Sumário

Citogenética Animal

ID - 5

SATELLITE DNA EVOLUTION IN EDESSA STINKBUGS PEST SPECIES

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Abstract:

The suborder Heteroptera, which includes stinkbugs, has relevant economic interest. The Pentatomidae family is one of the most diverse among it, grouping pest species that cause severe damage to South American crops, many of which belong to the genus *Edessa*. Studies focused on this genus lack information about its genomic architecture. Knowing that the largest portion of the nuclear DNA of most eukaryotic genomes is constituted by repetitive DNAs and that in many cases are involved in the composition of sex chromosomes, in the present work we investigate the evolutionary role of Satellite DNAs (DNAsat) in the genome of four species of *Edessa* genus with emphasis on sex chromosomes. For this, we use repetitive DNA recognition and analysis programs such as RepeatExplorer and RepeatMasker for bioinformatics comparative analysis and techniques such as Fluorescent in situ hybridization (FISH) for analysis at the chromosomal level. Our results showed different numbers of DNAsat families among the species, the highest of them being found in *E. loxallii* (25 families) and the lowest in *E. icteria* (5 families). Regarding the global abundance of DNAsat within the species, we calculated the average between male and female, resulting in about 1.33% for *E. icteria*; 5.15% for *E. loxallii*; 3.17% for *E. meditabunda*; and 3.62% for *E. rufomarginata*. Furthermore, we observed an increase in abundance of at least 20% in males relative to females for all, except for *E. meditabunda* that showed 1% decrease. Surprisingly, *E. icteria*, which showed the lowest number of families, also manifested the highest increase in male over female abundance (increased in about 5x), succinctly due to its high value in the three most abundant DNAsat families. *E. loxallii*, on the other hand, exhibited the highest number of families, highest mean abundance, and a smaller increase in male (about 24%) compared to *E. icteria*. The global average divergence of the DNAsat families were all lower in males than in females, evidencing evolution in concert acting on copies originating from the Y chromosome, except for *E. icteria*, probably due to its lower number of families and higher number of more variable ancestral copies in the male over the female. Although in *E. meditabunda* we did not observe this global increase in abundance in the male, three families (out of 13) showed a significant increase in the male genome, demonstrating the presence of DNAsat composition, as already mentioned for other insect groups. This can be visualized through FISH performed with probes from these three families of DNAsat in *E. meditabunda*, and a probe from a more abundant family in the female, reinforcing the data that point to strong patterns of bands located in the Y (3DNAsat) and X (1DNAsat). Despite the differences in abundance and divergence data between the four species, we noticed that the pattern of satellites predominantly harbored on sex chromosomes (Y) remains the same, which emphasizes the need for further studies in this category, since these species are pests of crops, and unraveling details about their genomes could help in futher researches.

Key-words: Repetitive DNA; Cytogenomics; Sex chromosomes;

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Special thanks to Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ, USP).

ID - 113

CHROMOSOMAL MAPPING OF MICROSATELLITE SEQUENCES IN THREE SPECIES OF THE GENUS *RHINELLA* (ANURA, BUFONIDAE).

Marlon Ramires da Costa Lima[1]; David Santos da Silva[3]; Samuel Santos Ribeiro[1]; Rodrigo Petry Corrêa de Sousa[3]; Ivanete de Oliveira Furo[4]; Edivaldo Herculano Correa de Oliveira[2,3]: Anderson José Baia Gomes[1]

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Abstract:
The amphibians of the Bufonidae family includes 8 genera and about 100 species. Cytogenetic studies have shown a slight chromosomal variation among its representatives, however cytogenetic data using different types of cytogenomic markers are still poorly addressed in this group. Thus, this work analyzed the karyotype of three species of the genus *Rhinella* (*R. marina*, *R. granulosa* and *R. margaritifera*) in order to reveal the proportion, location and organization of telomere, rDNA 18S and microsatellites sequence. The specimens were captured in Abaetetuba, PA (SISBIO license nº 78948, CEUA Nº 353929062). Chromosomes were obtained through bone marrow and intestine preparation. The karyotype was analyzed by conventional staining, C-Banding and Fluorescent In situ Hybridization (FISH) using four microsatellite sequences: $(CA)_{15}$, $(CAA)_{10}$, $(CAC)_{10}$ and $(CAG)_{10,}$ telomere and 18S rDNA probes. All analyzed species showed 2n=22 and NF=44. The three species showed constitutive heterochromatin in the pericentromeric region of all the chromosomes, with emphasis for a proximal block in the long arm of pair 3 in *R. marina* and in the short arm of pair 4 in *R. margaritifera*. FISH with 18S rDNA sequence corroborated previous data from silver staining, indicating NOR sites in pair 5 of *Rhinella granulosa*, pair 7 in *R. marina* and pair 10 in *R. margaritifera*. Hybridization with telomere probes showed only signals in the distal region of the chromosome arms. Experiments using the four microsatellite probes revealed a distal pattern of distribution of these sequences in *R. marina* and *R. granulosa*, in which only the $(CAA)_{10}$ probes have shown more discrete marks. On the other hand, these telomeric distributions were not evident in *R. margaritifera*, which has shown intensive signals in the interstitial region of a large metacentric chromosome, not observed for $(CAG)_{10}$ probe. Considering recent phylogenies and the closer relationship between *R. marina* and *R. granulosa*, these patterns of microsatellite distribution may be a chromosome signature for these two species and its interstitial distribution a specific feature for the margaritifera group. Thus, despite the conserved appearance of their karyotypes, a clear divergent process involving the repetitive portion of genomic is ongoing in these species, revealing the importance of the use of unusual cytogenomic markers in studies focusing on chromosome evolution and cytotaxonomy in frogs.
Key-words: Repetive DNA; Microsatellite; Amphibians;

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ID - 149
KARYOTYPE AND GENOMIC COMPARISON OF LION TAMARINS (*LEONTOPITHECUS*, CEBIDAE, PLATYRRHINI)

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Abstract:
The genus *Leontopithecus* is composed of four species, all considered endangered by the IUCN and endemic to the Atlantic Forest. The four species of the genus have the same diploid number 2n = 46 and fundamental number of autosomal arms FN = 74. *L. rosalia* and *L. chrysomelas* have been karyotypically better studied than *L. chrysopygus* and *L. caissara*. In order to obtain more information about this endangered genus, we comparatively analyzed the karyotypes of *L. chrysopygus*, *L. rosalia*, and *L. chrysomelas* after GTG-, CBG-banding, silver staining of the nucleolus organizer regions (Ag-NORs), fluorescent *in situ* hybridization (FISH) with telomeric sequences and rDNA 18S as probes, and genomic *in situ* hybridization (GISH). The chromosome preparations used in those experiments were obtained from fibroblast cultures started from skin samples of seven lion tamarins - four *L. rosalia* (three females: PRI 19, 20, 21 and one male: PRI 79), two *L. chrysopygus* (a female: PRI 54 and a male: PRI 53) and one *L. chrysomelas* (a female: PRI 75) - from the Fundação de Parques Municipais e Zoobotânica de Belo Horizonte, Minas Gerais, Brazil. The genomic DNAs were obtained from blood and skin samples. Our results revealed very similar karyotypes, with some differences between the CBG banding patterns, Ag-NORs distribution and FISH with rDNA 18S probe. Some of the differences found with CBG banding were the presence of signals in the distal portion of the long arms of chromosomes 6, 16 (in *L. rosalia*); 10,13, 17 (in *L. chrysomelas*); in the distal portion of the short arm of chromosomes 15, 19 (in *L. rosalia*); 8, 14 (in *L chrysopygus*); 7 (in *L. chrysomelas*); the whole short arm of chromosomes 3, 14, 15, 17 (in *L. chrysomelas*); and in the middle of the long arm of chromosome 10 (in *L. chrysomelas*). In regards to Ag-RON, the number of chromosome pairs that showed signals varied from four (in *L. rosalia*) to six (in *L. chrysomelas*) with *L chrysopygus* having five pairs. GISH experiments between *L. rosalia* and *L. chrysopygus* revealed species-specific heterochromatic regions on the centromeric or pericentromeric portion of chromosomes 4, 16, 20 in *L. rosalia* and chromosomes 4, 16, 20-22 and heterochromatic regions of the Y chromosome in *L. chrysopygus*. We also investigated two satellite DNAs, the alpha and the CarB present in the genome of *L. rosalia*, which could be related to the chromosomic differences observed between the *Leontopithecus* species. Our results indicate that a more detailed analysis of the repetitive sequences shall provide a better understanding of the chromosome evolution in the genus *Leontopithecus*.
Key-words: *Leontopithecus*; Banding Patterns; GISH;

