders *Peucetia rubrolineata*. **Methods:** In the present study, two types of prey were offered to spiderlings of *P. rubrolineata*: newborn crickets (*Gryllus* sp.) and fruit flies (*Drosophila* sp.). The prey preference was tested using a choice test. Three trials were performed and each one with four combined variables: type of prey (crickets or flies), animal's age (five or 15 days), time of exposure to prey (five or ten days) and the amount of prey offered (one or two). **Results and Discussion:** Among the variables used, animal's age (15 days after leaving the egg sac) and time of exposure (ten days) were the most efficient parameters in the establishment of prey preference. When well fed, spiderlings prefer adult flies (*Drosophila* sp.) over crickets (*Gryllus* sp.). However, when spiders are starved, the preference is for the first type of prey offered. In the second case, we observed a primacy effect, an important factor in imprinting determination. The prey preference based in learning is advantageous in case of changes in the original environment of species, colonization of new habitats, as well as exploring new sources of food. This learning process involved in the establishment of food preference can lead to an optimization of feeding behavior, ensuring rapid adaptability to environmental conditions. It happens early in the development of these animals and certainly is important in the survival of these spiders.

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8.06 Evolution of maternal care in spiders of the superfamily Lycosoidea

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Introduction: Spiders show a high diversity of parental care. These behaviors include from the construction of the egg sac to complex behaviors of extended care in social groups. In the superfamily Lycosoidea, females take care actively of the egg sac and spiderlings until dispersion. Categories of parental behavior in these spiders have been used as characters in some phylogenetic reconstruction studies. There is no consensus in the literature about the homology of these characters. Some authors use all parental behavior as states of one character, while others use just some aspects of maternal care divided into several characters. There is also confusion about the definition of the behavior of building a nursery web for spiderling care. Due to the complexity of this type of character, its delimitation should be made carefully and based on specific studies about these behaviors. In Lycosoidea, parental care has a high internal variability, which makes this group interesting for character delimitation. **Objectives:** Thus, this study proposed new characters to study the evolution of maternal care. **Methods:** By gathering literature data and also laboratory observations of maternal care, we propose 4 characters: care with the egg sac, construction of a silken retreat for the egg sac, helping the spiderlings to emerge from egg sac, and care with the spiderlings. We propose the homology based on the female behaviors and the structures built by her. **Results and Discussion:** The reconstruction of evolutionary history of these characters shows to carry the egg sac under the chelicerae, help the spiderlings to emerge from egg sac, make a silken retreat for the egg sac care and to guard the spiderlings until dispersion are plesiomorphic to superfamily Lycosoidea, and all these behaviors are found in *Pisaura mirabilis*. A synapomorphy of *Trechalea* sp. is to carry the spiderlings on the empty egg sacs and in Lycosidae (*Aglaoctenus* sp. and *Lycosa erythrognatha*) is to carry young on the female abdomen. Parental behavior has been important to phylogenetic studies of the Lycosoidea group. The characters proposed are conservative and there are low rates of homoplasy, so they can be suitable to the study of evolution and phylogenetic analysis.

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8.07 *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wolbachia* sp. (Alphaproteobacteria, Rickettsiales): influence of infection on reproduction and morphological phenotype of the mosquito

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Introduction: *Wolbachia* is an intracellular alpha-proteobacteria endosymbiont capable of infecting various arthropods and nematodes. They are transmitted maternally through the cytoplasm of the egg. These bacteria can alter reproduction of their hosts, inducing feminization of males, parthenogenesis, male killing, cytoplasmic incompatibility and changes in reproductive fitness. The effects of infection may be advantageous or disadvantageous, depending on the host and the strain of the bacterium. One of their hosts is *Culex quinquefasciatus*, a nocturnal mosquito, cosmopolitan, urban, very common in São Paulo city and competent vector of filaria and arboviruses. Despite the medical importance of this mosquito and the biological relevance of *Wolbachia*, little is known about the association between these two taxa. **Objectives:** In this study we evaluated whether this endosymbiont influences the reproductive fitness and wing form of the mosquito. **Methods:** A colony of *Culex quinquefasciatus* naturally infected by *Wolbachia* was started with mosquitoes collected on the banks of the Pinheiros River in São Paulo city. Some mosquitoes were treated with antibiotic, tetracycline hydrochloride, in two consecutive generations in order to eliminate the bacteria. After obtaining a colony of uninfected mosquitoes, comparisons were made between reproductive fitness of infected and uninfected mosquitoes. These two groups of mosquitoes were also compared for the wing geometry to assess whether the phenotype of wing shape changes would suffer after elimination of bacteria. **Results and Discussion:** We found that infected mosquitoes laid fewer eggs and the viability of eggs was lower than that of mosquitoes uninfected. Furthermore, data from wing morphometry showed that the presence of bacteria does not modify wing shape of the following generation. The presence of bacteria does not interfere in the determination of wing shape of *Culex quinquefasciatus*, although it appears to influence the reproductive fitness of mosquitoes. How this bacterial endosymbiont causes such disturbance in this mosquito is still being investigated.

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8.08 A curious case of duplication in spermathecae of *Acanthoscurria suina* Pocock, 1903 (Araneae, Mygalomorphae, Theraphosidae)

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Introduction: Some anomalies are described in the Theraphosidae, as in *Eurypelma triepii* Dresco, 1985 based on a single male which shows a tubercle on both chelicerae, character considered by Bertani as an anomaly and transferred to *Nhandu triepii*. Other examples are the occurrence of siamese twinning or conjoining in 14 specimens of *Poecilotheria regalis* Pocock, 1899 found in a single egg sac, and a probable case in the specimen described by Schmidt in 1997 as *Nhandu tripartitus*, since he based his description on only one female holotype which shows three receptacles on the spermathecae. **Objectives:** The aim of this work was to present a case of duplication in spermathecae of *Acanthoscurria suina* Pocock, 1903. **Methods:** The specimens studied are deposited in Instituto Butantan, São Paulo (curator: I. Knysak), Museu de Ciências Naturais (E. H. Buckup) and British Museum of Natural History (Janet Beccaloni). Female epigynum was dissected and cleared in 85% lactic acid at 100°C for observation of internal structures. The multifocal photos were taken using a Leica MZ 16A stereomicroscope with a Leica DFC 500 digital camera attached. **Results and Discussion:** The spermathecae of *A. suina* normally shows two lobes involved in a common base. Studying the material from Museu de Ciências Naturais, we found a curious duplication of the spermathecae. This fact notes the importance of having a large number of exemplars analyzed, since just one specimen with one or more different characters can be an anomaly and not a new species.

