# II KARYO KINESIS symposium

Sharing and multiplying knowledge on cell, molecular and developmental biology

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SHARING AND MULTIPLYING KNOWLEDGE ON CELL, MOLECULAR AND DEVELOPMENTAL BIOLOGY

# II Karyokinesis Symposium 2020

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Kathleen Yasmin de Almeida
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Carla Eliana Davico
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# SUMÁRIO

# A REVIEW OF CONSERVATION GENETICS

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Planet earth is currently in time of great environmental changes. The expansion of human-related activities has taken a toll on natural habitats, influenced climate changes and became decisive factors in evolution and decline of natural populations. With this decline of natural populations happening in a scary fast pace, there has been an urge of working in practices where conservation of threatened species is a primary goal. Even though ecological, political and economic efforts are at high concern when trying to avoid extinction of threatened species, in order to create resistance at long term, genetics and its related considerations must be another focus point in conservation plans. Conservation genetics utilizes tools and concepts from evolutionary and molecular genetics to solve problems in conservation biology. Conservation genetics deals with the genetic factors that affect extinction risk and the genetic management plans required to low these risks. We can name major genetic issues in conservation biology, such as: deleterious effects of inbreeding on reproduction and survival, loss of genetic diversity and ability to evolve in response to environmental change, fragmentation of populations and reduction in gene flow, accumulation and loss of deleterious mutations, genetic drift overriding natural selection as the main evolutionary process, and many others. *Ex situ* conservation programs have as main objective to maximize genetic diversity for endangered populations, within regulated spaces, inside collaborating institutions (such as Zoos and Aquariums), some of them have as final goal the reintroduction in nature of individuals born in captivity. To achieve better results in captive breeding programs, a lot of institutions are now aiming to manage their populations as a bigger group, including different individuals from different institutions as part of the same group, increasing the genetic diversity, population size and geographic origin of the founders. Genetic management *in situ* are programs where we work with wild populations in their natural habitats. In wild populations, the most important challenge is to address genetic issues in fragmented and threatened species. The blue and yellow macaw (*Ara ararauna*) recent studies in wild populations are a great indicative of the necessity to study and manage wild populations before they decline to alarming numbers. We can work on increasing gene flow between fragmented populations of the same species, and attempt to increase genetic diversity and decrease genetic erosion before it is too late. Substantial progress was accomplished in conservation genetics since its foundation in the late 1970s. The Golden Lion Tamarin (*Leontopithecus rosalia*) project is a good example of all conservation efforts working together to reestablish an extremely endangered population. They were first studied in wild declining

populations, to have, later on, its captive populations managed together in *ex situ* reproductive programs. After that, more than 150 individuals were reintroduced to nature, and currently the genetic structure of the population is strong, although it still suffers from human-related activities. There's still a lot to be done. With recent technology advances, combined with the use of different tools on the field (GPS, mathematical analysis, genetic approaches, population studies, individual information, between many others), conservation biologists often collect a lot of data that need immediate attention. Genomic technologies are in the limelight as an efficient way to deal with data acquisition storage and analysis, bringing improved precision and helping making decisions on conservation genetics. Besides that, the integration of genetic information with other biological and non-biological factors are still a current challenge on successful conservation programs.

**Key-words**: Threatened species, Conservation *ex situ*, Genetic variability, Conservation biology, Genetic management.

## ADULT NEUROGENESIS IN THE CENTRAL NERVOUS SYSTEM OF THE CRAB *Ucides cordatus*

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Neurogenesis occurs in adults in the most of organisms, both vertebrates and invertebrates. Decapod crustaceans, including *Ucides cordatus*, have a well-organized central nervous system composed of fused ganglia ('brain'): the protocerebrum, deutocerebrum, and tritocerebrum. In semiterrestrial crabs of the infraorder Brachyura, the deutocerebrum, where neurogenesis occurs, processes the olfactory sensory information from the antennae. The deutocerebrum is composed of a pair of olfactory lobes (OL) associated with cell clusters 9 and 10 (Cl 9 and Cl 10), containing proliferating cells. The location of the neurogenic niche in brachyuran crabs has not been defined. Therefore, the main objective of the study was to localize and describe the neurogenic niche in the central olfactory system of the semiterrestrial crab *U. cordatus*. Adult males of *U. cordatus* (5.9 to 8.3 cm carapace width) were collected from mangroves in Rio de Janeiro, Brazil. BrdU (5-bromo-2'-deoxyuridine) was injected into the circulatory system of crabs in order to observe cells in proliferation (S phase of cell cycle). Immunohistochemical assays were proceeded in their brains (n = 4) for some markers: mature neurons (NeuN), immature neurons (III Beta-Tubulin - Tuj-1), glial cells (glial fibrillary acidic protein

- GFAP), phosphohistone H3 (PH3, mitose phase) and propidium iodate for cell nuclei. Serotonin (5-hydroxytryptamine – 5HT) labeling was used to reveal neuroanatomical aspects of the central olfactory system and the neurogenic niche. The results showed a zone of proliferating neural cells labeled with PH3 within Cl 10, which also contains (Tuj1)+ immature neurons, associated with a structure that has morphological characteristics of neurogenic niche, with a central cavity lined with a ring labeled with 5HT, also within Cl 10. Glial cells were identified near the proliferation zone and were labeled with GFAP. The proliferation zone and the neurogenic niche have main similar characteristics with other crustacean species from different infraorders and were defined: 1) the niche contains a central cavity lined by a 5HT+ ring; 2) the niche cells surrounding the central cavity are morphologically different from those in the vicinity of the niche, being mostly elongated; and 3) the niche is close to the proliferation zone, where cells are labeled with BrdU. These striking similarities in both morphology and cell populations to maintain adult neurogenesis between *U. cordatus* and other crustaceans suggest a common strategy for the generation of new neurons in adult brains. We may infer that highly adaptive species that live in variable places, such as the aquatic/terrestrial habitat of *U. cordatus*, rely on adult neurogenesis, and therefore, the presence and the specific features of the neurogenic niche described are important for the capacity to adapt and exploit this environment. This new information about neurogenesis in a brachyuran crustacean may stimulate studies comparing these invertebrates with vertebrates, including mammals, since they may share some aspects regarding the mechanisms of neurogenesis and regenerative potential.

**Key-words**: Decapod crustacean, Neuronal cell, Olfactory lobe, Mangrove crab, Proliferation.

## ALTERNATIVE METHODOLOGIES FOR THE USE OF ANIMALS IN RESEARCH: ANALYSIS OF A USER-FRIENDLY TOOL

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Historically, the relationship between human and non-human animals is ethically questionable and has undergone major changes as our understanding of ourselves, other animals and the world evolved. Since science is part of social constructions, its development depends on the influence of the social environment, beyond that, on the agreements involved in our relationship with other animals and the environment. Animal experimentation became a daily practice in the scientific community and its predictability value and the ethics involved in its use have not been questioned with quality and

propriety by the scientific community, which sees it in its great majority as an irreplaceable part. However, this practice was based on the use of a model, more specifically, an animal model. This, like any model used in the scientific world, only approaches reality. The decision whether to use the animal model in research is generally based on practical reasons such as: ease of handling, storage and reproducibility, cost, feeding, breeding. Rats and mice represent 77% of vertebrate animals used in the scientific environment. And, as a consequence, the naturalization of their use generates the prevalence of the choice of these animals even in situations where they present significant disadvantages. Furthermore, causing the underestimated number that more than 100 million animals are used annually for scientific purposes. Therefore, alternative methods to the use of animals in research are essential for an interspecific relationship based on ethics and for advances in the way science is produced. *In silico* models have been allied tools to develop new possibilities of alternative methods. Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) models – collectively referred to as (Q)SARs – are models that can be used to qualitatively or quantitatively predict the physicochemical, biological (for example, an (eco)toxicological endpoint) and environmental fate properties of compounds from the knowledge of their chemical structure. Corroborating for this purpose, the following work seeks to analyze and disseminate the OECD QSAR Toolbox software, a free application that supports reproducible and transparent chemical hazard assessment, focusing on toxicological tests for human skin sensitization. The development of a manual in Portuguese was carried out to democratize the use of this software for scientists who are Brazilian or who communicate in the Portuguese language. Therefore, being an ally of the movement that seeks to reflect on the relationship between scientists and animals. In order to make it possible to discuss ways to reduce, and even abolish in the future, the use of animals as tools by the scientific community.

**Key-words**: Bioinformatics, *In silico* experimentation, Animal ethics.

## ASSOCIATION BETWEEN POLYMORPHISM IN THE IL-10 GENE WITH BREAST CANCER AND OBESITY

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Breast cancer is the most common type of cancer among women in Brazil and worldwide (except for non-melanoma skin cancer). Breast cancer has an important impact on public health policies in Brazil,

because about 60 thousand new cases are detected every year and it is the type of cancer that most causes the death of women, more than 17 thousand deaths per year. There are several risk factors for the development of this disease and many of them associated with inflammatory conditions, such as obesity. Wherefore, we look for genes involved with the immune/inflammatory response that may be associated with increased risk of the disease. Interleukins (IL) are proteins involved in the immune response (inflammation, differentiation, proliferation and secretion of antibodies) and, therefore, can influence both tumor escape and disease progression, as well as risk factors. In several studies, polymorphisms in interleukin genes have been associated with different types of cancer. The present study investigated the polymorphism located in the promoter region of the IL-10 gene, known as IL-10 -1082 G/A (rs1800896), verifying whether it is associated with an increased risk for breast cancer, seeking to relate it to increasing the expression of this gene and, so, the amount of IL-10 produced. DNA samples extracted from peripheral blood from 167 women diagnosed with breast cancer and 117 healthy controls were used, all patients at the University Hospital of the University of Santa Catarina - Florianópolis. Genotyping was done by allelic discrimination using the real-time PCR technique. The assays were performed using TaqMan® SNP genotyping sets from Applied Biosystem, using the C__1747360_10 probe. There was no difference in the distribution of genotypes between patients and control subjects for the analyzed polymorphism. However, a trend was observed for individuals with IL-10 -1082 G allele to develop obesity in the group of patients with breast cancer ($p = 0.059$), with obesity being an important risk factor for this type of cancer. IL-10 -1082 G/A polymorphism of the promoter region of the IL-10 gene did not contribute to an increased risk of developing breast cancer, however it may be associated with the obesity risk factor, requiring a better investigation of this association through the analysis of groups with obesity and without obesity. Furthermore, we intend to continue the analysis with two other polymorphisms (-592 C/A and -819 T/C) of the promoter region of the IL-10 gene for investigating the association of genotypes and haplotypes and increased risk of the disease. Thus, composing a more complete study of the relationship between the promoter region of IL-10, an important region in the regulation of transcription, with breast cancer.

**Key-words**: Inflammatory response, Risk factors, Tumor escape, Promoter region.

## CAUSES OF TRASTUZUMAB RESISTANCE IN PATIENTS WITH HER2+ BREAST CANCER

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Breast cancer remains a public health problem, being the second leading cause of cancer death among women worldwide. The search for more specific and efficient treatments has been important for the reduction of comorbidities and deaths. Breast cancer is classified into molecular subtypes with HER2+ breast cancer being rich in human epidermal growth factor type 2 receptors (HER2 or c-erbB2). HER2 is a cell surface glycoprotein, belonging to the family of transmembrane tyrosine kinase receptors and is involved in cell growth, differentiation and survival. Among the drugs recommended for the treatment of women with HER2+ breast cancer, trastuzumab, a monoclonal antibody, had a huge impact on the survival of these patients. However, about 30% of patients treated with trastuzumab demonstrate innate or acquired resistance to this treatment. Therefore, a systematic review was carried out to identify the causes of resistance to treatment with trastuzumab, aiming to better understand the efficacy and the mechanisms of resistance to the drug in search of possible therapeutic alternatives. A systematic review was carried out according to the PRISMA criteria (Preferred Reporting Items for Systematic Reviews and Meta-analysis). The PUBMED and Scielo databases were chosen to search for relevant articles until May 2020. The terms used for the search were “Trastuzumab”, “Breast Cancer”, “HER2+” and “Resistance”, in addition to all their synonyms and entry terms registered in the databases. A spreadsheet was created with data on the mechanisms of resistance, treatment and analysis performed on each article. 1553 articles were identified in databases through search engines. After screening the titles and abstracts, 1379 articles were removed and 174 were kept. During the evaluation of the articles in full, 124 studies were excluded, thus leaving the 50 articles included in this paper. In total, 86 resistance mechanisms were found, 59 of which are different from each other, and these can be classified into: I) Obstacles preventing trastuzumab binding to HER2; II) Upregulation of HER2 downstream signaling pathways (PI3K/MAPK/SRC); III) Alternate signaling (also increasing the downstream pathways of HER2); IV) Alteration of other proliferative pathways; V) Alteration of apoptotic pathways. Trastuzumab acts on cancer cells that express the HER2 surface protein, preventing its dimerization with other receptors and, consequently, the cancer proliferation pathways. Several mechanisms have been identified that prevent trastuzumab from binding to HER2, such as mutated p95HER2 receptors, high expression of CD44 proteins, which hide part of the HER2 protein, low levels of HER2 and false positive tests for HER2. In addition to these, mechanisms of overactivation of HER2 downstream pathways were also identified, such as overexpression of HER2 itself, mutations in PIK3CA proteins and loss of PTEN, all leading to overactivation of the PI3K/AKT/mTOR pathway. These pathways may also undergo activation in an alternative way due to the high expression of the other receptors in the HER group (HER1, HER3) or IGF-1 (insulin-like growth factor 1). Other unusual and poorly understood mechanisms, such as long non-coding RNAs,

micro-RNAs and transcription factors, which are involved in transcriptional regulation and in processes such as autophagy, can interfere in cell signaling pathways, generating resistance to treatment with trastuzumab. Trastuzumab can sometimes be non-responsive or insufficient to prevent the proliferation of HER2+ breast cancer. The alternative is to use trastuzumab in combination with other drugs that act on the mechanisms of resistance, such as Lapatinib or Pertuzumab. In addition, other alternatives would be to seek new methods of testing HER2 to prevent false positives and the creation of a panel of genes that predict resistance to trastuzumab treatment.