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

CYTOGENOMICS STUDIES REVEALS A NEW FAMILY OF LONG SATELLITE DNA IN THE FROG *PROCERATOPHRYS SCHIRCHI*.

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Abstract:
The species Proceratophrys schirchi, family Odontophrynidae, is found in southeastern Bahia, Espírito Santo, northeastern Minas Gerais and Rio de Janeiro, Brazil. It has a diploid number of 2n=22 chromosomes, a nucleolus organizer region in pair 8 and blocks of constitutive heterochromatin in the centromeres of the chromosomes. Furthermore, an atypical accumulation of heterochromatin was detected in a pair of chromosomes in P. schirchi females, pointing to the possibility of sexual heteromorphism.With the objective of initiation and deepening cytogenomic analyzes in this species of frog, especially the presence and influence of repetitive sequences, in this work, we carried out analyzes in P. schirchi using genomic data from Illumina sequencing in bioinformatics programs, such as RepeatExplorer, TAREAN and RepeatMasker to specifically characterize and quantify satellite DNAs (satDNAs) abundant in both sexes of this species. SatDNAs are defined as non-coding sequences organized in tandem, which can form heterochromatin regions, and then impact on the transcriptional activity of several organisms.The analyzes demonstrated the presence of several families of putative satDNAs. Among these, one drew attention due to its high abundance, monomer size and shared evolutionary patterns between the sexes. Named PscSat01-831, it is the most abundant satDNA among all putatives, has a monomer of 831 bp and an A+T ratio equivalent to 61.9%. Comparing the sexes, PscSat01-831 is more abundant in the male than in the female genome, with 0.6% and 0.4% respectively. The divergence values ??were similar, with 4.61 and 4.0, respectively. Thus, in this work it was possible to identify a new relatively long satDNA family (831 bp) in relation to others already described for amphibians, using high-throughput tools. The satellite PscSat01-831 emerged relatively recently in evolutionary history and shows similarity in both sexes, with a greater abundance in the male genome, leading to believe in a greater amplification due to heterochromatinization processes.Our work provides for the first time cytogenomic data with description and identification of a satDNA family for the species P. schirchi and demonstrates the need for further analyzes for a complete characterization of the satellitoma of this frog species.
Key-words: cytogenomics; satDNA; Proceratophyrs schirchi; anura; chromosome

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ID - 225

DRAFT GENOME AND SATELLITE DNA CHARACTERIZATION OF THE FISH *HYPHESSOBRYCON HETERORHABDUS* (CHARACIFORMES) FROM AMAZON BASIN

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Abstract:
Hyphessobrycon is the most diverse genus of the Characidae family comprising about 160 species distributed widely across the Neotropics, with records from southern Mexico to Rio de la Plata in Argentina. The great diversity and wide geographic occurrence indicate that this group can be an interesting model to explore evolutionary and environmental issues. In order to provide the bases for genetic, evolutionary, and toxicological studies, among other issues, was carried out the karyotypic description and genomic sequencing and assembly of *H. heterorhabdus* from the Amazon (Gunma Ecological Park, 1°13'00.86"S, 48°17'41.18"W, Santa Bárbara, PA, Brazil). Moreover, characterization of repetitive sequences focused on satellite DNAs was conducted. Karyotypes were obtained by conventional coloration of chromosomes with Giemsa. Genomic libraries were constructed with TruSeq Nano DNA kit and sequenced using NovaSeq6000 system (Illumina). Nuclear genome assembly and annotation were carried out with SPAdes-3.14.0 and Braker-v28.2 pipeline. Mitogenome was assembled using NOVOPlasty-4.3.1. Repetitive DNA sequences were characterized with RepeatMasker and RepeatExplorer/Tarean, and mapped by Fluorescent In Situ Hybridization (FISH). Sequences were amplified by Polymerase Chain Reaction (PCR). The karyotype of *H. heterorhabdus* has 48 chromosomes (KF = 25m-sm/22st-a) without morphologically differentiated sex chromosomes. The male and female draft genomes generated presented, respectively, scaffold N50 of 8,334 and 8,276, and 23,233 and 23,181 protein coding genes annotated. The circular mitogenome of male and female was 16,717 bp and 16,474 in length, respectively. RepeatMasker showed that 50.44 % of the male genome and 50.26 % of the female genome are composed of repetitive sequences. TAREAN software identified sequences organized as satellite DNA, which were mapped by FISH: Sat-1 is located on the short arm of pairs 15 and 16; Sat-2 is distributed on the centromeric region of all chromosomes; HHESat-3 is dispersed located along all chromosome pairs, Sat-4 is located on the short arm of pairs 23 and 24; Sat-5 and Sat-6 could not be amplified via PCR and therefore they were not mapped. Genetic annotation allowed the identification of coding and regulatory sequences, enabling their amplification by PCR. The present data indicate that the generated draft genome is useful for recovering coding and regulatory genomic sequences, and the knowledge generated here has great potential to be explored in several evolutionary and environmental studies.
Key-words: fish; repetitive DNA; cytogenomics; ;

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ID-105

CHROMOSOMAL STUDIES IN TWO SALTICID SPIDERS (ARANEAE, SALTICIDAE), *MENEMERUS BIVITTATUS* AND *CORYTHALIA* SP. INDICATE A CENTRIC FUSION IN THE *CORYTHALIA* X CHROMOSOME ORIGIN

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Abstract:

Salticidae is the largest spider family, comprising 6.522 species, and also the most studied by cytogenetics (171 species). However, this total corresponds to only 2,6% of the described species, and several clades are not sampled in the chromosomal studies, generating gaps that difficult evolutionary inferences. The aim of this study is to cytogenetically describe the salticid species *Menemerus bivittatus* (tribe Chrysillini) and *Corythalia* sp. (tribe Euophryini), contributing to fill these gaps on salticid cytogenetics and discussing karyotype evolution. The preparations were obtained from the gonads of eight specimens of *M. bivittatus* (six males and two females), and six specimens of *Corythalia* sp. (two males and four females). Both species were collected at Campo Grande city, in the state of Mato Grosso do Sul, Brazil. The gonads were dissected in insect physiologic solution (7.5 g NaCl, 2.38 g Na_2HPO_4, 2.72 g KH_2PO_4, in 1 l of distilled water), immersed in colchicine solution during 2 hours (0,16% in insect physiologic solution), hypotonized in tap water during 15 minutes, and fixed in Carnoy I (3:1 Methanol: Acetic Acid) for at least one hour. The gonads were dissociated in a drop of 60% acetic acid on the surface of microscope slides. The preparation was dried on a metal heating plate (35-40 ºC) and stained (3% Giemsa solution). The analysis of 99 cells of *M. bivittatus* revealed spermatogonial and oogonial mitotic metaphases composed by 2n(male) = 28 and 2n(female) = 30 telocentric chromosomes, respectively, that gradually decrease in length. Spermatocytes I in diplotene and metaphase present 13 autosomal bivalents and two sex chromosome univalent (X_1X_2) that appear side-by-side or close to each other and are positively heteropyknotic. Metaphases II have n = 13 and n = 13 + X_1X_2. It is possible to recognize the sex chromosomes due to the positive heteropyknosis. The karyotype of *M. bivittatus* (2n = 28, X_1X_2, telocentric) is the same found in all eight Chrysillini species karyotyped up to now, belonging to the genera *Menemerus*, *Phintella*, *Pseudicius* and *Siler*, except *Menemerus illigeri* (2n(male) = 14, X_1X_2, metacentric autosomes). The karyotype 2n(male) = 28, X_1X_2 is also the most frequent in Salticidae as a whole. The analysis of 61 cells of *Corythalia* sp. revealed spermatogonial and oogonial mitotic metaphases composed by 2n(male) = 27 and 2n(female) = 28. All autosomes are telocentric and the X chromosome is metacentric and the largest element of the complement. Spermatocytes I in diplotene and metaphase I have 13 autosomal bivalents and one sex chromosome univalent (X) that often appear positive hetepyknotic and also in a "V shape", bent at the centromere region. Among Euophryini, chromosomal data are available only in *Coryphasia*, *Euophrys*, *Jotus* and *Thyenula*, all species presenting the standard salticid karyotype 2n(male) = 28, X_1X_2. Thus, the presence of a large metacentric X chromosome in *Corythalia* sp. suggests the occurrence of a centric fusion between the X_1 and X_2 chromosomes found in other Euophryini. It is noteworthy that *Corythalia* belongs to the *Anasaitis-Corythalia* group, a clade that is sister to all other Euophryini.

Key-words: Chrysillini; Eouphryini; meiosis; mitosis; chromosome morphology

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ID -116

CHROMOSOMAL EVOLUTION IN NEOTROPICAL DEER: PATTERNS AND IMPLICATION FOR SPECIATION, TAXONOMY, AND CONSERVATION.

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Abstract:

The family Cervidae is recognized for its high rate of chromosomal evolution. The extensive karyotypic diversity is present in very similar morphological groups, which results in the existence of cryptic species complex and taxonomic uncertainties. Despite the great interest in the cytogenetic study, the expressive chromosomal evolution has remained little understood for Neotropical deer. Aiming to explore the new world deer evolutionary history, we used molecular cytogenetics techniques to map the evolutionary chromosomal pathway from a hypothetical ancestral of 2n = 70 karyotype using Bacterial Artificial Chromosome probes derived from *Bos taurus* and previously characterized in *Subulo gouazoubira*, that retained the basal karyotype, in species of the genus *Mazama*. Our results showed complex rearrangements driving the chromosomal changes in the group, being the main mechanism the tandem fusions, followed by centric fusions, centromere shift, inversions, and fissions. The cryptic species complex *Mazama americana* (2n = 42 to 2n = 52) shared three fusions and one inversion from the basal karyotype and were divided into three chromosomal lineages. Each lineage comprises enough chromosomal differences to be unquestionably separate into different species. However, chromosomal variations within a lineage, such as one tandem fusion of difference, indicate potential subfertility of the offspring from crossing two different cytotypes, due to the impact of this rearrangement associated with substantial postzygotic reproductive barrier. *Mazama jucunda* (2n = 32 to 2n = 34) and *Mazama nana* (2n = 36 to 40), otherwise, did not share fusions between them or with *M. americana* lineages. That is quite impressive, considering that mitochondrial phylogenetic three placed these two species as a sister group, together with some *M. americana* lineages in one clade, and the other *M. americana* lineages in a different clade. These results showed that karyotypic reorganization between species is considerable for a short divergence time and raises the question if the rearrangements were the main driving force leading to speciation in *Mazama*. Besides, it highlights the incredible and rapid chromosomal evolution of Neotropical deer, in which cytogenetic characterization considerably contribute to the species concept delimitation and clarification of the complex taxonomy of the group.

Key-words: Cervidae; molecular cytogenetics; chromosomal rearrangement;

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ID-149

FINDINGS ON THE KARYOTYPE OF THE TIGER-STRIPED MONKEY FROG (*CALLIMEDUSA TOMOPTERNA*, PHYLLOMEDUSINAE, HYLIDAE) IN CENTRAL AMAZON

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Abstract:

There is no data on the *Callimedusa tomopterna* karyotype, nor for the entire genus, which comprises six species. This absence of cytogenetic data makes difficult the understanding of the chromosomal evolution and the cytotaxonomic integration of the genus in the subfamily Phyllomedusinae and the family Hylidae. The present work describes for the first time the karyotype of *C. tomopterna*, presenting its chromosomal number (2n), the morphological classification of chromosomes and the location of the nucleolus organizer region (NOR). The specimens (3 males and 1 female) were collected in the region of Balbina, Presidente Figueiredo, Amazonas, Brazil. Chromosomal preparations were obtained from the intestinal epithelium, that were submitted to conventional staining with Giemsa 5% and silver nitrate impregnation. Chromosomal counts revealed that individuals of both sexes have 2n = 26 chromosomes. This data is consistent with the chromosomal number found for most Phyllomedusinae species. Exceptions are restricted to one species: *Phyllomedusa tetraploidea*, which underwent a polyploidization event and presents 2n = 52. The pairs 2, 3, 4, 5, 7, 9, 11, 12, 13 were classified as metacentric, pairs 1, 6 and 10 were submetacentric, and pair 8 presents a polymorphism, with chromosomes varying between acrocentric and submetacentric morphology. Silver nitrate impregnation revealed simple NOR, allocated in the proximal interstitial region of the long arm of the pair 8. For Phyllomedusinae, NOR descriptions indicate simple sites such as observed in *Phyllomedusa* and *Phasmahyla* or multiples as observed in some *Pithecopus* species. The present study reinforces that 2n = 26 is the most frequent chromosome number in Phyllomedusinae. The chromosomal microstructure of the species belonging to the subfamily presents a diversity not yet fully known, especially when it comes to less diverse genus, as the one discussed here. The description of these cytogenetic characters may contribute on the understanding of the evolutionary dynamics of the karyotype of this subfamily and may base future studies on the genus.