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8.09 Twinning, triplets and bicephaly in a clutch of the green snake, *Philodryas olfersii*

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Introduction: There are numerous reports of twinning and bicephaly in oviparous reptiles. Despite this, such phenomenon appears to be rare and most reports deal with isolated cases in captivity. However, reports of recurrence of twinning within a single clutch are virtually unknown. **Objectives:** Herein, we document the occurrence of twinning, triplets and bicephaly in a single clutch of the green snake, *Philodryas olfersii*. **Methods:** A clutch of 13 eggs was laid by a wild-caught female of *P. olfersii*. Eggs were measured, weighed and incubated in moistened vermiculite with temperature ranging from 22-28 °C. After the incubation period, 10 out of 13 eggs hatched and babies were measured, weighed and sexed. The three unhatched eggs were fixed and then dissected. Extra-embryonic membranes were excised and submitted to standard histological procedures. **Results and Discussion:** Dissection of the three eggs revealed that all contained dead, late-term embryos, each with varying amounts of residual yolk remaining. However, egg no. 1 contained two dead late-term dead embryos (twins) and egg no. 2 contained three dead late-term embryos (triplets). Both twins and triplets were connected to the same yolk sac through their own umbilical cords. Amnion and chorioallantoic membranes were observed in histological analysis. Moreover, twins and triplets were smaller than their normal siblings. All triplets showed cephalic abnormalities and one had the anal slit fully closed. Twins of egg no. 2 were seemingly biting each other inside the egg. Egg no. 3 contained just one dead late-term embryo showing bicephaly almost complete. At the dorsal region, two heads fused close to the eye position deforming the majority of supra-labials scales. At the ventral region, several abnormalities were observed, which makes it difficult to distinguish the separation of the two heads. Although twinning in oviparous snakes was already reported in the literature, the occurrence of more than one twinning within a single clutch is virtually unknown. Twinning in oviparous snakes is not an effect of incubation conditions because early embryonic segmentation (when twins are formed) occurs inside maternal uterus, and at oviposition embryos are well developed. The smaller sizes of twins and triplets in relation to their siblings are clearly explained by the partition of the same yolk during embryogenesis. Despite our twins, triplets and bicephalic dying before hatching, this is not a phenomenon incompatible with life. There are several reports of successful hatching of twins and bicephalic reptiles. The cause of hatching failure may be related to the several abnormalities present in the embryos.

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8.10 Skin morphology and cutaneous secretions support a case of müllerian mimicry involving the dart-poison frog *Ameerega picta* and the frog *Leptodactylus lineatus*

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Introduction: Cutaneous secretion of toxins plays a central role in amphibian defense against predation. Amphibian skin presents high densities of granular (or poison) glands, whose products and morphology vary among species. Highly toxic secretions are often associated with conspicuous color patterns, which are recognized and avoided by predators. This is the case of many dendrobatid frogs, such as *Ameerega picta*. Its color pattern is very similar to that of the presumably non-toxic *Leptodactylus lineatus*, assumed to attain protection through mimicry. **Objectives:** The aim of this work was the morphological and biochemical study of the skin of these two species, in order to assess the hypothesis of mimicry. **Methods:** Dorsal skin fragments were removed, fixed in paraformaldehyde and embedded in paraffin and historesin. For a histological study, paraffin sections were stained with hematoxylin-eosin, and historesin sections were stained with toluidine blue-fuchsin. For histochemical description, we used bromophenol blue for proteins, PAS for polysaccharides and alcian blue pH 2.5 for acid polysaccharides. Skin secretions were manually extracted in aqueous solution, lyophilized and submitted to SDS-PAGE and RP-HPLC monitored at 214, 280 and 339 nm. **Results and Discussion:** *A. picta* possess elliptical granular glands filled with non-protein granules rich in carbohydrates. *L. lineatus* shows spherical granular glands possessing protein granules. Accordingly, SDS-PAGE indicates great amounts of proteins in the poison of *L. lineatus* but not in that of *A. picta*. Chromatographic profiles further suggest marked differences in poison composition between these species. Moreover, both differ greatly in profiles of gland distribution: in *L. lineatus*, poison glands are organized in aggregates whose position coincides with colored elements of the dorsum, suggesting that poison disposition is announced to predators through skin colors. In contrast, *A. picta* shows glands distributed homogeneously and in lower densities than in *L. lineatus*. This simpler profile in *A. picta* suggests that its investment in chemical defense is rather qualitative than quantitative, in agreement with the high toxicity attributed to dendrobatids. In conclusion, our data suggest that both species studied are toxic, in contrast with assumptions that *L. lineatus* is non-toxic. Hence, *A. picta* and *L. lineatus* seem to transmit common warning signals to predators, probably benefiting from their resemblance. Rare among vertebrates, this class of mimicry is referred to as müllerian mimicry.

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8.11 Predatory behavior of *Chironius bicarinatus* (SERPENTS: Colubridae) WIED, 1820 in the exposition of the Museu Biológico of the Instituto Butantan

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Introduction: *Chironius bicarinatus* lives in the southern plateaus of Brazil reaching the northwest of Uruguay. It has as characteristic two keels in the vertebral scales, of green-bluish color, with black scales on the back and ventral side, with a black line appearing in zigzag on the subcaudal scales on the inferior side of the tail. It is non-venomous snake and it has average size reaching up to 1.80 m in length, and shows diurnal activity. Of arboreal habit, it inhabits bushes, orchards and gardens, where it feeds on amphibians, small lizards and birds, and it often goes down to the ground, and when threatened it quickly returns to the foliage of the trees. **Objectives:** The aim of this study was to observe the predatory behavior of the snake *C. bicarinatus* against prey species *Lithobates catesbeiana* offered in captivity. **Methods:** For a young specimen, female, *C. bicarinatus*, found in the Granja Viana - São Paulo, kept in a terrarium with environment with and not reflecting pool in favorable terms were offered frogs (n = 84) of the species *L. catesbeiana* adults and tadpoles, fortnightly. The specimen was fed during the period of 10 months, with 44% of adult frogs and 56% of the tadpoles. **Results and Discussion:** From a total supply of specimens of frogs of the species that were ingested, 33 of 35 adult frogs and 42 of the 49 tadpoles offered, of which there were three regurgitations of tadpoles and four tadpoles and two adult frogs offers not consumed. It was observed that the interest of the specimen, the food offered, was due to the movement that the tadpoles and frogs held in the water, where it was found a game based on the movement of prey. Its posture was predatory when more than half of the lower body attached to the branches of the enclosure, the other half semi-inflexed facing the water surface, where the pace of issuance of the tongue (dart) was 10-20 times/min. After the movement of tadpoles or frogs, the specimen strikes a boat for the most accurate in moving prey. In the subsequent capture of the tadpole, the specimen retracts the posterior portion of the body, performing a movement of spring toward the branches of the enclosure space. After ingestion of the tadpole, mostly by the tail or the head of the adult frog, the specimen rubbed its mouth on the trunk or leaves the room to clean the viscosity present in its mouthparts. Although frogs are one of the main food items of some Colubrids snakes, as in the case of *C. bicarinatus*, more detailed records about the interspecific relationship are scarce in the literature and are an extremely useful study in *ex-situ* and *in-situ* to elucidate the behavior of this species.