**Key-words**: Trastuzumab, Breast cancer, HER2, Resistance

## CELLULAR IMPAIRMENTS INDUCED BY METHYLMERCURY IN DEVELOPING HEART

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Organisms exposed to methylmercury (MeHg) generally show morphological, metabolic and behavioral damage, being the central nervous system the main target of the studies. However, the effect of MeHg on the development of the heart is still poorly understood. The aim of this study was to investigate the effects of MeHg on cell cycle and apoptosis in cardiomyocytes of the ventricular walls, using embryos of *Gallus domesticus* as experimental model. Fertilized eggs were incubated at 37.5ºC (± 0.5) and 65% humidity. At 33 hours of incubation (E1.5, heart with a single chamber), embryos were exposed *in ovo* to 0.1 µg MeHg/50 µL saline solution, being analyzed at 10$^{th}$ embryonic day (E10, heart with four chambers) (n = 45). Control embryos were exposed to 50 µL of saline solution at E1.5 and analyzed at E10 (n = 45) (Ethics Committee of the Federal University of Santa Catarina, CEUA - protocol 5843231018). Immunohistochemistry and flow cytometry were performed using anti-phosphohistone H3 (mitosis marker), anti-p53 and anti-p21 (cell cycle markers), anti-H2A.X and anti- proliferating cell nuclear antigen (PCNA) (DNA damage markers), anti-caspase3 (apoptosis marker). Additionally, TUNEL assay was performed to identify apoptotic cells. MeHg did not induces DNA damage in cardiomyocytes. However, a significant reduction in proliferating cells was observed after MeHg exposure, in right and left ventricles (17.5 ± 0.42 and 15.62 ± 0.75 PHH3+cells) in comparison to control (21.12 ± 0.39 and 19.12 ± 0.39 PHH3+cells; $p < 0.05$). Regarding some proteins

involved in cell cycle, a significant increase only on number of p53+cells was observed in MeHg-exposed embryos (688.8 ± 93.5), compared to control (414.4 ± 49.9; $p < 0.05$). In addition, a significant reduction in caspase3+ cells were observed after exposure to MeHg (579.1 ± 102.7 caspase3+ cells), compared to the control (382.5 ± 59.28 caspase3+ cells; $p < 0.05$). However, a reduction in apoptotic cells was observed after exposure to MeHg, only in the right ventricle (32.50 ± 19.8 TUNEL+cells), compared to the control (59.50 ± 18.8 TUNEL+cells; $p < 0.05$). The results presented demonstrate that a single dose of 0.1µg of MeHg administered *in ovo*, in the first 33 hours of incubation, was able to promote important cellular changes in the walls of the cardiac chambers. The reduction in cell proliferation and cell death by apoptosis demonstrated here, helps us to explain the morphological changes, such as the reduction in the thickness of the trabeculae, observed in previous analyses. Together, the reduction of proliferation and apoptosis compromise the modeling of trabeculae necessary for the development of the ventricular wall.

**Key-words**: Embryotoxicity, Cardiac ventricles, Cardiomyocytes, Cell proliferation, Apoptosis.

## CHARACTERIZATION OF MATERNAL CARE IN TWO RAT STRAINS

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The mother-infant bond in the periods of gestation and lactation represents a great interest in several areas of research, highlighting topics such as maternal behavior, postpartum depression, maternal fatigue and maternal-infant relationship. The use of rodents proves to be an important study tool for the area, as they already have behaviors well described in the literature. This type of interaction between mother and puppies is extremely important for the development and good establishment of the offspring, being a critical factor for the growth and development of a series of behaviors, in addition to modulating a wide range of neural circuits. Currently, the study of postpartum development has been a great importance to understand the appearance of a series of disorders in adults, such as anxiety and hyperactivity, present in Attention Deficit Hyperactivity Disorder (ADHD), for example. SHR (spontaneously hypertensive) rats are used in works related to arterial hypertension, in addition to studies of emotionality, is considered the "gold standard" among the models of ADHD. In addition to these, rats of the SLA16 strain (SHR.LEW-Anxrr16), have the same SHR genetic profile, except for

a part of chromosome 4. However, when they are compared with SHR rats, they present lower blood pressure, memory, anxiety, and higher locomotor activity. Thus, the present study aims to establish maternal care standards for the cited strains and to evaluate whether they are related to the behavioral differences found in adult individuals. For that, rats of the SHR and SLA16 strains in reproductive age were mated and the behavior of care for the offspring was evaluated in the initial 8 days of lactation. After weaning, the sexual development of the litter, weight gain, and behaviors related to emotionality were evaluated. These mothers went through another reproduction to identify possible differences for a second pregnancy. The evaluation of the results was verified by test-t or ANOVA of repeated measures. In the analysis of the preliminary results of maternal care, it was possible to observe that mothers of the SLA16 strain spend more time in the blanket position during the second lactation ($F(1, 32) = 9.6500$; $p = 0.00395$, SLA16>SHR). Also during the second lactation, both strains spent less time in the arched position ($F(1, 32) = 8.2254$; $p = 0.00725$) and doing self-grooming ($F(1, 32) = 18.163$; $p = 0.00017$), but spent more time in the passive position in the same period ($F(1, 32) = 5.6346$; $p = 0.02378$). For measures of exploration of the home cage, it is possible to observe that in general SLA16 mothers are more active ($F(1, 32) = 4.6835$; $p = 0.03802$, SLA16>SHR) and that during the second lactation this activity reduced for both strains ($F(1, 32) = 4.1585$; $p = 0.04975$). These results demonstrate that, during the second lactation, there is a change in the observed behavior pattern, with SLA16 rats spending more time engaged in behavior related to maternal care and SHR I try to reduce this care. In the monitoring of the development of the litter, no differences were observed, indicating that these differences in care may not be directly influencing these characteristics. Thus, it is possible to conclude there are differences between the first and second lactation, suggesting the importance of learning in this process. Finally, SLA16 mothers, during the first lactation, were more active and, during the second, spent more time in the blanket position than SHR mothers.

**Key-words**: SHR, Development, Genetic models, Maternal-infant relationship.

# COMPARATIVE ANALYSIS OF THE FREQUENCIES OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) LINKED TO THE PIGMENTATION OF HAIR, SKIN AND EYES IN DIFFERENT LATIN AMERICA POPULATIONS

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DNA phenotyping is a relatively novel approach within the field of Forensic Genetics. The essential concept of this tool consists of predicting Externally Visible Characteristics (EVCs) through the study of single nucleotide polymorphisms (SNPs). Among the various uses of this type of prediction within police investigations, it is worth highlighting its potential to elucidate cases of disappearance and eyewitness testimonies. EVCs can be divided into two major groups: pigmentation characteristics and non-pigmentation characteristics. Most of the research and developments in the area are related to the first as predictive models have already been created to estimate eye, skin and hair color from DNA samples. The HIrisplex-S system is perhaps the most remarkable of these models, and its current version is based on data collected from 51 global populations concerning 36 SNPs of 16 genes. Further studies have attempted to predict EVCs in specific populations with the help of HIrisplex-S since the publication of its first version. Several different results were obtained, especially in admixed populations. This study's primary goal is to annotate frequencies for nine SNPs used in HIrisplex-S and other predictive models in Latin American populations and the first step to do this was select the point mutations to be analyzed. Three SNPs related to eye color were chosen: HERC2 rs12913832 and rs916977 were previously mentioned in different studies as having significant roles in the iris pigmentations. SLC24A5 rs1426654 provided substantial results in predicting blue eyes in the admixed Brazilian population. Another set of three-point mutations were evaluated in hair pigmentation, both MC1R rs1805008 and rs1805007 have been linked to natural red hair, and OCA2 rs1800407 is one of the SNPs used in the HIrisplex-S. Finally, TYRP1 rs1408799 and OCA2 rs1448484 were selected, taking into consideration several studies that correlate these SNPs to skin pigmentation. Frequencies to each of the aforementioned SNPs were obtained with the help of the webtool Allele Frequency Calculator (available at Ensembl CRCh37). The webtool generated a table to each of the SNPs imputed with frequencies from multiple populations, according to the study by 1000genomes. Four groups of individuals with tracked biogeographic origin in Latin America were present in the study mentioned above: Mexican Ancestors from Los Angeles (MXL), Puerto Ricans from Puerto Rico (PUR), Colombians of Medellin, Colombia (CLM), and Peruvians of Lima, Peru (PEL). The preliminary results show an above-average frequency of HERC2 rs916977 in all four populations, whereas the other HERC2 variant was not present. In the PEL group, the frequency SCL24A5 variant was 0.72, much higher than in Latin American populations. Hair color-related SNPs had low frequencies in all groups, this particular outcome was expected for natural red hair-related mutations, since it is not a common feature in Latin American mixed populations. Another point worth mentioning is the 0.51 frequency of the TYRP variant in PUR. Comparative analysis such as this are important to assist in elucidating the genetic components involved in the pigmentation of structures in populations that have not been thoroughly studied yet, such as in the cases of Central and South

American populations. Further studies should attempt to broaden the sample populations within the cultural area known as Latin America.

**Key-words**: Forensic genetics, DNA phenotyping, Externally visible traits, FDA, Appearance.

# COMPARATIVE GENOMIC ANALYSIS FOR PRE-DISPOSAL TO CANINE HIP DYSPLASIA

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Due to many years of artificial selection, dogs (*Canis lupus familiaris*) express several phenotypes. They were selected according to their breeders' preference to maintain a characteristic that benefits humans in their daily activities or even to reproduce desired traits for the offspring. With this practice, in addition to the desired characteristics, some susceptibility mutations are also selected, which often can be harmful to the health and welfare of the animal or even be incompatible with the animal's life. A disease of great interest in veterinary medicine is canine hip dysplasia, a multifactorial genetic disorder that causes the hip joint to malform, mainly affecting large dogs and possibly different genes across different breeds. Because of the complexity and high incidence of this disease, this study aims to identify genomic regions related to the affected phenotype so that veterinary medicine can intervene early to improve the animals' welfare. Identification of susceptible dogs could allow for surgery, which is impossible when classical diagnosis by radiography is used at a later age. For the analyzes, available genomic data was obtained for Basenji, Boxer, German Shepherd and Great Dane breeds from the NCBI GenBank database. According to the literature, some chromosomes related to canine hip dysplasia were selected, and the global alignment of such samples was performed utilizing the MUMmer (v. 4.0.0) software, using 70% minimum identity as criteria. For example, one of our preliminary results indicate that Basenji and Boxer's chromosome 1 presented 77.34% shared identity when aligned. This result was filtered to match an identity threshold value greater than 99%, which showed that the aligned chromosome shared 70.55% identity. Precise comparative genomics in the chromosomes involved with canine hip dysplasia shows a low degree of variation in identity and

synteny; however, regulatory regions could be present in the non-shared part of the genome. We will perform a global alignment among all the selected breeds and chromosomes in the same fashion for the remaining analysis, identify syntenic regions, and functional genetic variants in these breeds chromosomes. The preliminary analysis carried out here allows a succinct view of the degree of identity shared between dogs' chromosomes, opening the possibility for future research on the subject. Between-breed variation is estimated at 27.5% in the literature, while genetic variation between human populations is only 5.4%. Comparative analysis is a useful tool when guiding studies about disease predispositions, which can be applied in veterinary medicine to further our understanding of canine hip dysplasia in different breeds.

**Key-words**: Veterinary medicine, Disease, Dogs, Alignment.

## *DE NOVO* TRANSCRIPTOME ASSEMBLY OF CETACEANS SPECIES OF THE BRAZILIAN COAST

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In genome analysis, an important part of it is the identification of transcripts. In addition to providing information about which genomic sequences are being expressed in a given situation and which cells or tissues are being exposed. It is set as an emerging field due to the increased concentration of emerging pollutants released into aquatic environments, thus initiating a chain of response mechanisms modulating gene expression and physiological changes in underwater organisms. *De novo* sequence assembly is a method that makes it possible to determine a transcript when there is no other known transcriptome or genome available for use as a reference, as in this case, and thus, presents a greater complexity of genomic data available for analysis. As shown throughout this text, the identification of transcriptomes in tissues for two sentinel species of cetaceans: *Balaenoptera brydei*, and *Stenella longirostris*, provides more data of great importance for a significant role in the control of contaminants on the coast. The transcriptomes' sequencing was performed using the Illumina HiSeq 2500 sequencer (Illumina Inc.), using the paired-end methodology, resulting in up to 100 bp sequences. For the initial analysis of the transcripts' qualities, the raw sequences were submitted to FastQC v.0.11.8 and subsequently by using the Trimmomatic program, artifacts and low-quality bases were eliminated. The transcriptomes' assembly was performed with the trimmed reads using the *de novo*

methodology through the program Trinity v2.8.5. The assemblies' quality evaluation was carried out through the set of tools indicated by the Trinity program, with N50 contig values. After trimming, the high-quality score results for the reads obtained a logarithmic precision of 99.94% on average in the Quality Score scale for both species. The initial sets generated from Trinity ranged from 37 to 45 million transcripts. In the search comparison surveyed, an evaluation of the quality metrics given by the N50 based on all transcripts by their size, led with 2180 and 2316 transcripts for each species respectively, with more than 95% of the reading mapping, considered an indication of a good quality set. The identification of transcripts with high coverage by homology was analyzed by BLAST +, and the mapping of reads using the transcriptome constructed as a reference was constructed by Bowtie2 mapper. The integrity of those encoding protein's gene pool was assessed using the BUSCO pipeline, revealing that most of the significant genes of the species were retrieved, specifically, from the 4104 single-copy ortholog dataset, only between 14 and 17% of the total ortholog were classified as absent in our assemblies, a good coverage of the transcriptomes for these species. The ortholog search recovery for 'complete' and 'duplicate' was 76% on average of BUSCOs completely recovered, of which 41.9% were duplicated for *Balaenoptera brydei*, and 39.5% for *Stenella longirostris*. In addition to providing inference about the integrity of the assembly, this study presents pioneering sets of protein-encoding transcriptomes and its comparison across these specific groups, reinforcing the importance of ongoing studies of the Brazilian ecosystem's sentinel species.