Key-words: Anura; animal cytogenetics; chromosomal number; nucleolus organizer regions;

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ID -24

KARYOTYPES, HETEROCHROMATIN AND RIBOSOMAL DNA LOCATION ON MOTH PEST SPECIES OF THE FAMILY NOCTUIDAE

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Abstract:

Noctuidae moths includes more than 11,700 species and thus represents one of the largest families of the insect order Lepidoptera. Besides species richness it also includes a number of serious agricultural pests causing severe economic losses through the world. Noctuidae species are highly polyphagous and with wide distribution in different cultures such as cotton, corn, rice, beans, among others in North and South regions of Brazil. Although with high importance, basic features of Noctuidae genomes are poorly know, including the diploid chromosome numbers. In this work we make the first advance on the better characterization of the chromosomes of twelve Noctuidae (*Acyclania tenebrosa, Agrotis ipsilon*, *Anticarsia gemmatalis, Chrysodeixis includens*, *Helicoverpa armigera*, *Helicoverpa zea*, *Mocis latipes*, *Spodoptera albula*, *Spodoptera cosmioides*, *Spodoptera eridania*, *Spodoptera frugiperda* and *Rachiplusia nu*), by description of general karyotypes, sex chromosomes and location of ribosomal DNA (rDNA). Diploid chromosome numbers and sex chromosome system was determined by analysis of mitotic metaphase obtained from wings imaginal disks of male and female. Analysis of polyploid cells from Malpighian tubules was performed to identify the sex chromatin that is associated with the presence of the W chromosome in females. We mapped through Fluorescent *in situ* Hybridization (FISH) probe from the major ribosomal DNA 18S on pachytene cells of males. The diploid number of all species, counted by analysis of mitotic metaphase, was 2n=62 with WZ (female)/ZZ (male) sex system. A single conspicuous sex chromatin body of regular spherical shape was observed in each polyploid nucleus of Malpighian tubules from females, except for *A. tenebrosa*, *A. gemmatalis* and *C. includes* in which the corpuscle was absent. No sex chromatin was found in males. On most species no heterochromatin blocks were detected, indicating non-enrichment of the repetitive DNA fraction in certain regions of the genome, except for *A. tenebrosa*, in which the heterochromatin was distributed as small dots along the entire length of all chromosomes and *A. ipsilon* with terminal heterochromatic blocks. FISH with 18S ribosomal DNA (rDNA) probe applied to male pachytene nuclei, revealed one rDNA cluster at the interstitial region of one bivalent for all species, that is a common pattern for Lepidoptera. In contrast, only in *A. tenebrosa* three terminal rDNA clusters in three different bivalents was found. Our data suggests, that although the karyotypes of Noctuidae is highly conserved, some divergent patterns are observed in specific species, evidencing repatterning for repetitive DNAs. This data contributes to the understanding of the genomic organization in Lepidoptera and about the evolution of their karyotypes and sex chromosomes that are important for future genomic studies.

Key-words: pest species; repetitive DNAs; evolution;

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ID - 128

KARYOTYPIC DESCRIPTION OF TWO SPECIES OF BIRDS FROM THE ORDER PASSERIFORMES.

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Abstract:
Passeriformes are the largest order of birds. Their distribution is cosmopolitan and the identification of species uses mainly morphological traits. However, a comprehensive understanding of phylogeny and evolution requires the integration of both genetic and phenotypic information. Concerning their genomic organization, there are still few karyotype descriptions of Passeriformes, especially applying molecular cytogenetics. Therefore, this work aimed to describe for the first time the karyotype of species belonging to two different families: Thamnophilidae (*Taraba major*) and Dendrocolaptidae (*Xiphorhynchus guttatus*), by means of classical and molecular cytogenetics. Individuals were collected in rural areas from Abaetetuba (PA) using mist nets mounted on trails in regions with few bushes. Chromosome preparations were obtained directly from bone marrow. Giemsa-stained chromosomes were used to define the diploid numbers and standardize the karyotype. C-banding was used to determine the distribution of constitutive heterochromatin blocks. In addition, fluorescent in situ hybridization (FISH) techniques were performed using probes corresponding to telomere and 18S rDNA. Both species showed the typical diploid number of (2n=80) observed in most species of birds. Both *T. major* and *X. guttatus* were observed 12 pairs of macrochromosomes, and 28 pairs of microchromosomes. C-banding and FISH experiments produced similar results in both species. Hence, constitutive heterochromatin blocks were found in the pericentromeric regions in all chromosomes in both species. Telomeric sequences hybridized only in the tips of the chromosome arms, without any interstitial locations, and 18S rDNA produced signals on only one pair of large macrochromosomes in *X. guttatus* and two pairs of microchromosomes in *T. major*. These results showed that, despite the great diversity of species within Passeriformes, and a conserved karyotype observed by the number and morphology of chromosomes, a classical cytogenetic marker, such as NOR, proved to be variable. Indeed, other cytogenetics tools are necessary to clarify these karyotypic similarities even in species phylogenetically distance.
Key-words: Cytogenetics; Passeriformes; Birds;

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ID- 7

NEW INSIGHTS ON THE SATELLITOME AND CENTROMERES OF THE TWO CLOSELY RELATED SPECIES, *DROSOPHILA SERIDO* AND *D. ANTONIETAE*

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Abstract:

Satellite DNA (satDNA) is a type of highly repetitive DNA, tandemly organized, typically found forming long arrays in the heterochromatic regions of chromosomes. SatDNAs can make up a significant portion (>25%) of the genomic DNA. SatDNAs are known to evolve rapidly and may contribute to the establishment of genetic incompatibilities between incipient species. *Drosophila serido* and *D. antonietae* are closely related cactophilic species, endemic to South America, that belong to the *buzzatii* cluster. Their satDNAs were first investigated in the pre-genomic era, when studies revealed the presence of only two satDNAs, pBuM and DBC-150. FISH (Fluorescent *in situ* Hybridization) experiments revealed that pBuM was likely the main centromeric component of all chromosomes except the Y, while DBC-150 was restricted to microchromosomes. The recent availability of sequenced genomes of these species (*D. serido*, strain R37, Lajes Pintadas, RN and *D. antonietae*, strain R73, Sertãozinho, SP) provided an opportunity to revisit and expand our acknowledge of their satDNAs. To identify and quantify satDNAs of each species, we used whole-genome sequencing Illumina reads and RepeatExplorer2. We constructed maximum likelihood trees with satDNAs copies from both species. We extracted gDNA from *D. serido*, amplified satDNAs by PCR, and conducted FISH using probes for pBuM and CDSTR138 in *D. serido* metaphase and polytene chromosomes. Our results revealed that both species share the same collection of five satDNAs. Three of them (pBuM, DBC-150 and CDSTR138) have been previously described. The remaining two are novel and were named SAT197/230 and SAT109/104. We also identified CDSTR198, a euchromatic minisatellite DNA, in the two species. The satDNA contribution to the total gDNA of *D. serido* (2.9%) and *D. antonietae* (3.9%) is relatively low compared to other *Drosophila* species (often exceeding 20%). Such low satDNA content may indicate possible selective constraints on genome size/heterochromatin content. The ML trees showed four satellites (pBuM, DBC-150, CDSTR138 and SAT197/230) with a species-specific clustering of repeats, indicating a concerted mode of molecular evolution. The calculated interspecific divergence for each satDNA ranges from 0.58%-14.4%. Such low levels of satDNA divergence might have been one of the factors that contributed for the establishment of a hybrid zone between the two species, as revealed in previous studies. Surprisingly, we found that CDSTR138 is likely the main centromeric satDNA in *D. serido*, while pBuM is slightly more distally located. Accordingly, we found that CDSTR138 is also enriched for dyad symmetries and expected to form stable secondary structure, features considered important for centromeric function. We confirmed that CDSTR198 is only a minisatellite in *D. serido*, because it is exclusively located in the euchromatic arms of polytene chromosomes. We will expand our work performing FISH with the two novel satDNAs in the metaphase and polytene chromosomes. The present study on the satellitome of the two closely related species provides insights into genome evolution and centromere structure in these species and contributes revealing new genetic markers for species identification, which will be particularly useful in studies involving the hybrid zone.