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8.12 Diet and feeding frequency of a fossorial snake (*Phalotris lativittatus*)

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Introduction: *Phalotris lativittatus* is a rare, fossorial and medium-sized snake distributed endemically in Cerrado vegetation areas of São Paulo State. Data on feeding habits are unknown, but like congeners, its diet probably consists of other elongated fossorial vertebrates. **Objectives:** This work aimed to characterize the diet and feeding frequency of the snake *P. lativittatus*. **Methods:** We dissected 51 preserved specimens of *P. lativittatus* to examine the gastrointestinal contents. Specimens were categorized as 'fed' or 'not fed' and stomach content was collected only when prey identification was possible. In parallel, newborn (both live and dead) dipsadid snakes were offered as food to some captive *P. lativittatus* hatchlings to verify the acceptance and ingestion of this item. **Results and Discussion:** We found that 96.1% (n = 49) of the analyzed specimens showed gastrointestinal content. Taken separately, it was observed that 96.4% of the females, 92.8% of the males and 100% of the hatchling/young had gastrointestinal content. It is a strikingly very high and uncommon frequency of fed individuals rarely observed in snakes. To date, the diet of *P. lativittatus* was unknown and it was hypothesized to be constituted of amphisbenids and other elongated fossorial vertebrates. Our observations suggest that *P. lativittatus* diet may be specialized in amphisbenids. Although we were able to identify stomach contents only in three specimens, amphisbenids were observed in all cases. In two females, we observed only skin pieces of *Amphisbaena* sp. and in one hatchling we found an intact prey, identified as *Amphisbaena roberti*. In addition, in all captive feeding trials individuals always refused to feed on dipsadid snakes. Feeding on *Amphisbaena* sp. also suggests that predation events occur underground, because amphisbenids show marked fossorial habits. In conclusion, amphisbenids as food item is also present in other elapomorphini snakes known to date indicating a conservative feeding habit diet in the group. Additional data both in *P. lativittatus* and other elapomorphini are necessary to test this assumption.

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8.13 Comparative reproductive biology of Amphisbaenidae (Squamata, Amphisbaenia) from Brazil

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Introduction: Amphisbaenia are a group of fossorial Squamata reptiles, whose most representative family is the Amphisbaenidae. There are currently a limited number of studies available on their reproductive biology. The reproductive cycle was studied only in eight species, and the histology of genital ducts was studied in three species. The family Amphisbaenidae is represented by 65 species in Brazil. **Objectives:** This study aimed to characterize the reproductive cycle, morphology and anatomy of the genital tract of eleven species of the Brazilian genus Amphisbaena. We also aimed to compare the data obtained from different species and to discuss the results comparing it with morphological and molecular phylogenetic trees. **Methods:** The analyses were performed using museum specimens. The reproductive cycles of males were analyzed from the variation of testis volume and vas deferens diameter during the year. Histological analysis of the testis and vas deferens furnish data on spermatogenic cycle and sperm storage. The reproductive cycles of females were analyzed considering the variation in follicle length during the year, the observation of primary or secondary vitellogenesis and the occurrence of eggs. The oviducts were analyzed by light microscopy, for anatomical description and observation of morphological changes during the reproductive cycle. All data were compared among the eleven species, regarding the different ranges and biomes occupied by each one. **Results and Discussion:** All reproductive cycles observed until now are seasonal, with a short period of spermatogenesis and the occurrence of sperm storage by males in the vas deferens. Females' follicles usually show a major yolk deposition and size increase during the two or three months before ovulating. One to eleven eggs are laid by the female, which varies according to the species. In most species, the eggs are arranged in a line in the female's abdominal cavity. The exception is *Amphisbaena alba*, in which eggs can be arranged side by side.

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8.14 Morphology and natural history of rattlesnake populations (*Crotalus durissus*, Viperidae) from Brazil

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Introduction: Rattlesnakes (*Crotalus*) originated in North America and subsequently spread throughout Central and South America. The species show a wide variation in body size and color patterns. In addition, there is a similar variation in reproductive patterns across the genus with litters of 5 to 8 neonates in smaller species to litters of 15 to 40 neonates in larger species. These snakes generally eat vertebrates with smaller species feeding on lizards and larger species feeding on lizards when they are young, switching to small mammals and birds, when adults. In Brazil, *C. durissus* occurs throughout the country, except in the states of Acre and Espírito Santo. Some populations show considerable variation in morphology, reproduction and diet composition even with neighbor populations differing drastically from each other. **Objectives:** Our aim was to study the relationship between *C. durissus* populations of Brazil, characterizing their morphology (morphometry, pholidosis and pattern coloration), reproduction and diet, to determine the relative influence of environmental parameters such as altitude, temperature and precipitation on these morphological and ecological patterns, such as the importance in the taxonomic diagnosis of the group. **Methods:** A total of 620 specimens of *C. durissus* housed in the Instituto Butantan (IB) from Southeast and Central-West regions from Brazil were examined. Several measures, counts of scales and pattern coloration of the body and the head were recorded for each individual. Fecundity (clutch size), sexual maturity, testicular activity (volume of the testicle), vas deferens diameter and uterine muscular contraction were analyzed. Histological analysis was performed to detect sperm storage structures in males and females. Snake stomachs were dissected to examine the percentage of occurrence of each prey category. **Results and Discussion:** Significant differences were not detected in body size ($F = 3.25$; $p = 0.07$). There were significant differences in the shape variables (MANOVA; Wilks' Lambda = 0.86; $p < 0.001$) and pholidosis variables (MANOVA; Wilks' Lambda = 0.72; $p < 0.000$). Female reproductive cycle for Southeast and Central-West regions was seasonal. The reproductive cycle of male for the same regions was annual. Ontogenetic variation in diet was not detected, which was mainly composed of rodents.