**Key-words**: Bioinformatics, Ecotoxicogenomics, Biomonitoring.

# ECOTOXICOLOGICAL EVALUATION OF COMMERCIAL NANOSILVER-BASED PRODUCTS VS BIFUNCTIONALIZED SILVER NANOPARTICLES: TOWARDS NEW ENVIRONMENTALLY SAFE NANOSILVER-BASED PRODUCTS

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Silver nanoparticles (AgNP) are one of the most produced and used nanoproducts in the entire world due to their unique properties, especially as a biocide. AgNP have been widely applied against

bacterial and fungal proliferation and they were proved to have a synergistic effect when mixed with antibiotics. Moreover, in recent years other applications were developed thanks to their highly tunable properties, for instance, AgNP as plasmonic nanosensors for the selective detection of heavy metals in water. However, as the number of nanoparticle applications increases, also are the chances of uncontrolled release and discharge into the aquatic environment, with the consequent interaction with inorganic and organic compounds and biota leading to possible deleterious effects. Therefore, ecotoxicological assessments must be carried out to understand, and in a best-case scenario prevent, the vulnerability of the ecosystems. This study aimed to evaluate and compare the ecotoxicity of novel AgNP bifunctionalized with citrate and L-cysteine (AgNP Cit/L-cys) (5 nm-sized) with a commercial formulation containing AgNP functionalized with polyvinylpyrrolidone (PVP) (AgNP PVP) (nanArgen®) (20-40 nm-sized). Laboratory assays were carried out employing two test species: the microalgae *Phaeodactylum tricornutum* and the crustacean *Artemia franciscana*. The microalgae were exposed to 0, 1, 5, 100, 500, and 1000 $\mu g.L^{-1}$ of AgNP Cit/L-cys or AgNP PVP (with six replicates per treatment), and after 72 h, the growth inhibition was analyzed. The assays were carried out with standard medium (F/2 prepared with natural seawater -NSW-) at controlled temperature (21 ± 1 °C) and in constant light. *A. franciscana* was exposed to 0, 0.1, 1, 10, and 100 $mg.L^{-1}$ to AgNP Cit/L-cys or AgNP PVP (with three replicates per treatment), and the mortality was evaluated after 24 and 48 h. The assays were performed using stock dispersed in NSW, at controlled temperature (25 ± 1 °C), and in dark conditions. The results showed no significant toxic effects to both test species in case of exposure to AgNP bifunctionalized formulation while, on the other hand, the commercial product containing AgNP PVP caused a dose-dependent growth rate inhibition in *P. tricornutum* starting from 100 $\mu g.L^{-1}$. In the case of *A. franciscana*, mortality was observed already after 24 h at 100 and 1000 $mg.L^{-1}$ of AgNP PVP formulation, which was exacerbated after 48 h at the highest concentration. The obtained data suggested the ecosafety of AgNP Cit/L-cys, in comparison with the commercial nanoproduct (AgNP PVP), probably due to the coating composition. AgNP Cit/L-cys coating design, in fact, probably played a key role in reducing the release of Ag ions, which represents one of the most important toxicity mechanisms of AgNP in aquatic biota. Currently, the use of AgNP is not strictly regulated by the authorities even if their frequency of use and application in commercial products is increasing. This situation has generated an increasing load of AgNP with unknown toxicological implication to enter the aquatic systems since no attention was given to their chemical design. The importance of this study lies in the need for development of more ecofriendly nanomaterials. Overall, we highlight the importance of nanoparticle design and encourage further studies to evaluate ecofriendly alternatives to take advantage of the increasing nanotechnological developments.

**Key-words**: Nanoecotoxicology, Ecosafety, *Phaeodactylum tricornutum, Artemia franciscana*.

# EFFECT OF THE CONDITIONED MEDIUM OF ABDOMINAL ADIPOSE STEM CELLS IN COMBATING HUMAN FACIAL ADIPOSE ATROPHY, *IN VITRO*

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Subcutaneous adipose tissue (AT) is important for nutrition, controlling the hair follicle cycle and the skin repair process. In aging, the volume and activity of AT are reduced due to the gradual degeneration that it suffers with age. Environmental factors, such as ultraviolet A (UVA) radiation, cause photoaging, intensifying AT atrophy. This UVA radiation induced atrophy occurs in many exposed skin regions, especially on the face, one of the most exposed to the environment. The reduction in the AT layer is attributable to the lower potential of adipose stem cells (ASC) or adipocyte precursors to generate adipocytes (adipogenesis) and to the latter cells not being able to accumulate triglycerides in their cytoplasmic (lipogenesis). High levels of oxidative stress, common in aging, induce subcutaneous AT lost. The ASC conditioned medium (CM) obtained from the abdominal subcutaneous region proved to be able to protect fibroblasts from photoaging due to its antioxidant properties that combating the effects of oxidizing agents such as UVA radiation. Our objective was to evaluate the ability of the abdominal ASC-CM to mantain he adipogenic potential of facial ASC against UVA radiation. Material and Methods: Facial ASC (fASC) obtained from 5 female donors with an average age of 55 years old were previously treated for 24 hours with abdominal ASC-collected from 3 female donors with an average age of 40 years old. Afterwards, fASC were irradiated with UVA radiation (10 J/cm$^2$) and induced to differentiate to the adipogenic lineage *in vitro*. The DCFH-DA assay was also performed, which quantifies reactive oxygen species (ROS) by detecting the fluorescent compound 2'–7'–diacetate dichlorofluorescein (DFC) by fluorescence spectroscopy at 529 nm. Our results demonstrate that the abdominal ASC-CM treatment had no effect on triglyceride accumulation during adipogenesis of facial ASC irradiated with UVA radiation *in vitro*. However, cells previously treated with ASC-CM were able to reduce the accumulation of ROS when compared to non-irradiated cells. Discussion: UVA radiation generates photoaging by provoking oxidative stress, which, in the long term, induces elevated levels of cell death and senescence in tissues. When in unfavorable conditions, stem cells tend to reduce their cell activities, exiting the cell cycle in a

reversible (quiescence) or irreversible (senescence) manner. In both situations, cell differentiation is compromised. Therefore, therapeutic strategies to increase cell resistance against oxidative damage are sought. However, non-pathological levels of ROS are important for the transduction of intracellular signals related to adipogenesis and lipogenesis. Adipogenic differentiation is accompanied by increased levels of intracellular ROS. Although the CM of abdominal ASC reduced the accumulation of ROS in fASC, it did not maintain the adipogenic differentiation against UVA radiation. We hypothesize that UVA radiation might inhibit adipogenic differentiation by an ROS-independent pathway, since CM-treated cells showed approximate levels of ROS levels in relation to non-irradiated cells. More experiments are needed to confirm our hypothesis. Abdominal ASC maintains normal levels of intracellular ROS but does not mantain the potential for adipogenic differentiation of facial ASC against UVA radiation.

**Key-words**: Anti-aging, Regenerative medicine, Mesenchymal stem cell, Fat.

## EFFECT OF THE HERBICIDE ROUNDUP WG® ON THE CONTENT OF PROTEINS AND CARBOHYDRATES IN GERMINATIVE CELLS FROM *Macrobrachium potiuna* OVARY

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Roundup® formulations are among the most used glyphosate-based herbicides (GBH) and are used to control the growth of undesirable plants in agricultural and urban areas. Due to the runoff and leaching of agriculture areas, the herbicide can reach aquatic ecosystems and compromise environmental quality and non-target organisms, such as the freshwater prawn *Macrobrachium potiuna*, endemic to Brazilian fauna playing an important role in energy cycling and the flow of nutrients. Studies with crabs have shown fluctuations in the synthesis and deposition of carbohydrates, lipids and proteins in the cytoplasm of oocytes when exposed to GBH, interfering in ovarian maturation and, consequently, in the availability of energy for developing embryos. Considering (i) the increase in the use of GBH in agriculture; (ii) the species differentiated sensitivity to toxic agents and that (iii) the ovaries of crustaceans, when exposed to GBH, presented an impairment in the reproductive cycle; this study aimed to investigate the effect of Roundup WG® on the content of vitellin protein, total proteins, acidic and neutral polysaccharides present in the germ cells of *M. potiuna*. Females in early,

intermediate and advanced stages of ovarian maturation were collected at Cachoeira do Poção in Florianópolis/SC and acclimated for 7 days at the Laboratório de Reprodução e Desenvolvimento Animal. Then, prawns were exposed for 14 days to the concentrations of 0.065 and 0.28 mg/L of GBH, and a control group (dechlorinated water only). The ovaries were dissected and processed for semi-quantitative analysis of the total proteins (Coomassie Brilliant Blue) acidic polysaccharides (Toluidine Blue) and neutral polysaccharides (Periodic Acid-Schiff) present in the oocytes. The vitellin protein was immunostained with anti-vitellin antibody. The profile of reactions in pre-vitellogenic, vitellogenic and mature oocytes was verified through the Integrated Density analysis, available in the Image-J software. The analysis of pre-vitellogenic oocytes showed an increase in the content of acid polysaccharides ($p < 0.0001$) in prawns exposed to the concentration of 0.065 mg/L, whereas neutral polysaccharides ($p < 0.0001$) and total proteins ($p = 0.0070$) increased in both concentrations tested. In vitellogenic oocytes, the acid polysaccharides content increased in the concentration of 0.065 mg/L ($p = 0.0052$), there were no differences in the neutral polysaccharides content ($p = 0.6372$) and the total proteins content increased in both GBH concentrations ($p < 0.0001$). In mature oocytes, a reduction in the acid polysaccharides content at the highest concentration of 0.28 mg/L ($p < 0.0001$) was observed, while the neutral polysaccharides content ($p < 0.0001$) and total proteins ($p < 0.0001$) increased at the lowest concentration tested. Similarly, an increase in vitellin protein was observed in pre-vitellogenic oocytes at both GBH concentrations ($p < 0.0001$), whereas in vitelogenic oocytes this increase occurred only in females exposed to 0.065 mg/L ($p = 0.0342$). Such results suggest that Roundup WG® was able to accelerate ovarian maturation, which may have been caused by a possible action of the herbicide as an endocrine disruptor.

**Key-words**: Crustaceans, Ovarian maturation, Oocytes morphometry, Reproduction, Glyphosate.

# EFFECTS OF EXPOSURE TO 2,4-DICHLOROPHENOXYACETIC ACID HERBICIDE ON MORPHOMETRIC AND HISTOPATHOLOGICAL PARAMETERS IN ADULT ZEBRAFISH GILLS

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The pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) was the first synthetic herbicide introduced on the market in the early 1940s and continues to be marketed and used in many countries. There are over

1500 pesticides on the market that use 2,4-D as an active ingredient. It is used in terrestrial and aquatic environments and has the potential to spread beyond the applied site and contaminate surface and groundwater, which can directly affect aquatic organisms. According to the Agência Nacional de Vigilância Sanitária of Brazil, this compound is a little toxic, however, studies have shown that 2,4-D can cause toxic effects in non-target organisms. Thus, the objective of this work was to investigate the effects of 2,4-D on morphometric and histopathological parameters in zebrafish *Danio rerio* gills. Adult zebrafish were divided into 4 groups: Control (0.0 mg/L); 0.03 mg/L; 0.3 mg/L and 3.0 mg/L of 2,4-D (n = 3/group) and exposed for 7 days. After exposure, the animals were euthanized, the gills were dissected, processed, and stained with hematoxylin and eosin for morphometric and histopathological investigation. Morphometry and histopathology were performed by analyzing 30 micrographs per group (10/animal), a total of 120 micrographs. The parameters analyzed using the Image-J software were the length of the lamellae, length of inter-lamellar space, filament width, and basal width of the lamella. Histopathological changes were determined based on what has already been described in the literature. The histopathology scoring index (HSI) was calculated from the frequency and severity of each histopathological alteration. Statistical analysis was performed using the Kolmogorov-Smirnov normality test, followed by a one-way analysis of variance (ANOVA) and Dunnett's post-test. The gill morphometry showed that 2,4-D caused a decrease in filament width in groups 0.3 mg/L and 3.0 mg/L [$F(3,221) = 29.05$; $p < 0.001$] and in the length of interlayer space in all groups treated [$F(3,927) = 20.11$; $p < 0.001$]. Zebrafish exposed to concentrations of 0.03 mg/L and 0.3 mg/L showed an increase in lamella width [$F(3,874) = 13.45$; $p < 0.001$]. The concentration of 0.3 mg/L generated an increase in the length of the lamellae [$F(3,987) = 27.71$; $p < 0.001$]. Histopathological analysis showed that the group exposed to 0.03 mg/L showed an increase in the number of lamellar dilations and hypertrophies, but the presence of aneurysms was also detected, however, infrequently. The 0.3 mg/L group showed similar results about the frequency of lesions, however, a moderate frequency of epithelial detachments was detected in this group. In the group exposed to 3.0 mg/L of 2,4-D, an increase in the frequency of lamellar dilations, hyperplasia, hypertrophy, epithelial displacement, and aneurysms was observed. The HSI demonstrated that all groups exposed to 2,4-D presented moderate to severe lesions, with emphasis on the 0.3 mg/L and 3.0 mg/L groups that presented greater severity in the branchial epithelium compared to control [$F(3,8) = 4.95$; $p < 0.05$]. This work was a pioneer in investigating the effects of 2,4-D on the branchial morphology of adult zebrafish. Because gills are in direct contact with water, they can undergo morphological changes if they are subjected to a considerable degree of stress. In this study, it was observed that the concentration of 0.03 mg/L of 2,4-D is already capable of inducing morphometric and histopathological changes, and these effects are aggravated according to the increase in concentration. Thus, the morphological and histopathological changes found in this work are possibly an adaptive response mechanism that seeks to hinder the entry of 2,4-D into the body. This study

contributed to the understanding of the toxic effects of 2,4-D in aquatic organisms, providing evidence for its reclassification by regulatory agencies.