Key-words: satellite DNA; Genome evolution; FISH; *Drosophila*; Repetitive DNAs

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ID - 202

PHYSICAL MAPPING OF REPETITIVE SEQUENCES IN THE CHROMOSOMES OF THE CARPENTER ANT *CAMPONOTUS RUFIPES* FABRICIUS, 1775 (HYMENOPTERA, FORMICIDAE)

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Abstract:

Ants of the genus *Camponotus* present great ecological diversity. This group, possibly not monophyletic, has the largest number of species and subspecies described, with 1496 taxa inserted in 43 subgenera with wide geographic distribution. One of these subgenera is *Myrmothrix* which includes 14 species, one of which is *Camponotus rufipes*. Cytogenetic studies with a population approach in *C. rufipes* showed a characteristic numeric chromosomal polymorphism in the species, with individuals presenting 2n=40 chromosomes and others with 2n=39 chromosomes, possibly resulting from a Robertsonian fusion. This hypothesis involves the fusion of the long arms of two medium-sized subtelocentric chromosomes that resulted in a long rearranged metacentric chromosome, which can be seen in the 2n=39 karyotype. In this study, chromosomal mapping of the telomere motif $(TTAGG)_6$ and of the microsatellite $(GA)_{15}$ was performed in order to generate information that supports the Robertsonian fusion hypothesis for *C. rufipes*. Larvae of *C. rufipes* were collected in Ponte Nova, Minas Gerais, Brazil. In order to obtain metaphase chromosomes, the cerebral ganglia of post-defecating larvae were extracted and submitted to a hypotonic solution of colchicine and fixatives. Chromosomal mapping was performed by fluorescent *in situ* hybridization, and probes $(TTAGG)_6$ and $(GA)_{15}$ were directly labeled with Cyanine-3 during synthesis by Sigma. $(TTAGG)_6$ sequences were located in the telomere region of all chromosomes in 2n=39 and 2n=40 individuals, which is a common feature in Formicidae. Interstitial markings with $(TTAGG)_6$, which could be remnants of chromosomal fusion by telomeres, were not observed in *C. rufipes* of 2n=39 and 2n=40. This supports the Robertsonian fusion hypothesis, since part of the telomeres of the chromosomes involved may have been lost. The microsatellite $(GA)_{15}$ was observed in the long arms of the chromosomes in the karyotype of 2n=39 and 2n=40, which corresponded to euchromatic regions, a pattern similar to the ant species already studied. However, in the rearranged metacentric chromosome, it is possible to observe that $(GA)_{15}$ was present along both arms, which is in agreement with the Robertsonian fusion hypothesis in this species, since this chromosome would be derived from two long arms of distinct subtelocentric chromosomes from the 2n=40 karyotype. In addition, in some chromosomes the distribution of $(GA)_{15}$ in the long arms was not continuous, since in these chromosomes it is possible to observe interstitial heterochromatic bands in their long arms, evidenced by the C-Band technique previously performed for this species. Therefore, the data obtained in this study incorporate new molecular cytogenetic knowledge for the species and support the Robertsonian fusion hypothesis as the origin of the karyotype with 2n=39 chromosomes.

Key-words: Hymenoptera; Cytogenetics; Telomeres; Microsatellites;

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ID -152

UNVEILING THE EVOLUTIONARY DIVERSITY OF CHROMOSOMES IN PELECANIFORMES (AVES): A COMPARATIVE ANALYSIS

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Abstract:
The order Pelecaniformes represented by five families, Threskiornithidae, Ardeidae, Scopidae, Balaenicipitidae and Pelecanidae, includes a diversity of waterbirds, totaling approximately 120 species. Cytogenetic data on Pelecaniformes indicates a derived karyotype when compared to the modal diploid number of birds (2n=80), showing a variation from 2n=50 to 2n=74 chromosomes. Studies on chromosomal organization in Pelecaniformes indicated the occurrence of distinct rearrangements, where the main events were fusions between macro and microchromosomes.The objective was to investigate the chromosomal organization of three species of Pelecaniformes, Cochlearius cochlearius (CCO) and *Syrigma sibilatrix* (SSI) from the Ardeidae family, and *Eudocimus ruber* (ERU) from the Threskiornithidae family. Skin biopsies were used to establish fibroblast cell cultures and the chromosomes were obtained by standard methodology, using colcemid, hypotonic treatment, and cell fixation in methanol/acetic acid. FISH experiments were performed using whole-chromosome probes of GGA (1-10) generated by flow-sorting at Cambridge Resource Centre for Comparative Genomics, for each probe, at least 10 metaphase spreads per individual were analyzed to confirm FISH results. The species studied showed karyotypic differences, both in chromosome number and morphology. C. cochlearius with 2n=74 chromosomes presents the 1st to 6th pair and the Z chromosome submetacentric, the 7th and 10th metacentric pair, and the 8th and 9th telocentric pair. *S. sibilatrix* (2n=62) has the 1st, 2nd, 3rd and 8th submetacentric pairs, the 4th, 5th, 7th, 9th, 10th pair and the metacentric Z-chromosome, the 6th telocentric pair and the acrocentric W-chromosome. *E. ruber* (2n=66) has the 1st, 4th, Z and W submetacentric, the 2nd, 3rd, 6th and 7th acrocentric, the 5th, 8th and 9th metacentric pairs. The remaining pairs of the complement in all three species are microchromosomes. The GGA probes produced signals on 11 pairs of chromosomes in the species studied. In *C. cochlearius* and *S. sibilatrix* species the GGA6 probe hybridized on chromosomes CCO4q and SSI4q while, the GGA7 probe hybridized on CCO4p and SSI4p, indicating a fusion event in both species. The probe GGA4 hybridized in two different pairs in each species, CCO5, CCO10 and SSI5, SSI10. Unlike the other species, in ERU two fusions occurred, one in the ERU5 pair where GGA7 and GGA8 hybridized, and another in the ERU4 pair by hybridizing GGA6 and GGA1, the latter which also hybridized in ERU2. GGA4 hybridized in ERU6 and ERU9. Previous studies reinforce what has been found here in families of Pelecaniformes, a decrease in diploid number during the evolution of this group resulting mainly from fusions. Besides the rearrangements mentioned above, other fusions of microchromosomes must have occurred to explain the low chromosome number of these species, highlighting the importance of these elements for the evolution of the group. We speculate that species of these orders have some genomic characteristics that facilitated the occurrence of interchromosomal rearrangements involving microchromosomes. Thus, new studies should be conducted to investigate this hypothesis.
Key-words: Chromosomal rearrangements; chromosome painting; comparative cytogenetics;

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ID - 83

FIRST CHROMOSOMAL ANALYSIS ON THE WEBSPINNER FAMILY ARCHEMBIIDAE: *PARARHAGADOCHIR CONFUSA* AND *PARARHAGADOCHIR FLAVICOLLIS* (INSECTA, EMBIOPTERA)

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Abstract:

The webspinners (Embioptera) is one of the smaller and poorly studied insect orders, comprising around 400 species, in which the morphological identification is often problematic and based mainly on males. Cytogenetics can be an additional source of data to help in taxonomy of this group. Up to now, only nine Embioptera species were karyotyped, none of them belonging to Neotropical region. The aim of this study is to chromosomally characterize *Pararhagadochir confusa* and *Pararhagadochir flavicollis* (Archembiidae), contributing to the cytotaxonomy of this group. The preparations were obtained from the testes of two subadult males and two whole embryos of *P. confusa*; and the testes of seven subadult males of *P. flavicollis*. Both species were collected in the state of Mato Grosso do Sul, Brasil. The gonads and embryos were dissected in insect physiologic solution (7.5 g NaCl, 2.38 g Na_2HPO_4, 2.72 g KH_2PO_4, in 1 l of distilled water), immersed in colchicine solution during 2 hours (0,16% in insect physiologic solution), hypotonized in tap water during 20 minutes, and fixed in Carnoy I (3:1 Methanol: Acetic Acid) for at least one hour. The whole gonads were dissociated in a drop of 60% acetic acid on the surface of microscope slides. The preparation was dried on a metal heating plate (35-40 ºC) and stained (3% Giemsa solution). Based on the analysis of 43 cells of *P. confusa* and 47 cells of *P. flavicollis*, the results were: spermatogonial mitotic metaphases composed of 2n(male)= 23 chromosomes in both species, and female embryonic mitotic metaphases of *P. confusa* composed of 2n(female)= 24 chromosomes. In *P. confusa*, all chromosomes are biarmed (mostly metacentric, with the exception of one or two submetacentric pairs, probably pairs 8 and 9). In *P. flavicollis*, four pairs are telocentric, mostly the smaller pairs, and the remaining chromosomes of the complement are biarmed, but much more diverse (metacentric, submetacentric, and subtelocentric) than in *P. confuse* (mostly metacentric). Based on the difference of chromosome number between male and female embryo (*P. confusa*), the chromosome length, and the Embioptera cytogenetics literature, we conclude that both species present an X(male)/XX(female) sex chromosome system. In *P. flavicollis* the X chromosome is easily recognizable, as the largest element of the complement (subtelocentric). In *P. confusa*, the X chromosome has a similar size to the first autosomal pair (metacentric). Thus, the presence of telocentric chromosomes and the size and morphology of the X chromosome in *P. flavicollis* prove to be informative for distinguishing this species from *P. confusa*. The karyotype 2n(male)= 23, X, found in both species in our study, was already described in one species of Embioptera, namely *Embia nuragiga* (Embiidae), but, in this species, the chromosomes are all metacentrics, possibly with the exception of one acrocentric pair. *P. flavicollis* prove to be an exception among the websppiners karyotyped up to now, because almost half of the chromosomes of the complement are telocentric. It is also noteworthy that *P. flavicollis* is not closely related to *P. confusa* in a published phylogeny of Archembiidae.

Key-words: chromosome morphology; mitosis; cytotaxonomy;

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ID - 53

CYTOGENETIC CHARACTERIZATION OF THE STINGLESS BEE *FRIESEOMELITTA TRICHOCERATA* (MELIPONINI) THROUGH CLASSICAL AND MOLECULAR TECHNIQUES

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Abstract:
The Meliponini Tribe is a very diverse monophyletic group of eusocial bees with more than 400 described species, and in Brazil there are about 244 species distributed in 29 different genera. Popularly known as stingless bees, they play an important role in the pollination of cultivated and wild plants, mainly in tropical and subtropical areas of the planet. The enlargement of biological knowledge about these pollinators is fundamental for management and conservation strategies. Cytogenetic knowledge, for example, is important to identify species or chromosomal variations between populations. Several genera of the Meliponini have already been cytogenetically analyzed, including *Frieseomelitta*, an ecologically important genus in the Amazon. This genus includes 16 species, 13 of which have already been karyotyped. This study aimed to expand the cytogenetic knowledge of this genus, through the description of the number, chromosome morphology and location of 18S rDNA sites for *F. trichocerata*. A comparison with data already described in the literature was also performed. Larvae of *F. trichocerata* were collected in Redenção/PA. The cerebral ganglia were extracted and submitted to a hypotonic solution of colchicine to obtain metaphase chromosomes. For analysis of chromosome number and morphology, staining with 4% Giemsa was performed. The morphology of the chromosomes was performed according to Levan *et al* (1964). Chromosomal mapping of 18S rDNA clusters was performed through fluorescent *in situ* hybridization. Females and males of F. trichocerata had 2n=30 and n=15 chromosomes, respectively, with karyotypic formulae 2K: 24M + 6SM; K: 12M + 3SM. This chromosome number was also observed in a population of F. aff. trichocerata collected in Juína/MT, being the most common within the genus. Of the 13 species of this genus already karyotyped, only *F. longipes* has 2n=34, the most common pattern observed in Meliponini. The 18S rDNA genes were located in the subterminal region of the first metacentric chromosome. These genes are also located on the first metacentric chromosome pair in *F. varia* and *Frieseomelitta sp.* (in terminal and subterminal regions, respectively), which may indicate a characteristic of the genus. Thus, the data obtained in this study increase the cytogenetic knowledge about *Frieseomelitta* genus. Future studies with species of this and other Meliponini genera will help to understand the karyotypic evolution of stingless bees, in general.
Key-words: FISH; Karyotype; Ribosomal genes;

ID - 131

CYTOGENETIC AND GENETIC ANALYSES OF SPECIMENS FROM VILA BELA DA SANTÍSSIMA TRINDADE - MT REVEAL A NEW LINEAGE IN THE SPECIES COMPLEX *PHYSALAEMUS CUVIERI-PHYSALAEMUS EPHIPPIFER* (ANURA:LEPTODACTYLIDAE)

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Abstract:

Previously, studies based on mitochondrial DNA (mtDNA) sequences, 3RAD markers and cytogenetics showed that the frog species complex *Physalaemus cuvieri-Physalaemus ephippifer* is composed of, at least, five distinct genetic lineages with a notorious karyotypic variation. From all these lineages, *P. ephippifer* is the only one that exhibits heteromorphic sex chromosomes Z and W, which are probably homologous to chromosome pair 9 of its sister clade, known as Lineage 1 (L1). For Lineage 3 of "*P. cuvieri*" (L3), cytogenetic data are restricted to one site, namely Porto Nacional-TO and it differs from the other lineages by presenting multiple Nucleolus Organizer Regions (NORs) and high level of NOR polymorphism. In an analysis based on mtDNA sequences (not available in public databases), a sister clade of L3 was recognized, composed of specimens from Central-Western Brazil, for which there is no cytogenetic information. Here, we investigated four exemplars from Vila Bela da Santíssima Trindade-MT (VBST), a site located in the Central West region of Brazil, through cytogenetic and mtDNA analyses, thus contributing to a better understanding of the *P. cuvieri-P. ephippifer* species complex. For cytogenetic analyses, chromosome preparations were stained with 10% Giemsa and subjected to silver-staining using the Ag-NOR method to reveal the NORs. Also, chromosome markers, such as the repetitive DNAs PcP190 and PepBS, were mapped using fluorescent in situ hybridization (FISH). In addition, mitochondrial DNA sequences (i.e., H1 fragment, which included the 12S and 16S ribosomal RNA genes and the tRNA-Val gene) were PCR-amplified from DNA samples obtained from liver tissue. The amplified fragments were sequenced using the BigDye Terminator kit, and the resulting nucleotide sequences were aligned (using MAFFT v.7) together with all the sequences available in the GenBank for the *P. cuvieri-P. ephippifer* species complex. Representative sequences of the remaining species of the *P. cuvieri* group were also included. Phylogenetic relationships were inferred using RAxML, employing the GTRCAT model, and clustered all the VBST samples in a sister clade of L3. The karyotype of the VBST specimens showed NORs in chromosome 8, which co-localized with PepBS sequences. The long arm of chromosome 8 shows pericentromeric NOR, whereas its short arm has interstitial NORs. We found notorious variation in NOR size and number, which enabled the recognition of five different morphotypes of chromosome 8. Depending on such variation, chromosome 8 shows a metacentric or a submetacentric morphology and the chromosome arm bearing the pericentromeric NOR become the short arm. Such NOR patterns differ from those found in L3, in which multiple NOR-bearing chromosomes are present but none of them resembles chromosomes 8 of the specimens from VBST. Another difference between L3 and the VBST clade refers to the distribution of chromosomal clusters of the PcP190 satDNA. In VBST specimens, this satDNA was mapped to the pericentromeric region of 2p and 3p, whereas almost all chromosome pairs of L3 showed chromosomal clusters of PcP190 sequences. Altogether, our results raise the hypothesis that VBST is a new genetic lineage in the species complex.