8.15 Geographical ecology of the rattlesnake *Crotalus durissus* (Squamata, Viperidae) in the Brazilian Cerrado

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Introduction: Geographical variation is intimately associated with the ecological characteristics of a species; however, few species of snakes have been adequately studied to determine the existence of geographical variation in morphology, diet and the reproductive cycle. In general, environmental parameters of latitude, altitude and temperature possibly reflect a general pattern of geographic differentiation in these morphological and ecological patterns in some species of rattlesnakes. Two species of rattlesnakes are restricted to South America, *Crotalus simus* and *C. durissus*. There has been little research conducted on *C. durissus* in Brazil involving geographical variation. **Objectives:** We investigated patterns of geographical variation in morphology (morphometry and pholidosis), reproduction, and diet of the rattlesnake *C. durissus* in the Brazilian Cerrado, and the relative influence of environmental conditions on these patterns. **Methods:** A total of 507 specimens were examined, 228 males and 279 females from the Brazilian Cerrado. Several measures and counts of scales of the body and the head were recorded for each individual. The sex of individuals was determined by direct examination of the gonads. In females, the reproductive condition was determined by the presence of vitellogenic follicles and embryos in the oviducts, and in males the reproductive condition was evaluated by the presence of enlarged testes and convoluted epididymides. Snake stomachs were dissected and their contents analyzed. Prey items were identified to the lowest taxonomic level possible. **Results and Discussion:** *Crotalus durissus* shows a marked sexual dimorphism in body shape, with males having bodies, tails and paravertebral stripes relatively longer than females, while females having bodies relatively wider. Clutch size averaged 11 embryos and was significantly correlated with snout-vent length. The female reproductive cycle occurred in the transition at the end of the dry season and the rain season, with the beginning coinciding with the greater activity of small mammals and male reproductive cycle occurred throughout the year. Ontogenetic variation in diet was not detected, which was mainly composed of rodents, a habit related to an ancestral pattern in the group. Association between climatic structure and ecological parameters from rattlesnakes was not detected, probably because of the predictability of the climate in the Cerrado.

8.16 Reproductive strategies in the snake genus *Bothrops*: gain and loss of genetic diversity

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Introduction: Viperid snakes show an ancestral sexual reproductive pattern, in which delayed fertilization is ensured by uterine muscular twisting (UMT) sperm storage. Female parthenogenesis is rare and poorly recorded, mainly in South American taxa. **Objectives:** The aim was to discuss gain and loss of genetic diversity in snakes in nature. **Methods:** We discuss gain and loss of genetic diversity in snakes in nature based on the observation of five cases of *Bothrops* litters from 3 females in captivity kept isolated long-term from males one from birth and other two for 7 and 9 years, respectively, since arrival. **Results and Discussion:** Five birth cases, one in *B. leucurus* and 4 in two different adult female *B. moojeni* in Laboratório de Herpetologia, were recorded. The female *B. leucurus* arrived in 2001 and laid 31 atretic follicles (af) and 2 stillborn females in 2010. The offspring of the first case of *B. moojeni* (mother born in captivity/January 2002) were observed on three occasions (2005, 2006, 2010). In April 2005, the snake laid 6 af and 1 neonate male which died some hours later. In March 2006, the same *B. moojeni* mother, laid 10 af and 1 stillborn male. The third time, this female laid 23 (af) and 1 teratogenic male. Second case *B. moojeni* offspring female arrived in 2003 and laid 14 (af) and 1 female (sexed by dissection) which survived 18 days. The literature points out 2 previous cases of possible parthenogenesis in *Bothrops* (*insularis* and *moojeni*). In all of them, there were doubts about total isolation from males which could characterize offsprings as a result from sperm storage, differently from the first case *B. moojeni* described here. Facultative automictic parthenogenesis (FAP) in the sexual determination system (ZZ, ZW) has explained the production of normal diploid males. However, here we had 4 males (only one teratogenic) and one female fully developed. Assuming parthenogenesis or alternative sexual mode of reproduction, besides FAP (which originates males) other meiotic events could produce females. Parthenogenetic snakes have been reported to survive longer in captivity. In the wild, despite very rare, we believe parthenogenesis would drastically reduce genetic variability, though vital for species survival, mainly when colonizing isolated places. Different genetic mechanisms could produce males and females and could represent restoration in opportunities of mating that combined with mutations would result in an increase in genetic diversity at least during the period of total isolation. Multiple copulation and sperm storage would contribute to accelerating gain in diversity in periods when contact with other specimens occurred. We carried out population studies through molecular analysis to evaluate polymorphism in natural populations as well as to understand the role of asexual reproduction in the natural history of the *Bothrops atrox* species complex.

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8.17 Predatory behavior of *Oxyrhopus guibei* (Squamata, Dipsadidae) Hoge & Romano 1977 in captivity

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Introduction: *Oxyrhopus guibei* is an opisthoglyphous dipsas, which occurs in most parts of southern, southeastern and central-western Brazil, and also in Paraguay, Bolivia and Argentina. This species is well adapted to the human environment and it feeds on lizards and small rodents. The choice of prey subjugation tactics, which can be constriction, poison or swallow while still alive, varies according to the risk of retaliation the prey shows.

Objectives: The objective of the present study was to improve the knowledge of feeding behavior of this species while in captivity by offering different prey. **Methods:** Three types of prey were offered: *Mus musculus* (mice), *Rattus norvegicus* (rat) and *Meriones unguiculatus* (gerbils). During the observations, we recorded the time spent eating (stopwatch seconds), the tactics of subjugation of prey (alive down, poisoning and/or constriction), the body site at which prey ingestion began (anterior, posterior or lateral region) and the amount of prey consumed. **Results and Discussion:** Thirty-seven predatory behaviors were observed. The type of the prey, as well as its size, affects the snake's predatory behavior. The amount of prey offered sequentially did not interfere with the choice of subjugation tactics used. Regarding small preys (0-5 g), the tactics of subjugation were swallowing while still alive, while for bigger preys (over 10 g), the tactics varied between constriction and poisoning/constriction. *O. guibei* has a Duvernoy's gland, which is responsible for toxin production and secretions in opisthoglyphous snakes. However, the poison of this snake has low toxicity, and apparently is used to complement constriction. We observed a tendency to start swallowing the prey from the anterior region, which is possibly related to reduction of the ingestion time and the energy invested. The ingestion time for different prey with the same mass illustrated no significant differences in their means, either between mice of different masses. However, the statistical analysis showed significant differences in ingestion time in three comparisons: between mice from 0 to 5 g and from 20 to 25 g; between mice from 0 to 5 g and rat from 20 to 30 g; and between mice from 0 and 5 g and gerbil from 20 to 30 g, probably due to the snake's greater energy spent in swallowing larger prey.