**Key-words**: Ecotoxicology, Histology, Xenobiotic, Fish.

# EFFECTS OF MECHANICAL STRESS IN ZEBRAFISH (*Danio rerio*) SKELETAL MUSCLE DEVELOPMENT

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Many pathologies associated with human aging are strictly related to the decrease in skeletal muscle tissue, including metabolic syndrome and chronic diseases. Considering this perspective, there are evidences correlating the number and volume of skeletal muscle fiber, as well as the proportion between slow twitch and fast twitch types, with the associated risks to these pathologies. With a high degree of similarity to the human muscular system, the zebrafish (*Danio rerio*) model fits the purpose of this project, in order to investigate the relationship between the parameters of mechanical stress in microgravity environment (2D clinostat) and hypergravity (vibrating platform), with possible alterations in the development of fast and slow muscle fibers of zebrafish embryos. Fifty zebrafish embryos will be used in this experiment from fertilization up to seven days post-fertilization. The embryos will be randomly divided in five groups as follows: Control Group (CG): embryos will be kept under regular breeding conditions; Microgravity Group (MG): embryos will develop inside a rotational wall vessel (2D clinostat), a device capable of mimicking the International Space Station (ISS) environment, once the upward hydrodynamic drag force compensates the downward gravitational force; Vertical Vibration Platform Group (VG): embryos will be stimulated for 10 minutes, every eight hours, with a 0.6 g sinusoidal mechanical wave in parallel to the gravitational force vector, produced by a vibration platform; Horizontal Vibration Platform Group (HG): embryos will be stimulated for 10 minutes, every eight hours, with a 0.6 g sinusoidal mechanical wave perpendicularly to the gravitational force vector, produced by a vibration platform; Multivectorial Vibration Platform Group (UG): embryos will be stimulated for 10 minutes, every eight hours, with a 0.6 g multivectorial mechanical wave in multiple directions, produced by a vibration platform; at 7 dpf, the larvae will be analyzed histologically using fluorescent microscopy. The hypothesis considers a directly proportional relationship between mechanical stress and the volume of skeletal muscle tissue, as follows (from lower to highest): MG, CG, VG, HG and UG. The upregulation of skeletal muscle

development (as hypothesized) may be explained through mechanosensors signaling as stretched-activated ion channels, cytoskeleton (microtubules) and the extracellular matrix Young's modulus. Confirmation of the hypothesis may outline a new approach for the treatment of sarcopenia, as well as its associated pathologies.

**Key-words**: Myogenesis, Teleost, Mechanotransduction.

# EFFECTS OF TRICLOSAN ON THE MODEL ORGANISM ZEBRAFISH (*Danio rerio*)

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Triclosan (TCS) is an antibacterial and antifungal agent widely used in personal care products, such as creams, deodorants and toothpastes, among others. One of the main sources of incorporation of TCS into the environment is through the waters of treatment plants and untreated. The zebrafish (*Danio rerio*) is a model organism widely used in ecotoxicological tests to assess the effect of TCS on non-target organisms. The aim of this review was to analyze the state of the art on the effects of TCS on zebrafish in the last 20 years. To do this, a bibliometric analysis and literature review was performed using Google Scholar (https://scholar.google.com/) and Science Direct (https://www.sciencedirect.com/) and articles in English published online were considered. The bibliographic search was carried out using the following combination of keywords: "triclosan-zebrafish" and "triclosan-*Danio rerio*" from 2000 to 2020. We found 33 papers that were included in the database. Of the total analyzed works, 87% were published in the period 2016-2020, where 68% of the studies were developed in early fish stages (embryonic and larval). The concentrations ranges tested were 8600 to 0.1 µg TCS $L^{-1}$, with 300 to 10 µg TCS $L^{-1}$ being the most widely used range (56%). The orientation of the effects evaluated was gene expression and ecotoxicogenomics (37%), followed by physiological and endocrine disruption studies (21%). Few studies have focused on prolonged exposure in adults (12%), compared to prolonged studies in early stages of development (44%). This review allows knowing the progress made in the last 20 years on the effects of the TCS in zebrafish, whose studies are primarily aimed at understanding changes in gene expression involved in physiology and metabolomics analysis of lipids and carbohydrates in early fish stages. Although most of the work uses sublethal concentrations, there are still few studies that evaluate environmentally relevant concentrations to study the effects of TCS. This review also highlights the need to increase ecotoxicological research involving adult organisms in zebrafish because they provide a more realistic

scenario of the potential risks that fish face due to emerging pollutants such as TCS and allow for the evaluation of differential effects in organs levels on fish. The literature analysis showed an increase in the interest in the adverse effects of TCS using zebrafish as a model species and the importance of continuing to investigate other relevant biological parameters in this species.

**Key-words**: Ecotoxicology, Review, *Danio rerio*, Emerging pollutants, Triclosan.

# ESTABLISHING ORTHOLOGY RELATIONSHIPS BETWEEN NUCLEAR RECEPTORS WITH POTENTIAL FOR APPLICATION AS CONTAMINATION BIOMARKERS IN AQUATIC SPECIES

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Due to anthropogenic activity, there is a significant increase in the concentration of pollutants in aquatic ecosystems, it originated mainly from industrial, agricultural, and domestic waste. These residues are made up of several potentially toxic chemical compounds - i.e., emerging contaminants and polycyclic hydrocarbons. Once in the aquatic environment, these compounds can interact with different species' biological systems and may induce a physiological disequilibrium in these individuals. Nuclear receptors (NRs) are characterized by the ability to act as toxicological sensors, since, for the most part, they are activated in the presence of a ligand molecule. Some pollutants can mimic this native ligand, modulating gene expression, and causing changes in homeostasis. Also, NRs constitute a protein class with highly conserved tertiary structures and nucleotide sequences. These characteristics make them important for evolutionary-comparative studies in the environmental monitoring of a heterogeneous group of species. Therefore, identifying NRs conserved between different aquatic species is of great interest for biomonitoring, since the molecular responses to environmental contaminants may exhibit similarities, revealing possible orthologous genes as candidates for biomarkers. Thus, to identify sets of NR orthologous genes within a group of vertebrates and invertebrates, proteomes of 11 aquatic species were obtained: three species of cetaceans, three of mollusks, two of fish, one of crustaceans, one of echinoderm, and one of brachiopod, through the Ensembl database. The *Homo sapiens* proteome was also included as a reference set. Subsequently, the protein sets were analyzed by the OrthoFinder program to define the

orthology relationships. After forming groups of orthologous genes, their annotation was performed using the Biomart tool (Ensembl) and TREMBL (UniProt). The orthologous groups were analyzed to identify the NRs family's genes and, once identified, they were organized according to the classification described in the literature, using *H. sapiens* as reference. From the results of OrthoFinder, it was possible to establish groups with consistent orthology relationships, being formed by only one subfamily of NR and shared by all or most species. We identified 48 groups of NRs orthologs within this criterion, nine groups including the 11 species, five groups with 10, and one group with 9. Although the number of identified NRs for each species varied between 13 (Crustacean) to 48 (*Homo sapiens* and *Danio rerio*), it was possible to categorize the nuclear receptors found for the seven subfamilies already described as receptors (NR0-NR6). The emerging contaminants mentioned above formed a class of pollutants composed of drug by-products, such as hormonal origin molecules, known as endocrine disruptors. When present in aquatic ecosystems, these by-products can cause a series of problems in the development and sexual maturation of different organisms when they bind to specific NRs, such as the estrogen receptor (ER). This receptor formed an orthologous group with nine species out of the 11 analyzed, having the potential to be studied more carefully and applied as a biomarker in environmental monitoring.

**Key-words**: Ecotoxigenomics, Xenobiotics, Proteomes, Orthologous Genes.

## EXPOSURE TO TAMOXIFEN INDUCES RELEVANT CHANGES IN GLYCOSPHINGOLIPID METABOLISM PATHWAYS IN OYSTER *Crassostrea gigas*

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Different studies have shown that xenobiotics affect the homeostasis of organisms, inhibiting or stimulating their gene transcription. Tamoxifen (TMX) is a selective estrogen receptor modulator commonly used to treat breast cancer. Metabolization is hepatic and the body does not fully absorb by-products. Its residues are eliminated in feces and urine and thus excreted in the environment. If there is no efficient waste treatment, contamination by TMX may occur, interfering with the balance of the environment. Different studies have already shown harmful actions in rats, humans, and some aquatic organisms, but the effect on bivalve mollusks is unknown. Bivalves are excellent sentinel organisms,

and *Crassostrea gigas* is an important species for monitoring xenobiotics. The cultivation of *C. gigas* is one of the most economically essential activities of aquaculture, making it even more necessary for the monitoring of biochemical and molecular alterations due to xenobiotic exposure. In this study, we intend to assess the impact of TMX on oysters of the species *C. gigas*. Six oysters were equally separated into control and exposed groups. The exposed group was submitted to 100 $mg.L^{-1}$ of TMX for 24 h. Then, RNA samples of digestive glandular tissue (GD) and gills (GL) were collected in each group and analyzed through the RNA-seq method. The quality of the sequencing of the samples was verified using FastQC. The STAR program performed the mapping with the genome *C. gigas* version v9. Counts of read mappings were obtained using htseq-count, and differential expression analysis was performed with DESEq2. Differentially expressed genes (DEGs) were defined by adjusted p.value < 0.05. The enrichment analysis was done with the topGO package for Gene Ontology (GO) (p.value < 0.05), and for the KEGG metabolic pathways, the clusterprofiler package was used (adjusted p.value < 0.05). After analysis, 2,942 DEGs were obtained for DG, of these, 1,701 genes were downregulated and 1,240 upregulated. In GL, 154 DEGs were obtained, of these, 100 genes downregulated and 54 upregulated. The main terms enriched in DG refer to transmembrane transport, cellular communication, and carbohydrate metabolism. In GL, the enriched terms refer to cell adhesion and intracellular movement. Four metabolic pathways have been enriched in DG, two of which are related to sphingolipid metabolism. It was demonstrated that TXM acts mainly by downregulating genes from the metabolism of *C. gigas* in both analyzed tissues. It is also evident that DG metabolism suffers more significant effects due to its characteristic of metabolizing compounds. Previous research on mollusks has shown that exposure to TXM causes signs of oxidative stress, including increased lipid peroxidation, increased activity of antioxidant enzymes such as Glutathione S-transferase (GST) and Glutathione reductase (GR). Among the DEGs found, GST1 isoform D and Glutathione-requiring prostaglandin D demonstrated an overexpression in glandular tissue, corroborating the evidence presented in previous studies. In humans, TMX has been shown to inhibit sphingolipid metabolism, glycosylation, and hydrolysis of ceramide. It was possible to observe that *C. gigas*, when exposed to tamoxifen, presents a reduction in the lipid and sphingolipid biosynthesis and, consequently, its transport. The enriched Glycosphingolipid biosynthesis (GB) pathways - lacto/neolacto and globo/isoglobo, showed downregulation in most of the genes involved, as observed in humans. The GB lacto and neolacto pathway are related to reproduction control. This may indicate long-term problems in the species reproduction, which has already been observed in fish exposed to TMX. It was possible to infer that oysters exposed to TMX caused disturbance of their homeostasis. There is an effect on genes, critical pathways for their balance, survival, and reproduction. However, more studies are needed to assess the impact.

**Key-words**: RNA seq, Ecotoxicology, Xenobiotics.

# FORENSIC GENETICS: AN EFFICIENT STRATEGY TO COMBAT BIOPIRACY

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Law 9605/1998 characterizes criminal actions against the environment and defines as a crime against fauna "to kill, persecute, hunt, catch, use specimens of wild fauna, native or in migration route, without the proper permission, license or authorization of the competent authority, or in disagreement with the obtained one" with increased penalty if the crime is practiced against rare species/risk of extinction or as an exercise in professional hunting, among other aggravating factors. Consequently, biopiracy can be described as the exploitation, manipulation, export or commercialization between international territories of biological resources, genetic knowledge and associated traditional knowledge. The *Phyllomedusa bicolor* anuran known as "kambô frog" or green frog is a perereca of the Phyllomedusidae family found in the Amazon, with restricted distribution. The Katukina Indians (Acre) are the holders of the traditional knowledge of the "frog vaccine" that uses the secretion of this animal with the promise of giving strength, resistance and even the cure of diseases such as cancer or depression. Some Amazonian tribes make medicinal use of this substance in ritualistic ceremonies and the therapeutic or medicinal use outside the cultural context is prohibited by ANVISA (Agência Nacional de Vigilância Sanitária). The animal's venom is scraped from the body with bamboo reeds and later applied in a series of superficial burns that can cause a poisoning reaction of about 15 minutes. Although no study has proven the effectiveness, the traffic of the substance and the use of the poison is increasing internationally, putting at risk not only the species but also the genetic patrimony of possible patents. Since 2019, in partnership with IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis), the group of Forensic Genetics of UFSC (Universidade Federal de Santa Catarina) has been identifying samples from inspection actions. Using mitochondrial markers as COI (subunit I of Cytochrome Oxidase) it is possible to rescue DNA from epithelial cells that are shaved in the collection of the secretion and identify with similarity rates between 98% and 100% even if they are two species of the same genus. Considering the restricted distribution of the species, seizures of biological material in Santa Catarina are treated as potentially resulting from trafficking. The utilization of molecular tools in support of justice has increased in the last few years, due the profile changes of the animal smugglers and strategies used by them. In the absence of the animal, in order to affect the association with trafficking, the molecular characterization of the seized biological sample is essential to formalize the accusation so the fiscalization authorities can realize their due

penalties. It is necessary to change the economic logic of trafficking: people need to understand that it is much more advantageous to protect biodiversity than to lose it. This information allows the creation of designatory associations for the sharing of benefits. The financial resource should be an invitation to vulnerable populations that preserving is an excellent business. The maintenance of genetic heritage should be a priority for governments, thus valuing the combined action of inspection agencies with academic research.