Key-words: Leiuperinae; Chromosome; Evolution;

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ID -170

CHROMOSOMAL ORGANIZATION OF HISTONE H1-H3 AND U2 SNDNA MULTIGENES FAMILIES IN THREE SPECIES OF LORICARIIDAE (SILURIFORMES), AND DESCRIPTION OF A NEW CYTOTYPE FOR GENUS *SPATULORICARIA* FROM THE BRAZILIAN AMAZON

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Abstract:
Loricariidae comprises more than 900 neotropical species. Cytogenetic analyses involving histones genes and snDNA U are limited to a few members of this family. We performed the physical mapping of histone genes (H1 and H3) and snDNA U2 in *Scobinancistrus aureatus*, *Scobinancistrus pariolispos* and *Spatuloricaria* sp. The results showed a new karyotype for the genus *Spatuloricaria* sp. (2n=66, NF=82, 50m-10sm-6m). The histone H1 gene mapping in both species of *Scobinancistrus* showed scattered signals along the chromosomes, while in *Spatuloricaria* sp. clusters histona H1 were observed in pairs 1, 2 and 3; in relation to histone H3 gene, both *S. aureatus* and *S. pariolispos* carry a cluster in pair 4, in contrast to *Spatuloricaria* sp. which presents histone H3 only in pairs 1 (only one homologue) and 2 snDNA U2 presented distinct distributions in the three species: in *S. aureatus* it was observed in only one homolog of pairs 3 and 18; in *S. pariolispos* in the pair 15; in turn, *Spatuloricaria* sp. showed signals on pair 1. In *Scobinancistrus*, associations of Histones genes sequences to transposable elements (TEs) may explain the dispersed genomic organization of these multigenes. Additionally, the new cytotype described for the genus *Spatuloricaria* may indicate occurrence of cryptic species.
Key-words: Repetitive DNA; Chromosome; Histone; Loricariidae; U2 snDNA

ID - 223

HIGH CHROMOSOMAL REORGANIZATION AND PRESENCE OF MICROCHROMOSOMES IN CHACTIDAE SCORPIONS FROM THE BRAZILIAN AMAZON

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Abstract:
Scorpions are of particular interest in cytogenomic studies, as they can present a high incidence of chromosomal rearrangements heterozygous in natural populations. In this study, we cytogenetically analyzed four species of Chactidae. In *Brotheas*, 2n = 40 was observed in *Brotheas silvestris*, 2n = 48 in *Brotheas paraensis*, and 2n = 50 (cytotype A) or 2n = 52 (cytotype B) among populations of *Brotheas amazonicus*. Our results showed a bimodal karyotype in *Neochactas parvulus*, 2n = 54, with microchromosomes and a concentration of constitutive heterochromatin in macrochromosomes. The 45S rDNA is located in only one pair of the karyotype, with different heteromorphisms of clusters of this rDNA in the cytotype B of *B. amazonicus*, with NOR-bearing chromosomes involved in multi-chromosomal associations during meiosis I. The U2 snDNA was mapped in the interstitial region of distinct karyotype pairs of three Chactidae species. Our results indicate the possible formation of cryptic species in *B. amazonicus*; the different 45S rDNA configurations in the genome of this species may result from amplification and degeneration. We suggest that the bimodal karyotype in *N. parvulus* results from fusion/fission events and that the unequal distribution of repetitive DNAs between macro and microchromosomes contributes to the maintenance of its asymmetry.
Key-words: Scorpiones; monocentric chromosome; meiotic multi-chromosomal associations; NOR; Bimodal karyotype

ID - 190

THE PAIRING OF A AND B CHROMOSOMES DURING MEIOTIC PROPHASE I IN SPECIES OF THE GENUS MOENKHAUSIA (CHARACIFORMES: CHARACIDAE)

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Abstract:

Neotropical freshwater fish of the genus *Moenkhausia* are commonly known as Piabas or Tetras. Cytogenetic studies in species of this genus show a low variation in the diploid number (2n) from 48 to 50 chromosomes, with the presence of B chromosomes (B+) in seven out of fourteen karyotyped species. However, meiotic behavior studies were developed in only a few of their representatives. Here we analyzed the meiotic chromosomes of *Moenkhausia australis* and *Moenkhausia bonita* in the pachytene stage. Thirteen specimens of *M. australis* (four females and nine males), and ten specimens of *M. bonita* (one female and nine males) from the upper Paraguay River basin were analyzed by direct mitotic chromosome preparation and meiotic surface-spreading techniques. The synaptonemal complex protein 3 (SYCP3) was visualized by immunofluorescence, as well as the B chromosomes were detected by chromosome painting using probes obtained by microdissection and amplification in these species. Both species showed a diploid number of 2n=50 chromosomes and the karyotypic formula comprised 8 metacentric, 32 submetacentric and 10 subtelocentric in *M. australis*, with a fundamental number equal to 100, and 14 metacentric, 24 submetacentric, 8 subtelocentric, and 4 acrocentric in *M. bonita*, with a fundamental number equal to 96 in individuals of both sexes. In addition, an expressive inter-individual variation in the frequency of B chromosomes was observed in individuals of both species, with a variation of 0 to 3 small acrocentric B chromosomes in *M. australis* males, and from 0 to 2 small metacentric B chromosomes in *M. bonita*. Meiotic results of B+ individuals at the pachytene stage was identical in both species. Chromosomal painting of these preparations confirmed the presence of small-sized B chromosomes in the pachytene cells, as well as the fluorescent signals in chromosomes of the A complement, in both species. The SYCP3 signals revealed the presence of 25 bivalents (A chromosomes) and a small-sized supernumerary univalent element (B chromosomes) in fishes with 2n=51 chromosomes. We also found that all chromosomes, including the B chromosomes, form a regular synaptonemal complex structure. In this context, we can hypothesize that multiple B chromosomes may be pairing with each other during the pachytene stage, and through a regular synapsis process, these chromosomes can undergo chiasmata that hold the homologous chromosomes together, allowing them to share DNA segments among B chromosomes. Thus, we hypothesized that checkpoint mechanisms that detect unsynapsed chromatin segments are not activated, therefore ensuring the continuation of spermatogenesis. In this case, the regular synapsis occurring among the B chromosomes is potentially essential to maintain their transmission process and ensure the evolutionary success through the generations.