8.18 Psalistopoides Mello-Leitão, a new synonymy of the genus Rachias Simon (Araneae: Nemesiidae)

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Introduction: The genus *Psalistopoides* was proposed by Mello Leitão in 1934 to include *P. fulvimanus*, described from Alto da Serra de Paranapicaba, Santo André, São Paulo, Brazil, based on a single male specimen. The last modification that occurred in the genus was proposed by Lucas and Indicatti in 2006, which considered *Psalistopoides* as a valid genus, redescribed the type-species and described a new species from Ribeirão do Pinhal, Paraná, Brazil. The diagnosis they provided was that the species of the genus *Psalistopoides* differ from *Neostothis* Vellard and *Prorachias* Mello-Leitão by the presence of keels close to the embolus; from *Pselligmus* Simon by the presence of tarsal scopulae on legs III and IV; from *Stenoterommata* Holmberg by the absence of a megaspine on tibia I and the aspect of the palpal bulb; from *Rachias* Simon by the absence of spines on all tarsi and from *Pycnothele* Chamberlin by the longer embolus. **Objectives:** The aim of this study was to synonymize the genus *Psalistopoides* with *Rachias* Simon and describe the females of *P. fulvimanus* and *P. emanueli* Lucas & Indicatti for the first time. **Methods:** The examined material was deposited in the Arachnida collection of the Instituto Butantan. The female spermathecae were dissected and submerged in clove oil to study internal structures. The illustrations and morphological observations were made using a Leica MZ12.5 stereomicroscope with a camera lucida. **Results and Discussion:** The study of *Rachias dispar* Simon, type-species of *Rachias*, that has the apical area of the embolus dilated, and of other species of the same genus that do not have spines on tarsi, and of the unknown females of the two species of *Psalistopoides*, led us to conclude that *Psalistopoides* is not a valid genus and that it should be synonymized with *Rachias*. This is because the diagnosis provided by Lucas and Indicatti does not differentiate *Psalistopoides* from *Rachias*, due to it having the same features. The females of the species that belonged to *Psalistopoides* are described for the first time. The female of *Rachias fulvimanus* (Mello-Leitão) comb. nov. differs from those of the other species of the genus by the basal dome of spermathecae being very high and narrow, and *R. emanueli* (Lucas & Indicatti) comb. nov. differs from those of the remaining species by the very reduced basal dome and by the thicker duct. In addition, new records on distribution range of both species are presented.

Supported by: CNPq

8.19 Chilopod biodiversity from Belterra (Pará, Brazil)

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Introduction: Studies about centipede biodiversity was begun during the project "Butantan Amazonia" in Belterra and Santarem cities. Chilopods are represented by superclass Myriapoda and includes Pauropoda, Symphyla and Diplopoda. They have a long and segmented body, a pair of antenna and a pair of poison claws. There are poor studies about centipedes in Brazil and there is no specific research. **Objectives:** The aim of this work was to study centipede biodiversity from the Amazon Forest in Belterra and Santarem cities and specify the species involved in human accidents. **Methods:** Collections were made during December 2009 to February 2010 in the field and urban area in Belterra and Santarem cities. The collected animals were kept alive and sent to Butantan Institute where the animals were identified. **Results and Discussion:** Until now, four genera from family Scolopendridae have been identified, one from Newportidae and one of the order Geophilomorpha. Data from Belterra and Santarem show a high centipede diversity and these samples are similar to those of other studies previously conducted in the Amazon Forest.

8.20 Lifestyle and reproduction of captive Brazilian insular snakes

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Introduction: Insular habitats are important for evolutionary studies; in addition, the geographical reclusion favors endemic species. Two islands off the coast of SP are known to be important players in the Brazilian ecological scene: Queimada Grande Island, where we find *Bothropoides insularis* and Alcatrazes Island where *Bothropoides alcatraz* is found. Due to the ever increasing habitat destruction, both venomous species are threatened with extinction. The reproduction, lifestyle and venom production of a new third species *Bothropoides jararaca aff.*, which is still in the description phase, is being studied. This new species inhabits Moela Island in the southern coast of SP. Due to the absence of mammals in these three islands these species show different feeding habits. **Objectives:** Our aim was to study the reproduction and behavior of these snakes in order to develop methods to help in "ex situ" conservation projects and venom extraction for research. **Methods:** *B. alcatraz*, *B. insularis* and *B. jararaca aff.* kept in captivity were used. **Results and Discussion:** One male and five females of *B. alcatraz* have been kept for about 10 years in captivity with zero mortality rates. In captivity, these snakes spend most of the time wrapped around tree branches even feeding from this location. In the wild, *B. alcatraz* feeds on small lizards, centipedes and small amphibians. In captivity they have accepted rats as food besides the usual diet described. So far, three forms of mating have been observed; however, no female has completed conception. One female and one male of *B. insularis*, born in the Herpetology are now 12 and 10 years old, respectively. In the wild, the young feed on amphibians, lizards and centipedes, and the adults mainly on migratory birds. In captivity they also feed on rats, gerbils and hamsters and show preference for newly hatched chicks. Two matings have been observed, but the female did not complete conception either. From a mating registered in 2008, four snakes were born in March 2009, and in February 2010 another six were produced. All have been developing remarkably and accepting the food offered. Of the new species, *B. jararaca aff.*, 5 specimens are kept (two males and three females). Four snakes were born from a female already pregnant when collected. A mating in captivity was also observed and the behavior studied and described. While the species is still being described, preliminary results indicate that they also feed on small lizards, small amphibians and centipedes. In captivity they have already begun to accept rats as food. The success of the reproduction in captivity of this species is very important for their 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, may not be removed, therefore the success of these endeavors depend on the successful reproduction of these species in captivity.

8.21 Regeneration of the parotoid macroglands in the toad *Rhinella icterica* after mechanical compression

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Introduction: Toads have a pair of parotoid macroglands that secrete venom used in passive defense against predators. These macroglands are composed of juxtaposed alveoli filled with syncytial glands, which are connected to the exterior by ducts. When the parotoids are bitten, the venom is expelled on the predator's oral mucosa in the form of jets, causing several pharmacological actions. The empty secretory syncytia collapse in the interior of the alveoli.

Objectives: Using morphological methods, we aimed at the study of the parotoid regeneration after manual mechanical compression, simulating a predator's bite. **Methods:** Parotoids of male toads *Rhinella icterica*, were dissected after being compressed at successive times, from 2 hours to 105 days. Animals with normal non-compressed parotoids were used as control. Two positive control animals were also examined: one sacrificed 330 days after parotoid compression and another collected from nature with a defective parotoid. The samples were fixed in Bouin and prepared for histology using paraffin. The sections were stained with HE, Mallory trichrome and picosirius. **Results and Discussion:** The results showed that after compression, a considerable number of alveoli remained intact. The alveoli which were effectively affected were observed with their syncytia totally collapsed and with an extensive hemorrhagic area and inflammatory infiltrate around them. From the 5th to the 105th day, the syncytia gradually showed signs of recovery, showing conspicuous nuclei and amounts of secretion inside them. The hemorrhagic areas gradually diminished. In the last stages, together with the syncytia almost completely regenerated, there were a few which were only partially recovered and others which seemed to be still in the first phases of regeneration. The presence, in the first stages, of hemorrhage and inflammatory infiltrate around the syncytia, including a great number of macrophages, indicates that the glandular tissue, as well as the connective tissue around it, suffers injuries when compressed. In the last stages of observation, many alveoli were well regenerated. Regeneration, however, seems to occur in different grades, from complete to restricted to a few areas of the syncytia. The alveoli which suffered the worst injuries did not seem to recover their functional state. On the other hand, the fact that not all the alveoli are effectively compressed can be crucial for toad survival, since they do not lose all their venom stock in the case of a bite, remaining protected for new attacks.