**Key-words**: Biopitacy, Phyllomedusa, Bicolor, Kambô, Frog.

# IMPACT OF *IL18* -137G/C (RS187238) POLYMORPHISM ON SUSCEPTIBILITY AND CLINICAL MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies, formation and deposition of immune complexes, inflammation process in several organs and tissue damage. Patients can experience symptoms progressively or faster, oscillating between activity and remission phases. The most described symptoms include fatigue, mild fever, myalgia, weight loss, peripheral lymphadenopathy, photosensitivity, arthritis and skin lesions. SLE etiology is not yet completely understood, however some factors have been described for their influence on the disease's pathogenicity, such as hormonal balance, environment conditions, immunological state and genetics. Around 90 loci have been associated with the disease, many of them are single nucleotide polymorphisms (SNPs) and play a role in SLE susceptibility or in its pathogenicity. Interleukin-18 is a pleiotropic pro-inflammatory cytokine encoded by the *IL18* gene, and the SNP -137G/C (rs187238) - located on the gene's promoter region - has been studied in several populations showing divergent results. Thus, the aim of this study was to analyze whether this SNP is associated with SLE susceptibility and its clinical manifestations in a Brazilian population. This project was approved by the university's Ethics Committee on Human Research. A total of 153

patients fulfilling the American College of Rheumatology (ACR) classification criteria for SLE were recruited, as well as 147 controls without evidence of said disease nor any family cases. Every participant signed an informed consent form (ICF) prior to blood drawn, authorizing the use of their samples as well as their clinical information in this and future studies. All patients answered a questionnaire regarding their symptoms and had hematological information collected from their medical records along with data from established inflammatory markers. DNA extraction from blood samples took place before this project started, and all isolated DNA samples were stored at the laboratory's DNA Bank, in order to be used in various studies, such as this one. Genotyping was performed by sequence-specific polymerase chain reaction (SSP-PCR) followed by agarose gel electrophoresis. To assess SLE susceptibility a logistic regression test was conducted while clinical aspects were tested with a Poisson regression analysis. A positive association between the *C_ genotypes for the SNP rs187238 and SLE was found and data revealed that these genotypes are associated with a 127% increased chance of developing the disease. Similar results were obtained for an Indian population but not for Europeans or Asians, showing the important role played by ancestry on association studies. The *C_ genotypes were also shown to be associated with clinical manifestations such as photosensitivity, malar rash and Raynaud phenomenon. In addition, patients with said genotypes have a 27.2% increased chance of having the above cited clinical outcomes altogether. Therefore, the assessed SNP appears to be directly associated with SLE manifestation in the Brazilian population, moreover, exerting an effect in its clinical aspects. Investigating SNPs associated with autoimmune diseases is a slow and difficult process, due to the elevated number of said polymorphisms involved in the conditions and the strong influence of ancestry on results. Despite these obstacles, association studies are important for their contribution to develop areas such as pharmacogenetics and personalized medicine, improving diagnostics and treatment.

**Key-words**: Interleukin, Immunogenetics, Autoimmunity, Association Study, Cytokine.

## *IN SILICO* PREDICTION OF NEW *Phaseolus vulgaris* MIRNAS RELATED TO PLANT PATHOGEN

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MicroRNAs (miRNAs) are a class of non-coding small RNAs involved in the regulation of several cellular, physiological and stress-responsive processes in plants. MiRNAs are well characterized in the plant-pathogen interaction, where they perform an important role in the plant defense or in the pathogen establishment. The common bean (*Phaseolus vulgaris*) is an important crop for human nutrition and is hardly affected by the anthracnosis. This disease is caused by the *Colletotrichum lindemuthianum* one of the most damaging seed-borne fungi. Considering the importance of this pathosystem, the present work intends to detect new miRNAs related to plant diseases in *P. vulgaris* for future investigation during *C. lindemuthianum* assays. In this way, the current study was aimed to predict and annotate the new miRNAs families in *P. vulgaris* based on conserved legume miRNAs throughout the genome employing the comparative method. Our methodology relies on the homology-based *in silico* approaches. Due to the conservative nature of miRNAs at inter- and intra-species level, sequence information of previously validated legume miRNAs related to plant-pathogen and plant-microorganism interaction were searched across the *P. vulgaris* genome. Initially, all Fabaceae (Leguminosae) miRNAs deposited in miRBase and PmiREN databases were selected resulting in 1224 miRNA families from 13 species. The next step was to investigate which of those miRNAs were related to pathogens or symbiosis interactions. The search was made at the Web of Science, NCBI, and Research Gate databases, using the crossing of the keywords "miRNAs" and "plant pathogen", "biotic stress" and the 12 Fabaceae species (excluding *P. vulgaris*). Only miRNAs families that were not yet characterized in *P. vulgaris* were selected. A total of 25 miRNA families from *Arachis hypogaea*, *Cicer arietinum*, *Medicago truncatula* and *Glycine max* were filtered and searched against the *P. vulgaris* genome deposited on Phytozome v12.1. Perfect matches of mature sequences were observed for the miRNAs families miR828, miR1507, miR1512, miR1521, miR4413, miR5037 and miR9750. From those miRNAs, the candidate precursor's sequences were selected and tested for the secondary structure using the RNA-folding program Mfold. Two conserved miRNAs, miR828 and miR5037, were *de novo* identified in *P. vulgaris*. For miR828 was detected only one member localized at chromosome 11, and derived from the 3' arm of the pre-miRNA sequence; while miRNA 5037 was provided from two different loci at chromosome 8, and processed from the 5' arm of the pre-miRNA. Additionally, the potential miRNAs targets were predicted in the psRNA target database using the three identified miRNAs as queries. The results of the analysis showed that pvu-miR828 regulates a MYB- transcription factor, while pvu-miR5037a and pvu-miR5037b targets a family of three distinct Plastid Movement Impaired 1 (PMI1) transcripts. These findings point to new possible routes for common bean miRNAs during pathogen interaction. Experimental validation of these new pvu-miRNAs and respective targets, as well as, their expression levels will be investigated during anthracnosis disease in *P. vulgaris*.

**Key-words**: Common bean, Legume miRNAs, *In silico* prediction, Plant-pathogen.

# INTERLEUKIN-10 GENE PROMOTER REGION POLYMORPHISM -1082 A/G (RS1800896) ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic Lupus Erythematosus (SLE) is a chronic, multisystemic and autoimmune inflammatory disease, characterized by the production of autoantibodies. It has varied clinical manifestations in periods of exacerbations and remissions, showing a higher incidence in young women in the reproductive phase. Its etiology is not fully understood and it can affect individuals of different ethnicities, gender and ages. The development of the disease is related to environmental, hormonal and genetic factors. The higher rate of occurrence in monozygotic twins and the increase in disease risk in the same family indicate a role of genetic factors in the pathogenesis of SLE. In addition to this, the production of cytokines by immune cells is highly unbalanced, especially in those with polymorphic gene sequences, which may be associated with an exacerbated immune response and increased disease severity. These changes are observed in Interleukin-10 (IL-10), since its expression is regulated by several polymorphisms in the promoter region. Previous studies reported that the single nucleotide polymorphism (SNP) -1082 A/G (rs1800896) in the promoter region of the *IL10* gene may be responsible for changes in the IL-10 expression. This variant has also been associated with SLE susceptibility. We evaluated the role of this SNP in the SLE susceptibility in a mixed population, from Santa Catarina located in the south of Brazil. Peripheral blood samples were collected from 108 women with SLE and 107 healthy women to be used as controls. All the participants signed the Informed Consent Form and this research was approved by the Human Research Ethics Committee of the Federal University of Santa Catarina. DNA extraction had already been done and is part of the laboratory sample bank. The SNP was genotyped by allelic discrimination using the real-time polymerase chain reaction (PCR), following the manufacturer's recommendations. The adherence to the Hardy-Weinberg (HWE) equilibrium was assessed by Chi-square test. Continuous and categorical variables were analyzed using specific statistical tests, such as t test, chi-square and regression. Allele and genotype frequencies are in HWE. It was observed that homozygotes for the A allele have a higher risk of developing SLE (odds ratio (OR) = 2.564, confidence interval (CI) 1.48-4.5) p = 0.001 than those carrying the G allele. It was also found that women with SLE carrying the G allele had menopause years earlier (43.4+-4) than homozygous AA (47.9+-5.4), with p = 0.038. Several studies

have shown controversial and heterogeneous results depending on the population analyzed. It seems that the GG genotype and the G allele in Asian patients has been associated with SLE susceptibility. However, the populations evaluated have little or no admixture, which can be one of the explanations to this variance among the results. This also reinforces the importance of analyze populations as used in the present study. In addition, studies showed that the activity of the disease is higher in pre-menopausal women. Besides that, there is an increase in inflammatory cytokines such as IL-10, along with TNF-a, after menopause as a compensatory mechanism, which may be related to an apparent decrease in disease activity in the post-menopause stage. The present study revealed for the first time the association between SLE susceptibility and the SNP -1082 A/G (rs1800896) in Santa Catarina women. It was also observed a role of this variant in the menopause age in this population. This is the first approach analyzing this population and further studies should be done to understand the role of this gene in SLE.

**Key-words**: Autoimmune diseases; Genetic association; Cytokines.

## IS THERE A ROLE OF TNF-A POLYMORPHISMS IN THE RHEUMATOID ARTHRITIS?

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The involvement of the pro-inflammatory cytokines, such as the TNF-a (tumor necrosis factor alpha), is a well-known characteristic of the rheumatoid arthritis (RA). The TNF-a is coded by the *TNF-a* gene, that presents polymorphisms in its promoter associated with alterations on the levels of this cytokine. The polymorphism rs361525 (-238 A>G) already proved to be functional and associated with the development of symptoms in RA. The aim of this study was to relate this polymorphism with RA susceptibility and its clinical symptoms in a Brazilian population. Methodology: Peripheral blood samples from previously collected patients were used. The DNA of the samples was extracted and stored in the Sample Bank of the Laboratory of Genetic Polymorphisms (LAPOGE - UFSC). All individuals signed the Free and Informed Consent Term (TCLE) and this research was approved by the Human Research Ethics Committee of the Federal University of Santa Catarina (CEPSH/UFSC). It was analyzed 110 RA patients and 113 control samples through Real Time PCR (polymerase chain reaction) with Applied Biosystem's TaqMan® SNP (single nucleotide polymorphisms) rs361525 assay, sonda C___2215707_10. The genotypes were grouped in GG versus A carriers. The susceptibility was assessed by logistics regression and the clinical data were analyzed through Poisson's regression. Sex and age were used as co-variations. Both samples are in Hardy-Weinberg

equilibrium. The allelic frequencies founded in RA patients were G = 0.885 and A = 0.115. In the control sample, they were G = 0.920 and A = 0.08. It was not possible to observe any association between the polymorphism and the susceptibility to RA. The clinical data analyzed were disease's state, rheumatoid vasculitis, synovitis, rheumatoid nodules, cardiopathy, anemia, leukopenia, leukocytosis, thrombocytopenia, rheumatoid factors and anti-citrullinated peptide. It was not observed any significant relation between the polymorphism and the development of these symptoms. It was not observed any relation between the polymorphism and RA, as well as its clinical symptoms. We will enhance the sample amount, as well as analyze other polymorphisms on the same gene for a real perspective of its interaction with RA. Therefore, this study can add information about the importance of these variants to the susceptibility and the clinical condition on this very relevant autoimmune disease in an admixed population such as the Brazilians.

**Key-words**: Rheumatoid arthritis, Cytokines, TNF.

## IS THERE AN INFLUENCE OF POLYMORPHISMS IN INTERLEUKIN 10 GENE IN BREAST CANCER?

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Breast cancer is the second most prevalent cancer in women around the world and in recent years its incidence has increased, both in developed and developing countries, and it is the main cause of deaths due to cancer in women. The biological processes that lead to the development of breast cancer are still not well understood, but the role of cytokines in carcinogenesis and cancer immunity is well established. The interleukin 10 (IL-10) is the main cytokine acting in several immune response views and it seems to be related to breast carcinogenesis. However, associations between *IL10* gene polymorphisms and susceptibility to breast cancer, or its clinical characteristics, show inconsistent results in the literature. The aim of this study was to verify the influence of variants in the promoter region of *IL10* gene and susceptibility to breast cancer or its clinical characteristics in a Brazilian population. It was analyzed two polymorphisms named -819 (rs1800871) and -592 (rs1800872) by PCR-SSP multiplex and the haplotypes derived by them in 197 women, being 148 breast cancer patients and 49 women without the disease. Breastfeeding was associated with breast cancer susceptibility (p = 0.025) since it was observed a shorter breastfeeding time in patients than controls. Several analyses showed a p-value close to significance, indicating a tendency. Smoking habits analyses showed more smoking or ex-smoking women in breast cancer group than in control one (p =

0.088). When the haplotypes derived by the variants were categorized in relation to IL-10 expression level, it was observed that the majority of women in the control group showed haplotypes of low or intermediate IL-10 expression (p = 0.076). It might suggest that high IL-10 levels can be related to susceptibility to breast cancer. The cancer patients group showed a higher prevalence of relatives with some cases of cancer in comparison with the control group. Analyzing the clinical characteristics of breast cancer group, it was observed a relation between IL-10 haplotypes and IMC (p = 0.014). Women with low or intermediate expression haplotypes were more prevalent in overweight group while women with high expression haplotypes were more prevalent in eutrophic group, showing an IL-10 role in body weight. Therefore, this study demonstrated that IL-10 can have a role in the breast cancer susceptibility and its clinical and pathological characteristics in a Brazilian population. More studies are necessary to reinforce these findings.