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8.22 Tick karyotype test: methodology improvements and technical training

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Introduction: There are some tick species that are still not well studied. One parameter that may be taken into consideration for the better characterization of those species is the karyotype. **Objectives:** The aim of this study was to adapt cytogenetic techniques to be used as a complementary study in the characterization of different tick species **Methods:** Based on a published peer-reviewed methodology, an established cell culture strain from the tick *Ixodes scapularis* (ID8) was used. The cells were incubated in 0.01 µg/ml colchicine for 18 h. The detached cells were centrifuged, incubated in 0.075 M KCl for 30 min, fixed with 3:1 methanol/ acetic acid solution and stained with Giemsa, 10% or 2%. The slides considered suitable for analysis (based on the visual subjective size of the cell population, integrity and percentage of cells in division) were screened. **Results and Discussion:** A good number of metaphases were obtained, but some modifications must be introduced to obtain a better morphology of the chromosomes. Staining with 2% Giemsa worked better than the 10% concentration since it allowed a clearer distinction between chromosomes from the same cell. Future tests are needed to improve our karyotype test technique including KCl time/concentration and adjustment of chromosome dispersion on the slide for better visual conditions for chromosome counting and morphological analysis in order to confirm the identity of cell culture lines.

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8.23 The forgetful spider

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Introduction: Memory is considered by many scientists as the most fascinating product of biological evolution. Use of previous experience helps animals to cope adaptively with ever changing environmental conditions, in habitat selection, mate selection, etc. Animals such as web spiders which hoard food as part their predatory behavior depend on memory to retrieve hoarded items and maximize intake by keeping memory representation of hoarding sites. The Orbiculariae are a group of spiders, the synapormophy of which is to build orb webs. Orbwebs, in the course of evolutionary time, may undergo structural changes and give origin to sheet webs and cobwebs. When spiders capture more than one item of prey, and one prey is taken off from the web, spiders exhibit search behavior which includes locomotion on the web, plucking of web lines, etc. in attempt to recover the stolen prey. *Zosis geniculata*, although from this group of spiders, does not show, under normal conditions, prey searching.

Objectives: We examined in our study the behavior of *Z. geniculata* when captured prey is suppressed. **Methods:** In condition 1, a first prey (p1) was offered and afterward it was carried to the hub by the spider, a second prey (p2) was offered, and p1 was taken off from the web while *Zosis* was capturing p2. In condition 2, a single prey was offered and this prey (p1) was taken off when the spider was attracted to the periphery of web. **Results and Discussion:** *Zosis* did not search for p1 under condition 1, but did search under condition 2. Results show that memory of p1 capture is affected by the capture of p2. This may be due to the time spent by *Zosis* wrapping p2. *Zosis* belongs to the unique spider family which has no poison glands and thus needs to spend enough time wrapping its prey. According to another interpretation, the lack of searching of p1 in condition 1 is due to working memory constraints in *Zosis*. Other derived spiders and one ancestral (Desidae) of the Orbiculariae group studied do search for the first prey even when a second is offered, so it seems probable that this behavioral feature has been lost in the Uloboridae. It is possible that there is an adaptive advantage in forgetting p1, in *Zosis*, but this is still not clear and should be addressed in further research.

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8.24 *Badumna longinqua* (Araneae: Desidae): description of the predatory behavior and plasticity of the web

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Introduction: *Badumna longinqua* is an introduced spider species in Uruguay. It appeared in the 1960s, probably due to the arrival of *Eucalyptus* sp. Both spider and tree came from Australia. Spiders from this genus have been reported to build irregular ground sheet webs. **Objectives:** The aim of this work was to study two populations of *B. longinqua* in Montevideo (Uruguay) and to describe the predatory behavior and the web. **Methods:** We observed two populations of *B. longinqua* (n=60) at the campus of the IIBCE and in the park of the city zoo. **Results and Discussion:** These spiders build two types of irregular sheet web: a two-dimensional and three-dimensional. The aerial web uses leaves from trees as support, and its retreat is inside a group of leaves knit together. The trunk web can be either two- or three-dimensional, with the retreat always inside cavities of the cortex. The first sheet built by the spider is two-dimensional, but later she adds new capture areas and the structure eventually becomes three-dimensional. The differences in the webs can be the result of incipient speciation, of adaptive plasticity or of competition for web sites with large differences in the overall fitness. Predatory behavior has unique features in such spiders. They capture the prey by biting: they approach it, seize it in their chelicerae and bring it directly to the retreat to be wrapped. The wrapping is different from the usual pattern in which alternate movements of legs IV throw silk onto a prey suspended on the web. At the retreat, she releases the prey and starts to rotate over the prey, eventually fixing threads over its legs. While rotating, keeping the prey below her cephalothorax, the spider repeatedly dabs the spinnerets over the substrate and the prey, so as to immobilize it. There are variations in this wrapping technique; sometimes the spider fixes a few threads over the prey and sometimes they do not wrap it at all, and start the ingestion right away. Some scientists describe a similar kind of wrapping in the Theraphosidae, Tenebrionidae and Agelenidae. These spiders wrap the prey by moving their entire body and spinnerets, fixing threads over the dorsal portion of the prey. *Badumna* also wraps the prey by moving the body, but there are slight differences as to where the spider fixes the threads. Following the proposal of these researchers, the wrapping by moving the body is the ancestral condition in araneomorphae, and this behavior is a homology in all these groups, now including the Desidae.