**Key-words**: IL-10, Cytokines, Breast neoplasm, Promoting region, SNPs, Tumor.

## MOLECULAR MECHANISMS OF BREAST CANCER METASTASIS BY GENE EXPRESSION PROFILING OF CIRCULATING TUMOR CELLS

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Breast cancer (BC) is the second most affected cancer in women worldwide. It is a disease with complex mechanisms and underlying molecular factors, highly heterogeneous and accompanied by a gene expression high diversity of patterns. Circulating tumor cells (CTCs) are cells detached from the primary or metastatic tissue of cancer. They are present in the bloodstream and are commonly involved in cancer metastases. CTCs are related to worse survival in patients with metastatic and non-metastatic breast cancer. In BC, there is a high prevalence of metastasis, and for this reason, it is necessary to develop new diagnostic strategies. Knowing the heterogeneous condition and precise characterization of the tumor transcriptome and the microenvironmental gene regulation of both tumor and circulating cells that will generate metastasis can improve the identification of putative biomarkers and facilitate early and non-invasive diagnosis. This study intends to explore the relationship between expressed genes in the tumor and CTCs. RNASeq data from 12 breast cancer tumor samples in stages II and III, 16 CTC samples, and five healthy breast tissue samples were obtained from the Gene Expression Omnibus database. After sample alignment and processing, differentially expressed genes

(DEGs) were identified using the DESeq2 package from R statistical software, defining the statistical significance at adjusted p value < 0.05. The resulting data were used in path enrichment analyses to characterize molecular functions, both made in R, through the topGO package for gene ontology (GO) and clusterprofiler for the KEGG analysis of metabolic pathways. In total, 3163 tumor DEGs were obtained, 75 genes upregulated and 3088 downregulated. For CTC, 4003 DEGs were found, corresponding to 543 upregulated genes and 3460 downregulated genes. The results of the enrichment analysis of the CTC samples revealed that the negatively regulated DEGs were enriched mainly in the spliceosome pathways, pathways related to cell adhesion, p53 protein signaling, cell senescence, apoptosis and immune response. The samples DEGs were negatively enriched, especially for transcription factors, via EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, apoptosis pathways, and immune response. There were no enriched pathways for positively expressed DEGs in both samples, tumors and CTC in these analyses. The GO enrichment analysis suggested that the CTC DEGs are enriched in biological processes (BP) mainly to transport Golgi vesicles, nuclear transport, and macrophage. Enrichment in molecular function (MF) was mostly for the corrective and co-activating transcription activity, Ras-GTPase protein binding, and growth factor binding. In the tumor, the BP DEGs enrichment was mostly for cell-substrate adhesion, adherens junction, organization of the extracellular and matrix structure, negative regulation of protein amino acid phosphorylation and signal transduction mediated by Ras protein and enriched in MF for binding Rab-GTPase and Rho-GTPase, binding to the Hsp90 protein and growth factor. For metastasis to occur, there must be cell adhesion changes caused by the invasion of tumor cells. Therefore, it is suggested that changes in genes and proteins that participate in cell adhesion and related processes may influence metastasis. Another factor that can enhance mutations in the genes favoring tumorigenesis is the enriched spliceosome pathway, affecting cell cycle control, angiogenesis, invasion and metastasis processes and apoptosis. The DEGs and pathways enriched in the present study can help understand the BC progression's molecular mechanisms serving as potential biomarkers. Many of these are related to cell adhesion, proliferation, or immune response, influencing metastasis by regulating functions or entire pathways.

**Key-words**: RNA-seq, Gene ontology, Differentially expressed genes, Biomarker.

## MOLECULAR TECHNIQUES FOR BIRD SEXING

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Bird sexing, whether from an individual, a population or a community is crucial to studies of management, reintroduction, natural history and conservation of species. With these data it is possible to understand factors that affect population growth, its structure and other behavioral aspects in the life cycle of these organisms. However, few birds have external morphological differences and plumage that allow inference the sexes in a non-invasive way. In the order Passeriformes only 31% of the species have some level of sexual dichromatism. Some characteristics are very subtle or only expressed during the sexual period or reproductive maturity, in which the individuals present a brood patch in the abdominal region for the incubation of eggs (males and or females) or cloacal protuberance, in which they form a pocket that stores the sperm (males), making it difficult and generating doubts in sexing. Therefore, molecular techniques have been a crucial tool for the validation and determination of sex in this group, aiming to complement studies on conservation, reproductive biology and population. For example, manakin males take two to four years to acquire mature plumage, and many juvenile males have similar plumage to females, making it impossible to identify with certainty the age and sex of these individuals. Ornithologists, when realizing the difficulty in identifying the age and sex of Pipridae, apply their knowledge about the plumage and molt sequence, in addition to the polymerase chain reaction (PCR) technique to identify the age and confirm the sex of this family of birds. In these manakins, the green plumage seems to be the only reliable one to identify the male gender. The DNA has been frequently used in the molecular technique for bird sexing in the last decades, because it is a precise and safe method for the sexual identification of these organisms, considering that the chromo-helicase-DNA-binding (CHD) gene is conserved in this taxon. In this technique the allele-specific primer pair P2/P8 is used for the CHD gene, located on the sex chromosomes (Z/W) of birds. After electrophoresis, the samples must present two alleles for females (ZW) and one for males (ZZ), being heterogametic and homogametic, respectively. Considering the difficulty in identifying the sex of birds through external morphology, we are testing protocols for molecular sexing a group of Passeriform and non-Passeriform sampled in protected areas located on the coast of Santa Catarina State. Blood samples were collected from individuals that did not present sexual dichromatism and stored in 100% PA ethanol in 1.5 mL microtubes. Our next steps are the extraction, quantification and amplification using the PCR technique, using the universal bird primers CDH1F and CDH1R. Afterwards, the amplification will be confirmed by agarose gel electrophoresis. We have not yet obtained results, but we want to clarify the importance of using DNA in molecular biology techniques as a tool for sexing birds in field surveys. And in our case, to improve the monitoring and population parameters of the avifauna in protected

areas of the Atlantic Forest in southern Brazil, contributing to the understanding of traits of the bird community that may serve as indicators of local biodiversity.

**Key-words**: CHD, Molecular sexing, P2/P8.

# NUCLEAR RECEPTORS PHYLOGENY IN CETACEANS: A PRELIMINARY STUDY INVESTIGATING EVOLUTIONARY MOLECULAR RELATIONSHIPS BETWEEN GENES OF ECOTOXICOLOGICAL INTEREST

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In view of the ongoing increase in marine pollution levels, mainly due to anthropogenic actions, improving ecotoxicological techniques is of noticeable importance. Under this perspective, the nuclear receptors (NR), for their remarkable role in environmental pollutants response pathways and the high degree of conservation of their sequences, allow to elucidate the evolutionary relationships between molecular response mechanisms to xenobiotics in different species, possibly even revealing candidate biomarkers. With this in mind, the present study brings NR nucleotide sequences from the unpublished transcriptomes of two cetacean species, *Balaenoptera brydei* and *Stenella longirostris*, which make it possible to investigate such evolutionary relationships within this group of aquatic mammals. Noteworthily, cetaceans not only comprise several currently endangered species but are also important bioaccumulators due to their high trophic levels. It was decided to conduct, before performing the orthology prediction proper, a pilot study to evaluate how the NR genes get arranged in nucleotide phylogenetic trees, providing insights on their evolutionary relationships. The NR genes selected for this preliminary study were Estrogen-related receptor alpha (ERR1) and Rev-ErbA alpha (Rev-erba), whose sequences from the newly sequenced transcriptomes were aligned by Multiple Alignment using Fast Fourier Transform (MAFFT) program with the corresponding nucleotide sequences, found by BLAST hits, from the cetacean species *Balaenoptera acutorostrata*, *Lipotes vexillifer*, *Orcinus orca*, *Phocoena sinus*, *Physeter macrocephalus* and *Tursiops truncatus*. Each alignment included a marine bony fish sequence as an outgroup: *Takifugu rubripes* for ERR1 and *Myripristis murdjan* for Rev-erba. When there were at least two transcript variants for the same species, only the longest one was selected. The resulting alignments had their hypervariable regions removed by Gblocks Server and

were converted from FASTA to Nexus format by EMBOSS Seqret tool. Lastly, the phylogenetic trees were generated from the final alignments by MrBayes program, using the nucleotide substitution models indicated by TOPALi software. In the resulting nucleotide trees, neither of the bony fish sequences rooted, so they were replaced by ruminants' sequences from *Bos taurus* and *Ovis aries*. However, they also got misplaced, so the phylogenetic trees were rebuilt without outgroups. The two final nucleotide trees showed the nodes bootstrapping values all above 98%, and in both phylogenies the only polytomy was between *B. brydei* and *B. acutorostrata*. The Rev-erba tree followed the species phylogeny, and even the distances between its splits were similar to previous estimations with other genes. The only exception was the distance between the radiation of Delphinoidea and its split from Lipotidae's clade, which was estimated to be much shorter than in previous studies. In ERR1 tree, the main difference from species phylogeny was the placement of *L. vexillifer* as a closer relative of *S. longirostris* and *O. orca* than *T. truncatus* and *P. sinus*. This study findings suggest that both genes sequences evolved, in general, concomitantly with most of the genome in cetaceans. The *L. vexillifer* affinity for Delphinoidea, found in both trees, indicates that its NR sequences must have changed little from their last common ancestor, which is surprising since it is one of the cetaceans that migrated to freshwater habitats, coming in contact with several new substances. It is also impressive how highly different is *T. truncatus* ERR1 sequence from the other two delphinids, as they are closely related. In sum, ERR1 and Rev-erba phylogenies in cetaceans reinforce evolutionary relationships found by previous studies and raise questions for further analysis. Future studies comprehending these and other nuclear receptors may manage to assess significant biomarkers for environmental contaminants.

**Key-words**: Cetacea, Ecotoxicology, Nuclear receptors, Phylogeny, Transcriptome.

# POPULATION GENETICS OF *Anopheles bellator*, ONE OF THE MAIN VECTORS OF BROMELIAD-MALARIA IN THE AMERICAS

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Malaria is a tropical disease caused by parasites of the genus *Plasmodium*, which are transmitted through the bite of mosquitoes of the genus *Anopheles*. According to the World Health Organization, in 2018 there were around 228 million malaria cases in the world. In the Brazilian Atlantic Forest region, the main species involved in the malaria transmission are *Anopheles cruzii* and *Anopheles*

*bellator*. Some studies have suggested that *An. bellator* is a species complex distributed throughout Atlantic Forest, such as *An. cruzii*. The purpose of this review is to gather the information available in the literature about the genetic differentiation between *An. bellator* populations from Atlantic Forest and to correlate with other complex species of *Anopheles* genus. The researches were carried out in the Scholar Google and PubMed databases using the keywords "*Anopheles bellator*", "genetic", "Atlantic Forest", "complex species" and "*Anopheles*". Studies using isoenzymes showed that *An. bellator* population from the Trinidad Island (Trinidad & Tobago) is genetically distant from the other Brazilian populations analysed from São Paulo (SP), Santa Catarina (SC) and Bahia (BA), which suggested an incipient speciation process. Among the Brazilian populations, the samples from SP and SC are genetically closer to each other and distant from BA samples. The use of timeless and Clock genes as molecular markers in different Brazilian populations of *An. bellator* corroborated with the isoenzymatic analysis, and showed that the BA population is genetically distant from those of the South and Southeast Brazil (RJ, SP, PR and SC). Different studies based on isoenzymatic, molecular and cytogenetic analysis have also been used for the study of different *An. cruzii* populations from the Brazilian Atlantic Forest. These studies showed that populations from SC, SP and the coast of RJ are genetically closer to each other when compared to the BA population, a similar result found in the studies with *An. bellator*. Still, one of the analysed populations from the interior of RJ, more specifically from Serra da Mantiqueira, also presented population structure. The most studied species complex is the *Anopheles gambiae* sensu lato, which comprises the main Africa malaria vectors (*Anopheles gambiae* s.s., *Anopheles coluzzii* and *Anopheles arabiensis*), as well as species that are not considered vectors, such as *Anopheles quadriannulatus*. The study of this species complex allowed the correct identification of the true vectors of malaria in Africa and helped in the elaboration of specific measures to tackle the disease. The genome sequencing of different species from *An. gambiae* s.l. has already been carried out, which allowed more in-depth studies, such as the analysis of speciation islands, helping to understand this species complex. In the future, these studies may determine which genetic units of the likely *An. bellator* complex are true vectors of the disease here in Brazil and help to understand the epidemiology of malaria, in addition to assisting in the implementation of vector control strategies.

**Key-words**: Species complex, Atlantic Forest, Malaria.

## POPULATION GENOMICS OF *Anopheles cruzii*, THE MAIN VECTOR OF HUMAN AND SIMIAN MALARIA IN THE ATLANTIC FOREST REGION

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Malaria is an infectious disease caused by parasites of the genus *Plasmodium* that can be transmitted to humans by female mosquitoes of the genus *Anopheles*. This pathology still affects millions of people globally. Although in Brazil most of the cases occur in the Amazon region, there is still transmission in other regions of the country, as in regions of the Atlantic Forest. In this ecosystem, the main vector is the mosquito *Anopheles (Kerteszia) cruzii*, which is also accused of being the only mosquito capable of transmitting *Plasmodium* sp. from non-human primates to humans. Despite its relevance as a vector, there are still few studies related to this mosquito, especially regarding the genetic and population structure of this species. Our group is pioneer in the study of the genetic differentiation of *An. cruzii* using multilocus analyzes, in different populations throughout the Atlantic Forest. Our previous results suggest that *An. cruzii* is a complex of at least three cryptic species: (i) the first with wide distribution along the Atlantic Forest, from south to southeast of Brazil, and two others located in (ii) Itaparica (BA) and (iii) Itatiaia/Bocaina (RJ and SP, respectively). Still, Bocaina (SP) is a region of sympatry, with reduction of heterozygotes, which is a clear evidence of speciation. In this sense, clarifying the population genetic structure of these species' complexes, as appears to be the case of *An. cruzii*, is a prerequisite for determining which members of the complex are true vectors of malaria. In addition, knowledge of the existence of these species allows the implementation of appropriate strategies of vector control to those that actually have responsibility in the transmission of the disease. In this scenario, we have as main objective to clarify for once the existence of a complex of cryptic species within the *An. cruzii* taxon. For this, we sequenced 12 genomes (Illumina) from different populations of *An. cruzii* and also other species of the subgenus *Kerteszia* from Florianópolis (SC), Serra da Bocaina (SP), Guapimirim (RJ), Itatiaia (RJ), Ilha Grande (RJ), Santa Teresa (ES) and Itaparica (BA). Genome sizes ranged from 164,548,160 bp to 172,647,140 bp, similar in size to the genome of *An. darlingi*, the main malaria vector in the Amazon region. We will also carry out population studies looking for regions of high divergence between the genomes of the different populations of *An. cruzii*, in order to confirm the differentiation between them and estimate the introgression between the different genomic regions.

**Key-words**: Malaria, *Anopheles cruzii*, Genome, Species complex.

# PYRIPROXYFEN INDUCES NEUROTOXICITY IN THE FOREBRAIN AND MIDBRAIN DURING THE DEVELOPMENT OF CHICKEN EMBRYOS

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Pyriproxyfen (PPF) is a pyridine-based larvicide that acts as an insect growth regulator. In Brazil, PPF has been used since 2014 in drinking water-reservoirs, mainly to control the proliferation of disease-transmitting mosquitoes. In 2015, there was an increase in cases of microcephaly in newborns in the country and, although this increase was correlated to Zika virus infection, the possibility of an association between cases of microcephaly with the use of PPF was raised. Thus, to investigate the neurotoxic effects of PPF, this study characterized the morphology and cellular events involved in forebrain and midbrain histogenesis after PPF exposure, using *Gallus domesticus* embryos, as an experimental model. Then, fertile eggs were incubated at 37.5 ºC temperature and 65% humidity (CEUA-UFSC protocol No. 5843231018). Sublethal concentrations of 0.01 mg/L PPF (n = 18) and 10 mg/L PPF (n = 18) used in the study, were defined by a survival curve previously. Non-exposed embryos were used as controls (n = 18). PPF exposure was performed *in ovo* after 24 h of incubation, which corresponds to the embryonic age (E1), and the embryos were analyzed in E10. Dissected brain was submitted to the histological routine to perform morphometric analysis, such as determination of cell layers thickness and counting of the numerical density of cells per area. Immunohistochemistry and flow cytometry techniques were performed to investigate the effect of PPF on the protein content involved in DNA damage, cell proliferation, apoptosis, and neuronal and glial differentiation. Morphometric analysis of the forebrain indicated a significant reduction in the thickness and numerical density of cells per area of ependymal and cortical layers in the groups exposed to the two PPF concentrations. In the midbrain, a significant reduction in the thickness of some of its layers, such as the ependyma and in layers I, IV-V, VI, VII, and X, was observed in the groups exposed to the two PPF concentrations. In addition, the numerical density of cells per area in the midbrain also significantly decreased in the ependyma and layers I, VI, VII, VIII, IX, and X. In the group exposed to 10 mg/L PPF concentration, there was an increase in DNA damage (using anti-H2A.X), a reduction in cell proliferation (anti-phospho-histone H3, a mitosis marker), and an increase in apoptotic cells

(TUNEL assay). There was no reduction in neuronal differentiation (anti-ß3 Tubulin) and glial differentiation (anti-GFAP). From the integrative analysis of our results, it is suggested that the increase in DNA damage may be related to the interruption of the cell cycle, which reduced the proliferative capacity, and when the repair was not carried out, the cell was taken to apoptosis. These impairments can affect the brain morphology, interfering with cell density, and cell layers thickness in the forebrain and midbrain. Together, the results reveal that PPF caused damage to the cellular architecture of the brain vesicles and, therefore, can potentially interfere with brain development.

**Key-words**: Central nervous system, Morphometry, DNA damage, Cell proliferation, Apoptosis.

## PYRIPROXYFEN TOXICITY IN VERTEBRATE EMBRYOS

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Pyriproxyfen (PPF) is a potent insecticide used to control various insects. PPF acts as an analog of the juvenile hormone, resulting in the insect growth inhibition in its larval stage. In several countries, PPF is used in agriculture and horticulture for insect control, and it is also applied, with the approval of the World Health Organization (WHO), in drinking water reservoirs to control mosquitoes that transmit diseases. After its application, PPF remains biologically active for up to 8 weeks, adsorbed on organic matter or water, and its metabolites may last in soil up to one year. To assess the effect of exposure to PPF on non-target organisms during the early stages of development, some studies have used vertebrate models in toxicity tests, performed on fish, amphibian, and rodent embryos, using different doses and forms of exposure. In this context, this work aimed to gather the latest data on the effects of PPF on vertebrate embryos. For this purpose, a critical literature analysis and review was performed. The databases used for the research were PubMed and ScienceDirect, using the keywords “pyriproxyfen AND toxicity”, “pyriproxyfen AND vertebrate”, “pyriproxyfen AND embryo”, “pyriproxyfen AND toxicity AND embryo”. When quantifying the number of works available in the databases, it was found a small number of publications, ten articles mostly published in 2015. It is believed that most of these publications justify their studies due to the possibility of a correlation between the increase in cases of microcephaly in newborns in Brazil and its use for mosquito control. Survival curves have been described for some vertebrate embryos exposed to PPF, such as *Danio rerio* (0.66 and 1 mg/L), *Rhamdia quelen* (170 mg/L) and *Xenopus laevis* (300 ppm). These divergences in the LC50 can be explained by the way in which the exposure was carried out, organisms age, the time

and duration of exposure to PPF, and the detoxification mechanisms among the species. Studies with embryos and larvae of *D. rerio* exposed to PPF report delay in hatching, reduced survival, behavioral changes, cephalic and heart deformities, increased abdominal edema and abnormal body curvature, apoptosis, and DNA damage. In tadpoles of *X. laevis*, studies point to developmental delay, reduced head size, reduced tadpole mobility, in addition to the accumulation of metabolites found in the liver and gastrointestinal tract. In tadpoles of *Odontophrynus americanus*, reductions in enzymatic and hormonal activities and an increase in heart rate were observed. In mouse pups, the main results indicated a reduction in body weight gain whose mothers were treated with PPF during pregnancy. PPF also increased the stillbirth rate and decreased survival in a dose-dependent manner. The weights of the liver, kidney, heart, and brain of newborns were reduced, and histopathological changes in tissues such as cell degeneration, vacuolization, inflammatory infiltration, congestion, and hemorrhage were also observed. In conclusion, there is considerable information on the effects of exposure to different concentrations of PPF during the development of vertebrates, which suggests caution in the use and management of concentrations of this larvicide. However, it is important to note that this field of study is promising, and new approaches are needed to understand the myriad of effects of PPF, especially developmental neurotoxicity.

**Key-words**: Pyriproxyfen, Embryotoxicity, Non-target organisms, Development.

## RELATIONSHIP BETWEEN SPRINT AND GENETIC POLYMORPHISMS IN SOCCER PLAYERS: A BRIEF REVIEW

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Soccer is a sport with different physical demands, such as muscular endurance, sprinting ability, ball control, precision passing and finishing, and others. These characteristics are increasingly being related to the body's innate abilities, such as the genetic background of each individual. The purpose of this bibliographic review is to correlate the sprint capacity with different genetic polymorphisms of soccer athletes. The search for articles took place in the Pubmed database on October 7, 2020 at 9:30

pm, using the main terms: “soccer”, “genetics” and “sprint”, and their MeSH terms, with the Booleans logical operators "AND" and "OR". The pre-established criteria for inclusion of articles were: publications in English or Portuguese; research with professional athletes and football amateurs; studies that link sprint capacity with genetic polymorphisms; collection of sprint data by the authors. The exclusion criteria were the failure of inclusion and review articles. The data collected for each article were: year of publication, title, authors, polymorphism studied, type of sprint measurement, distance and sprint result. Nine articles were found. One was excluded for being with rugby players, and two others for not having sprint data collected by the authors. Six articles were analyzed, with publication date in the last five years, excepting one (2011). All articles performed the sprint test, a method that uses photoelectric sensors (barrier, gates or sensors in the track) that accurately calculate the time the athlete passes through them. In general, the athlete is positioned behind the first sensor and other sensors are located at 5, 10, 20 and/or 30 meters away, making possible to calculate acceleration and speed. Of the six reviewed articles, five mentioned the *ACTN3* R577X polymorphism. Although most studies present the direct relationship between the “R” allele and a greater capacity for muscle strength and, consequently, a better performance is expected in the sprint tests, this occurred only in one article where, in the 30 m, RR/RX genotypes ran faster compared to XX athletes. For athletes with DD genotype (*ACE* I/D polymorphism), in the Under-14 and Under-17 it was noticed the motor improvement in performance in the 30 m sprint. The other three articles that mentioned this gene, showed no significant difference. One article saw that *AMPD1* CC genotype was related to sprint capacity of 10 m. Another observed that the CC genotype of *BDNF* polymorphism showed a faster sprint (10 m) than the carrier of the “T” allele. Also related were the genotypes *COL2A1* CC, *COL5A1* CC and *NOS3* TT, with the carrier of the “T” allele, genotype CT and CC, respectively. At 20 m, the *COL2A1* CC genotype stands out when compared to the carrier of the "T" allele and *BDNF* GG genotype has an advantage over "T" allele. Finally, one article obtained rs55743913 polymorphism in the PTPRK gene as the most significant for the 5 m sprint and *SEM4A4* rs12401573, *NFATC2* rs4811192 and *TERT* rs33954691 polymorphisms the most significant for 20 m. Thus, some genetic polymorphisms studied in the set of articles showed a significant relationship with sprint in players, including less known polymorphisms like rs5574391 in the *PTPRK* gene, *SEMA4A* rs12401573, *NFATC2* rs4811192, among others. In order for these relationships to be better established, further studies are needed within the particularities of the sport. Although the physical effort, training, food, technique, environment, time of rest, physiological and psychological factors, influence physical tests, it is necessary that, increasingly, take into account the innate factors of sports performance.

**Key-words**: Genetics, ACTN3, ACE, Athletic performance, Football.

# REPRODUCTIVE OUTCOMES OF LIPID-LOWERING TREATMENT WITH ROSUVASTATIN IN MALE SWISS MICE SINCE PRE-PUBERTY TO ADULTHOOD

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Obesity is increasingly affecting children and adolescents worldwide, and one of its consequences is dyslipidemia. Thus, statins have been prescribed for those age groups to reduce serum levels of cholesterol and triglycerides. In order to analyze the possible adverse reproductive effects of exposure to rosuvastatin from pre-puberty to sexual maturity, pre-pubertal Swiss male mice were randomly divided into three experimental groups (n = 10/group), which received daily, voluntarily, from postnatal day (PND) 23 to PND 80, 0.9% saline solution (vehicle), 1.5 or 5.5 mg/kg of rosuvastatin diluted in the vehicle. Afterward, the reproductive and vital organ weights were evaluated, as well as body weight evolution and age of preputial separation, indicative of puberty installation in male mice. On PND 80, males were mated with non-treated female mice. Then, fertility and fetal parameters, such as fetus weight, number of resorptions and post-implantation loss rate were assessed. Exposure to rosuvastatin, in both doses, significantly delayed puberty onset ($p < 0.01$) and increased body weight at the age of onset of puberty ($p < 0.05$). Rosuvastatin, however, did not promote significant differences in body weight evolution, final body weight and vital organ weights ($p > 0.05$). Furthermore, the weight of the empty seminal gland was reduced in the group exposed to the lower dose of rosuvastatin, when compared to the control group ($p < 0.05$). The other reproductive organ weights did not present significant differences between the experimental groups, as well as fertility and fetal parameters, although both treated groups differed from the control group in the post-implantation loss rate ($p = 0.053$). The assessment of body weight variation provides information about systemic toxicity and represents an important aspect of the interpretation of reproductive effects. The similarity found in most fertility and fetal parameters, the evolution of body weight and final body weight of rosuvastatin-treated mice have also been previously described in the literature in studies with rats exposed to rosuvastatin, during the pubertal period, at the doses of 3 or 10 mg/kg/day. Additionally, there was a significant difference between the control group and the group exposed to 1.5 mg of rosuvastatin in the absolute and relative weights of the empty seminal gland. Chemical agents that significantly alter reproductive organ weights may indicate potential adverse effects on the male reproductive system. Since peripuberty represents a moment of fundamental transformations linked to hormonal action, the susceptibility to substances with harmful potential is increased, which

may provoke damage to the male genital system reflected in adulthood. Also, mice exposed to rosuvastatin showed increased body weight when compared to the control group, which can be justified by a somatic development that is consistent with the older age of these animals, due to the delay observed in the preputial separation of these experimental groups. Delayed postnatal maturation may be related to damage to the male reproductive system, which can be caused by xenobiotics, possibly associated with an androgen depletion that statins promote by interfering in the synthesis of cholesterol, a precursor of sex steroid hormones, and/or by decreasing sensitivity of androgen receptors to androgens. In summary, rosuvastatin may alter the time of puberty installation, increase post-implantation loss after natural mating and reduce the weight of the empty seminal gland, indicating possible androgenic impairment and damage to the structure of the seminal gland, considering that this organ is androgen-dependent and very sensitive to androgen depletion.