Supported by: CNPq

8.25 Ultrasonographic evaluation of pregnancy in the snake *Boa constrictor constrictor* (red-tail boa constrictor)

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Introduction: The red-tail boa constrictor (*Boa constrictor constrictor*) is a snake species found in the Neotropical region. They are viviparous and kill prey by constricting them with the robust body which may reach about 4 m in length (Gomes *et al* 1989). Since the physical examination is limited in reptiles, the semiological process of the internal organs is complicated. The ultrasonography is the easiest method to evaluate the internal organs, particularly those of the reproductive system. **Objectives:** The objective of this study was to describe the stages of embryonic development in the uterus of *Boa constrictor constrictor* through ultrasound. **Methods:** The *Boa constrictor constrictor* (MIB N° 3640) under study is kept in the Biological Museum of Butantan Institute with one male and one female of the same species. It is 2.5 m in total length with a weight of 10.5 kg. Between December 2009 and March 2010, three ultrasound examinations were performed: (1) with portable ultrasound Sukuba 4500, (2) with color Doppler ultrasonography in the IVI (Veterinary Institute of Image) and (3) with ultrasound My Lab 30 Gold Vet and My Lab Five Vet (Cimex-Esaote). **Results and Discussion:** In the spring, the female had an increase in abdominal size. In the first (Dec. 12, 2009) and the second (Dec. 15, 2010) ultrasonographies, 28 eggs were observed in uterine chambers. Sonographic images were hyperechogenic with hypoechogenic peripheral region, with no visualization of cardiac activities such as flickering motion. In the last ultrasonography (Mar. 26, 2010), many eggs were observed together, making it impossible to count individually. On Mar. 29, 2010, 25 offspring were born together with three atresic eggs. The offspring were born in early autumn of 2010. Therefore, we suggest that increased abdominal size was due to fertilization (ovulation) that occurred only in early spring (September). The female may have stored sperm in the reproductive tract during the winter for the fertilization and the pregnancy beginning in the spring with a higher temperature. This fact is corroborated by the high percentage of offspring and few atresic eggs in early autumn. Ultrasound is thus considered the easiest method to diagnose and demonstrate the gestational process.

8.26 Body plasticity and chemical communication: morphology of the pheromone glands among the gymnophthalmid lizards

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Introduction: Lizards make great use of pheromones for chemical communication with conspecifics and with the environment. These substances are, in general, secreted by epidermal glands located in femoral, cloacal and/or ventral regions. The small lizards of the family Gymnophthalmidae are distributed in different types of habitats, and some groups have as common feature progressive levels of appendicular reduction or even the total loss of limbs, associated with grades of adaptation to the fossorial environment. This phenomenon seems also to be correlated with the arrangement and functioning of the pheromone glands.

Objectives: The aim was to understand the role and morphology of these glands in chemical communication through the study of the pheromone glands in five psamphylic species of the tribe Gymnophthalmini, living in sandy environment, and with different levels of limb reduction. **Methods:** The glands of *Calyptommatus leiolepis*, *Nothobachia ablephara*, *Procellosaurinus tetradactylus*, *Psilophthalmus paeminus* and, *Vanzosaura rubricauda* were removed, fixed in Karnovsky solution and embedded in historesin. The sections were stained with toluidine blue-fuchsin for the general study of glandular morphology. **Results and Discussion:** The glands are present only in males. In *P. tetradactylus*, *P. paeminus* and *V. rubricauda* they are located in the femoral region, arranged in a row below the dermis, forming a glandular cord. In *C. leiolepis* and *N. ablephara*, which have a significant limb reduction, they are arranged side by side in the pre-cloacal region. In all species, the glands show an arborescent shape and are divided into lobules composed of peripheral germinative cells and secretory cells, in the internal region. At the end of their maturation, the secretory cells are totally filled with granules, die and eventually desquamate from the secretory epithelium, becoming part of the solid secretion plug, which permanently obstructs the duct. This process is typically holocrine and must generate an intense cellular turnover within the gland: at the same time that a dead cell desquamates, another germinative cell takes its place. The secretion is deposited on the substrate through a pore opening in the center of a scale. The general morphology of the glands seems to agree with the psammic environment in which these animals live. The brittle nature of the secretion plug must contribute to the rapidly pheromonal dispersion as the animal moves. Moreover, the primary role of chemical communication among these animals seems to be related to sex, since glands seem to be absent in the females.

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8.27 Ultrasound monitoring of male and female gonads in some species of venomous snakes kept in captivity at the Laboratório de Herpetologia - Instituto Butantan

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Introduction: The use of ultrasound examination in small animals as a diagnostic tool has increased in the last two decades, mainly because it is a non-ionizing examination, painless (does not require sedation of the animal) and noninvasive, and allows visualization of the internal architecture of organs. Although the use of this examination is being currently used on wild and exotic animals, few studies have been published. Studies on snakes are more restricted and are based mainly on the study of non-venomous snakes. Ultrasound examination can be extremely important in reproductive studies of snakes, indicating gonadal changes that occur in different seasons. **Objectives:** The aim of this study was to characterize the ultrasonographic image of the testes and ovaries and monitor changes occurring in these organs in four species of venomous snakes kept in captivity, throughout the seasons. **Methods:** From March to June/2010, six females and five males of the species *B.jararaca*, *B.fonsecai*, *B.atrox* and *Crotalus durissus terrificus* were examined monthly and had their gonads analyzed and measured with the aid of a portable ultrasound machine using a 6 – 13 MHz transducer. **Results and Discussion:** The testes and ovaries of the snakes are located in the final third of the body, a few inches below the gallbladder. The gallbladder has an anechoic content (easily located) and is a good reference point to locate the gonads. The testes are less echogenic than the adjacent tissues, have a homogeneous echotexture and an ellipsoidal shape. The testicular tunic is thin and hyperechoic. The ovarian parenchyma has an echogenicity similar to that of the surrounding tissues and also has a homogeneous echotexture. The ovarian follicles may have different echogenicity, depending on their stage: in phases I and II (<0.6 and 0.6 to 1.1 cm, respectively) the follicles are usually anechoic and in phases III and IV (1.2 to 2.0 cm and >2.1 cm, respectively) they are more echogenic, but hypoechoic in relation to the ovarian parenchyma. In March and April, the females of *B. atrox* and *B. jararaca* showed follicles in phases I and II, while in the months of May and June follicles in phases II and III were the most encountered. Females of *B. fonsecai* and *Crotalus durissus terrificus* showed follicles in phases I, II and III in March and April, and in the months of May and June follicles in stage IV were already observed. During the study period, no differences in testicle size was seen.

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8.28 Management of rattlesnakes in captivity: wood or plastic cages?

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Introduction: The Laboratório de Herpetologia at Instituto Butantan currently maintains about a hundred venomous snakes of the genus *Crotalus durissus* to yield poison for anti-venom production and research in animal biology. This species inhabits the open areas of central Brazil, the arid and semi-arid region of the Northeast, and the fields and open areas in the South, Southeast and North of Brazil. Before the reform of the Laboratory in 1993, all venomous snakes were kept in wood cages. From that year on these cages were replaced by plastic ones aimed at the improvement of the sanitary handling of the animals. Some months after the change the rattlesnakes began to have health problems such as pneumonia and attack by fungal dermatitis. *Aspergillus* sp, *Cladosporium* sp, *Fusarium* sp, *Trychophyton* sp, *Trichosporon* sp, *Geotrichium* sp and *Penicillium* sp were the fungi most commonly found. Most of these fungi are saprophytic but, when associated with the decrease in the host immune system, can become pathogenic. Six months after the change, some deaths occurred and all the rattlesnakes were again placed in the wood cages which, because of their porosity and wire mesh slide, provide a greater ventilation and lower humidity, thereby preventing the emergence of infections by fungi. However, the maintenance of rattlesnakes in wood cages hampers the appropriate health management of the snakes, since the porosity of the wood contributes to the development of microorganisms and their disinfection is not satisfactory.

Objectives: This experiment aimed to keep the snakes in plastic cages, improving their sanitary handling by increasing the number of ventilation openings. **Methods:** The room where the rattlesnakes are kept has 20 m² with six shelves. The average room temperature is 25 °C and the humidity is about 60%. In this experiment, started on February 2, 2010, 18 animals were divided into three groups of six animals each: a control group in wood cages, a experimental group in plastic cages (with an average of 50 holes of 3 mm diameter) and a third experimental group, also in plastic cages (with an average of 150 holes of 6 mm). All the animals of the experiment were kept on the same side of the room. All the snakes of the experiment received the same care as the others of the room. **Results and Discussion:** After five months of the experiment, fungi or other diseases in the snakes were not observed, even in the six specimens kept in common plastic cages, contrary to what occurred in 1993. This result is probably related to the higher frequency in the cleaning of the cages and its disinfection with sodium hypochlorite (4%). We can conclude that if appropriate conditions are provided, rattlesnakes can be kept in plastic cages without harm to their health.

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8.29 Aspects of the reproductive biology of *Crotalus durissus* Linnaeus, 1758 (Serpentes, Viperidae)

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Introduction: Brazil has only one species of rattlesnake, *Crotalus durissus*, and its accident is of great importance due to the high mortality it causes in humans. Reproduction is considered the main component of the life history of organisms; however, most of the work on *Crotalus* reproductive biology is done with North American species and works in Brazil are scarce, which is indispensable in future studies on the species. The litter size is one of the parameters of the reproductive biology, having the advantage of being compared between different populations and species. **Objectives:** The study aimed to determine whether there is any relationship between the litter and offspring size with maternal body length, compare the weight and measurements of young males and females and examine the reproductive effort of pregnant females, contributing to the knowledge of the reproductive biology of South American rattlesnakes. **Methods:** In this study 25 litters of *C. durissus* were used, with a total of 206 offspring. The 25 pregnant females were donated to the Instituto Butantan and had their biometric data and mass taken. At birth, the offspring were measured, sexed and their health conditions verified. Statistical analysis was performed using Fisher's exact test, t test and regression analysis and ANOVA. The level of significance was $\alpha = 0.05$ for all tests. **Results and Discussion:** The offspring's birth occurred mainly in January (n=11) and February (n=10). The average number of offspring per litter was 10.4 (2-28). Several authors suggest that litter size (number of offspring) in snakes is closely related to the body size of the females. The offspring were born with an average snout-vent length (SVL) of 31.23 inches (18-37 cm) and average weight of 24.15 grams (7 to 37.3 g). The ratio between males and females was 1:1. There was no significant difference in SVL ($t = 0.19$, $p = 0.84$) and weight ($t = 0.09$ and $p = 0.96$) for males and females, although males showed a greater relative length of tail ($t = 14.34$ $p < 0.0001$). Relationships were found between female SVL and litter size, and between maternal SVL and average size of their offspring. The relative reproductive effort (RRE) shows that the energy investment for reproduction in females is large (RRE= 42%). These snakes are born larger than other neotropical viperids and, in this way, they can feed upon larger prey in the early stages of life, which can promote a rapid growth and avoid predators quicker.

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8.30 Geographic distribution of the Neotropical pitvipers *Bothrops atrox* group (Linnaeus, 1758) (Viperidae: Crotalinae): A survey in the museum collection Alphonse Richard Hoge and troubleshooting systematics and conservation

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Introduction: The *Bothrops atrox* group is a very diverse snake taxon regarding morphological characters, macro- and microgeographic habitat occupation, sexual and ontogenetic variations, but low levels of mtDNA phylogeographic distinction. Depending on the author, *B. atrox* species complex includes the lineages of *B. atrox* (Amazonian distribution), *B. moojeni* (Central Brazil and surroundings), *B. marajoensis* (described for Ilha de Marajó) and Atlantic forest populations assigned as *Bothrops leucurus* (presently including *B. pradoi*). However, species boundaries are unknown. **Objectives:** The aim of this study was to define their boundaries in geographic distribution (through DIVA-GIS), searching for possible sympatric regions of occurrence and discuss phylogeography of *B. atrox* species group. **Methods:** We surveyed the registry books of the Museum Collection Alphonse Richard Hoge - IBU and scattered literature. **Results and Discussion:** Preliminary data pointed out approximately 54 localities for the presence of *B. leucurus*, 179 of *B. atrox*, 150 of *B. moojeni*, and 5 of *B. marajoensis*. Maps show sympatry areas between *B. atrox* and *B. moojeni* (northern Tocantins) where putative hybrids have been observed and collected. Three records of *B. marajoensis* on the continent were found, namely Monte Alegre – PA, Pinheiro – MA and Macapá – AP. *B. leucurus* including *B. pradoi* shows a wider geographic distribution along the Atlantic forest which should be taken into consideration when discussing its conservation status. We carried out population studies combined with molecular analysis to evaluate polymorphism as well as to check the status of those putative hybrids of the *Bothrops atrox* species complex.

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8.31 Occurrence of *Microsporidia* in larvae of black flies (*Diptera: Simuliidae*) in São Paulo State

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Introduction: Biological control of insect vectors has high efficiency because it is a more specific and has less environmental impact. Among the entomopathogenic agents, microsporidia may contribute to the management programs for the control of black flies, but little is known about its occurrence and efficiency in Brazil. The family Simuliidae includes vector species of pathogens, and biological control of this group is a challenge for public health. **Objectives:** The objective of this study was to investigate the occurrence and identify larvae infected by Microsporidia. **Methods:** Larvae were collected fortnightly during a period of four months (April-July 2010) in the municipality of Caraguatatuba, São Paulo. Larvae that showed symptoms of infection by microsporidian were dissected in distilled water, and fresh smears were made and stained with 10% Giemsa according to the method of Undeen and Vavra (1997). **Results and Discussion:** Of a total of 1213 larvae, 1210 were identified as normal and 3 larvae showed symptoms of infection by microsporidians, which were identified by morphological analysis as *Polydispyrenia* sp.