**Key-words**: Statin, Male reproduction, Fertility, Mice.

## THE ROLE OF PLANT MICRORNAS DURING *Colletotrichum spp.* INFECTION

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The response of plants to stress involves multiple gene regulatory mechanisms including the post transcriptional regulation of gene expression. MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in these regulatory mechanisms, which are crucial during development as well as abiotic and biotic stress responses. Previous evidence shows that miRNAs are inherent to plant immune systems during bacterial, viral and fungal attack. Species of the hemibiotrophic fungus *Colletotrichum* causes anthracnose, a highly destructive disease that affects several important crops around the world. The understanding of the molecular mechanisms involved in plant resistance response to this pathogen supports the development of biotechnological strategies to control production losses. Therefore, the present study analyzed the state-of-the-art of miRNAs related to plant immune responses to *Colletotrichum* species. We selected the studies from Web of Science, Scielo, PubMed, and Google Scholar databases, whose miRNAs and their respective targets were experimentally validated. Among the addressed studies, six conserved miRNAs families and one novel

miRNA were investigated, as well as their respective targets and their regulatory pathways were described. Four plant species (*Arabidopsis thaliana*, *Capsicum annuum*, *Manihot esculenta* and *Populus trichocarpa*) and three *Colletotrichum* species (*C. gloeosporioides*, *C. higginsianum* and *C. truncatum*) were investigated during their interactions. The validation of miRNAs targets was performed by RT- qPCR and 5'RLM-RACE. The miRNAs families' miR160 and miR393 regulate the Auxin Response Factor 10 (ARF10) and Transport Inhibitor Response 1 (TIR1), respectively, which are involved in the regulation of auxin signaling and related to biotic stress. The upstream regions of miR160/miR393 genes have cis-regulatory elements that are defense and stress responsive. The miR393 promoter contains defense and stress responsiveness (TC-rich repeats), MYB Binding Site involved in drought inducibility (MBS) and fungal elicitor responsive element (Box- W1), known to be the most prevalent cis-elements in fungi-responsive miRNAs. The miR396 regulates the family of Growth-regulating Factor (GRF) transcription factors and its overexpression is related to higher susceptibility to infection. The miR472 exerts a key role in plant immunity by affecting Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) transcripts. Actually, this miRNA triggers phasiRNAs which promote the silencing of NBS-LRR genes. Also, the reported data indicates that overexpression of miR472 results in plant susceptibility to *C. gloeosporioides*. The miR858 targets members of the V MYB myeloblastosis viral oncogene homolog (MYB) family of transcription factors that regulate genes involved in flavonoid biosynthesis. During the infection, this miRNA act as a negative regulator of plant immunity by controlling the accumulation of antifungal phenylpropanoid compounds. Additionally, another two miRNAs, miR773 and the new miRn37 were demonstrated to regulate the transcripts of Methyltransferase 2 (MET2) and Ethylene Response Factors (ERFs) genes, respectively, which modulates the hormone-dependent activation of plant defense responses against *Colletotrichum* species. Therefore, the available data suggests the important role of miRNAs during plant infection by *Colletotrichum* pathogens. The present study provides valuable information regarding the current status of miRNAs regulatory mechanisms in important crops. In addition, this review will assist in the selection of miRNAs in future studies involving anthracnose disease, since some of these miRNAs belong to conserved families.

**Key-words**: miRNA, *Colletotrichum* spp., Plant-pathogen interaction.

## THE STEP BY STEP USE OF WHOLE EXOME SEQUENCING ON DIAGNOSTICS AND CARE OF PANCREATIC DUCTAL ADENOCARCINOMA

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The pancreatic ductal adenocarcinoma, PDA, is a highly aggressive disease, responsible for 90% of pancreatic cancer cases. Its prevalence is 2% of all neoplasias in Brazil, 4% of the deaths, and the incidence increases with aging. The diagnosis is made by tissue biopsies, followed by TNM classification: T being the measurement of the extent of the primary tumor, N the extent of local linfonode metastasis, and M the extent of distant metastasis. The surgical removal of the tumor is the most used treatment, as Karnofsky performance status (TNM classification) recommends. Nevertheless, this approach does not access the patient and their tumor genotype, which is essential for a more specific diagnosis to select and exclude therapies. In this context, whole-exome sequencing (WES) appears as a low-cost solution to find PDA somatic and germline variations to, after analysis, propose better diagnosis and treatment for the disease. We used WES to demonstrate a step-by-step approach for PDA diagnosis and treatment, illustrating how to align properly, call, annotate, analyze, and use the identified somatic and germinative variants to care for PDA patients. At a glance, the alignment process rebuilds the patient's genome by merging the 150 base reads produced by Illumina sequencers. The calling process finds the patience's variants: genomic single nucleotide permutations, small insertions, and deletions. The annotation process identifies the metabolic and clinical consequences of these variants, and the analyses step aggregate all information to be examined in comparison to the specific scientific literature. This way, we will be able to build an individual and specific care plan. We took four tumor-normal (T-N) PDA sample pairs from 4 Caucasian patients who have signed the informed consent. All the tumor samples were localized at the pancreas head, with a T3N1M0 TNM classification, and the normal samples came from patients' white blood cells. All samples were sequenced and publicized by Texas Cancer Research Biobank. The resulting sequence files are in the public domain. For the alignment and variant calling process, we ran the SAP-DP package, a Bash pipeline that implements the Broad Institute Best Practices. For annotation, we used the software VEP (Variant Effect Predictor) and Funcotator. Results showed that, although TNM diagnosis classified all tumors as PDA, each patient and their tumor had specificities that changed the therapeutic possibilities. For example, two out of four patients had a wild type KRAS, and one had a P53 one. It showed a better prognosis (KRAS) and the possibility of radiotherapy-induced cancer cell apoptosis (P53). At the germline variants, 3 patients have an XRCC1 polymorphism, which means a decreased response to the platinum-based therapies. This example demonstrates the necessity of in-depth knowledge of the disease characteristics to maximize PDA patients' quality of life. In conclusion, this work showed a way to overcome the TNM limitations. WES improves specificity while keeping costs low, finds the tumor variants through analysis, allows a highly specific targeted treatment, which most likely will improve the patients' quality of life.

**Key-words**: Exome, Precision oncology, Somatic variant call, GATK.

# UVB RADIATION CAN INDUCE LOSS OF VISUAL ORIENTATION IN LARVAE OF THE FRESHWATER PRAWN *Macrobrachium olfersii*

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The radiation emitted by the sun is composed of electromagnetic waves of different lengths, such as X-rays, gamma rays, infrared, ultraviolet (UV) radiation, visible light, microwaves and radio. Among the components of the solar spectrum, UV radiation is important for several physiological processes of organisms. UV radiation can be classified, according wave lengths and to its photobiological effects, into ultraviolet A (UVA-I, 400-340 nm, UVA-II, 340-315 nm), ultraviolet B (UVB, 315-280 nm) and ultraviolet C (UVC, 280-100 nm). Although a lower incidence of UVB radiation manages to cross the ozone layer, this radiation is the most harmful for organisms. Most research related to UVB radiation is directed at humans, described as responsible for causing dermatological diseases, skin cancer, cataract and DNA damage. However, not only humans are affected, animals that live in aquatic environments can also be affected when exposed to this radiation. Several factors determine the amount of radiation incident on these aquatic animals, including turbidity and depth of the water column. Therefore, some species have developed forms of protection, such as shells and thorns, but in the case of embryos and larvae, they are more susceptible to damage caused by UVB radiation, as they do not have their defense systems fully developed. The prawn *Macrobrachium olfersii* has been used in studies that investigate the effects of UVB radiation on embryonic development, due to its reproductive features, such as eggs carried in an external brood pouch that allows the monitoring of embryos, which are naturally subject to UVB radiation. Females carry their eggs for a period of 14 days (24 ºC), with day 7 of development (E7) characterized by an increase in the optic lobe, defining the anterior part of the embryo. On the 14$^{th}$ day of development, the embryo hatch and then the visual system becomes essential for their orientation, being essential for survival, both in the search for food, and to assist in the escape of predators. Thereby, the present study aims to investigate possible behavioral changes caused by UVB radiation during the larval development of *M. olfersii*, in order to identify whether the damage caused by this radiation in the embryo persists until larval development. For this, ovigerous females with embryos in E7 were irradiated with a 6W UVB lamp, for 30 min. In

the first 24 h after the irradiation protocol, the females were kept in a dark aquarium and then they were relocated to another aquarium with visible light, in which they remained until the larvae hatched. Those larvae that were obtained from non-irradiated ovigerous females were used as controls. To evaluate the behavior of phototropism, larvae (n = 20) were placed in an apparatus that consisted of a small aquarium with two sides (light and dark) separated by a partition. Previously, the larvae were acclimated 10 min. by placing them all on one side of the partition, after, the aquarium partition was removed, and the light side of apparatus was illuminated with a lamp for 10 min. After, the partition was replaced, and the larvae were counted on both sides. As result, it was observed that the larvae of the control group recognized and migrated towards the light (18 ± 1.18), while larvae of UVB-irradiated group tended to remain in the dark side of apparatus (16.5 ± 1.18; $p < 0.001$) compared to the control. This suggests that UVB radiation can affect the development of the *M. olfersii* visual system. Considering that exposure to UVB radiation occurred during embryonic development, it is plausible to assume that damage occurred in the eye of the embryos and persisted until the larvae. Damage to the visual system can be assessed by the loss of positive phototropism behavior, which can affect the larval development as a whole (growth, feeding, flight of predators), as well as its survival.

**Key-words**: Embryonic development, Phototropism, Crustacea.

## ZIKA VIRUS DISRUPTS THE DEVELOPMENT OF THE TELENCEPHALON AND CRANIOFACIAL BONES

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In the years 2015 and 2016 scientists related zika virus (ZIKV) infection to congenital malformation of the Central Nervous System (CNS) and bones of the calvaria, classified as microcephaly. The reduction in the volume of the cerebral cortex, accompanied by advanced ossification of the calvaria are the two most prominent features of the so-called ZIKV syndrome. During embryonic development, the cerebral cortex is formed by the most anterior region of the neural tube, called the telencephalon. The bones of the calvaria are formed by neural crest (NC) and paraxial mesoderm cells. Interestingly, despite having very distinct origins, CNS and bones of the calvaria, both are specifically affected during development by ZIKV. Is there any mechanism common to these two structures that could be

being changed by ZIKV? During the initial development of the head, cephalic NC cells secrete Noggin, which blocks ectodermal Bmp, allowing the expression of Fgf8, the main factor related to the initial expansion of the telencephalon (future cerebral cortex). With the advance of this process, NC cells stop to express Noggin, allowing Bmp signaling to block Fgf8 activity, inducing bone differentiation of cephalic NC and mesoderm cells. Therefore, this study hypothesizes that ZIKV influences the axis of Fgf8-Bmp signaling during craniofacial development. To test this hypothesis, we used an *in ovo* infection model in chicken embryos (*Gallus gallus domesticus*), with the following methodological approach: 1) Infection *in ovo* with ZIKV at concentrations of 5 and 500 Plaque-Forming Unit (PFU) in chicken embryos with 2 days of development; 2) Confirmation of infection in 3-days embryos by RT-PCR; 3) Analysis of Fgf8 expression in 4-days embryos by *in situ* hybridization; 4) Measurement of the area of the telencephalon and midbrain in 3-days embryos with the Image-J tool; 5) Study of the bones of the calvaria from the diaphanization technique in embryos of 15 days, colored with alcian blue (cartilage marking) and alizarin red (bone marking). *In ovo* infection with ZIKV proved to be effective and variable among embryos of ZIKV 5 PFU infected groups (ZIKV 5 pV) and 500 PFU (ZIKV 500 pV). A positive correlation was observed between viral load and the severity of malformations. The survival rate of the 3-day embryos showed no difference, however, the 15-day embryos of the ZIKV 500 pV group showed a reduction of 60% compared to the control group. In the analysis of Fgf8 expression in the telencephalon, no differences were observed, however, the size of the telencephalon in the embryos of the ZIKV 500 pV group showed a reduction of 27%, while the midbrain region did not present differences. In the analysis of the cranial bones of the ZIKV 500 pV group, a reduction of 40% in the size of the parietal bones and 58% in the squamosal bones were evidenced. These data demonstrate a close relationship between ZIKV infection and the mechanisms responsible for CNS expansion and the skull ossification process, however, molecular signaling needs to be better explored. The next steps are: perform molecular analyses of Six2 and Noggin, as well as Fgf8 at other ages. These data will help to understand the influence of ZIKV on signaling that controls craniofacial development, especially concerning bone changes. In addition to improving our understanding of craniofacial development, these data may in the future help improve the quality of life of people presenting this clinical condition.

**Key-words**: Calvaria, Central Nervous System, Microcephaly.


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