Key-words: cytogenetics; SYCP3; chromosomal painting; meiosis in fish; synaptonemal complex

Acknowledgement

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ID – 219

CYTOGENETIC CHARACTERIZATION OF HYPOSTOMUS SERTANEJO ZAWADZKI, RAMOS & SABAJ PÉREZ 2017, (SILURIFORMES, LORIICARIDAE): AN UNPRECEDENTED CONTRIBUTION ON THE DIPLOID NUMBER IN THE GENUS

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Abstract:

Studies of identification and characterization of fish from the Brazilian ichthyofauna allow us to understand the evolution patterns and knowledge of the biodiversity of fish from rivers and streams of the basins of the Brazilian neotropical region. In this study, we employed cytogenetic techniques to identify and characterize Hypostomus sertanejo. Historically, the minimum diploid number of 54 chromosomes is accepted for the genus Hypostomus. However, we found that the species in question has 2n=52, which represents a new discovery for the genus. Our objective was to identify and characterize specimens of H. sertanejo, collected in the Carius River, in the state of Ceará. For this work, we performed cytogenetic analyzes of 6 individuals through Giemsa staining, C-banding, silver nitrate (Ag-NOR) impregnation and fluorescent in situ hybridization (FISH) using probes for the 5S and 18S ribosomal genes. The analysis protocols were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEUA 1968010719). H. sertanejo showed karyotype 2n=52 (16m+16sm+20st/a), multiple Ag-NOR with markings on two pairs of chromosomes, coincident with the marking by the 18S probe. Marking by the 5S probe was also observed in two pairs of chromosomes. However, in one of them, the marking was co-located with 18S suggesting that these two genes are close in the same chromosomal locus. C-banding showed blocks of heterochromatin with emphasis on the first pair of metacentric chromosomes. Knowledge about the chromosomal data of a species is essential for identification and taxonomic classification, allowing to differentiate it from other closely related species. Thus, the cytogenetic study carried out in this work provides an important contribution to a better understanding of the evolutionary history of the group. Furthermore, our data may also have important practical implications, especially for the conservation of the species, as the identification of chromosomal variation can help to identify isolated or distinct populations of a species, which may require specific conservation measures.

Key-words: Hypostomus; cytogenetic characterization; biodiversity;

ID - 197

GENETIC AND MORPHOMETRIC ANALYSES IN NEOAULACORYSSUS SPECIOSUS (COLEOPTERA, CARABIDAE): POSSIBLE EVIDENCE OF PHENOTYPIC PLASTICITY

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Abstract:

Neoaulacoryssus speciosus Noonan, 1985 is a ground beetle species that commonly presents an annual population boom in urban environments. This species appears to be of no formal medical concern, but when tight it can release a defensive secretion, causing skin burns. Remarkable differences in the dorsal color of this beetle have been observed among some Brazilian populations, in which the specimens exhibit two patterns: green pronotum - red elytra (GR) or red pronotum - green elytra (RG). Aiming to evaluate if this phenotype difference is caused by genetic variation or environmental influences, we performed cytogenetic, molecular, and morphometric analyses in two populations: Cuiabá (GR phenotype), state of Mato Grosso, and Montes Claros (RG phenotype), state of Minas Gerais. Chromosome preparations were obtained from testes of adult specimens and standard stained with Giemsa. For molecular analysis, we used a fragment of the Cytochrome Oxidase I (COI) mitochondrial gene. The five DNA sequences obtained for each population were edited in the Geneious 7 software, aligned using the MUSCLE method on MEGA X software. The genetic distance between the two populations of *N. speciosus* and other closely-related species (*Paraulacoryssus puertoricencis* - COI sequence extracted from Bold Systems) were calculated in MEGA X. The lengths of pronotum and elytra of male and female specimens from each population was measured in a Leica stereomicroscope using the MC 190HD image capture system. Values were compared using permutation and t-tests using the R package coin. The cytogenetic studies revealed the 2n=34, XX/XY. This diploid number was determined mainly by analysis of diplotene cells, in which 16 homomorphic and one heteromorphic bivalent were visualized. This last bivalent was formed by one medium and one small-sized chromosome, being interpreted as X and Y sex chromosomes. No cytogenetical differences were observed between the populations studied. The DNA sequences consisted of 591bp, including 41 variable sites. These sequences did not present genetic saturation, with the Iss (Index of Substitution Saturation) R2=0,019 and Iss.c - R2=0,740. The genetic distance between the populations from Cuiabá and Montes Claros was equal to 0%; however, when we compared both populations of *N. speciosus* with *P. puertoricencis*, the genetic distance was 7,28%. In both morphometrical variables, the measures obtained from specimens from Montes Claros were considered statistically larger than specimens from Cuiabá ($p<0.001$). Therefore, our results indicate that the differences in the coloration and body size between the *N. speciosus* populations could not be traced to genetic isolation, being either a response to environmental variables, i.e. an example of phenotypic plasticity, or a more complex genetic scenario than first thought.

Key-words: chromosome; cytochrome oxidase I; genetic distance;

ID - 150
KARYOTYPIC ANALYSIS OF TWO TYRANNIDAE SPECIES (AVES: PASSERIFORMES)

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Abstract:
The Aves class comprises approximately 10,900 species that display high levels of morphological, ecological, and behavioral diversity. Birds are currently divided into two major phylogenetic groups: the Palaeognathae (basal birds) and the Neognathae (modern birds, including Galloanseres and Neoaves). The Passeriformes order among Neoaves has the greatest species variety, and the Tyrannidae family, which contains about 600 species, is the most diverse and numerous one. This family has unusual cytogenetic traits, such as stability in the diploid number (between 2n=76 and 84), but with variance in chromosomal morphology, indicating the occurrence of different chromosomal rearrangements. Despite these remarkable features, cytogenetic studies of the Tyrannidae remain limited, with only 20 species having been karyotyped thus far. The objective of this study was to describe the karyotypes of two species from this family (*Myiopagis viridicata* and *Sirystes sibilator*), as well as to analyze the distribution of constitutive heterochromatin-rich regions (C-banding) and the location of telomeric sequences, 18S rDNA, and microsatellites through fluorescence in situ hybridization. The karyotype of *S. sibilator* is composed of 2n=80, with the first, fourth, fifth, and the sex chromosomes as submetacentric morphology. The second, third, sixth, seventh, eighth, ninth, and tenth pairs are acrocentric, while the other 29 pairs are likely telocentric. *M. viridicata* is also composed of 2n=80, with the first and fifth as submetacentric morphology, the nineth metacentric, second, third, fourth, sixth, seventh and tenth pairs acrocentric, and the other pairs are likely telocentric. The Z chromosome is acrocentric. In *M. viridicata*, the 18S rDNA probes hybridized to two pairs of microchromosomes, but in only one pair of microchromosomes in *S. sibilator*. According to this finding, *M. viridicata* underwent ribosomal sequence duplication and translocation, whereas *S. sibilator* preserved the ancestral trait of having just one pair of microchromosomes with ribosomal sequences. Only the telomere portions of each chromosome in both species were hybridized by the telomere sequence probes, ruling out interchromosomal rearrangements and the presence of interstitial telomeric sequences. The majority of the microsatellite sequences were located in the centromeric and telomeric regions of several macro- and microchromosomes in both species, which likely correspond to the heterochromatin-rich regions. Overall, these results shed light on important aspects of the karyotype of Tyrannidae members and contribute to a better understanding of chromosomal evolution in this group.
Key-words: FISH; Karyotype; Microsatellites; Probes;

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ID - 6

SEX CHROMOSOMES IS A CENTER OF SATELLITE DNA EXPANSION AND DIVERSIFICATION: THE CASE OF *EUCHISTUS* GENUS

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Abstract:

Part of the complexity of eukaryotic genomes is due to the large proportion of repetitive DNAs, in addition to the occurrence of sex chromosomes. Studies of repetitive DNAs and sex chromosomes, as they show accumulation of this class of DNA, are complex and neglected in most genome assembly projects. However, these studies can be an important tool in understanding the basic biology and evolution of species, being also useful for possible practical applications for the benefit, for example, in agriculture. In Brazil, despite being important agricultural producers, little is known about the genomic and chromosomal organization of the species that are pests of our crops. Among the most iconic pests in the country, mainly related to soyben are the stinkbugs of the genus *Euschistus* (Hemiptera, Pentatomidae). Here we advanced on the understand of the genomic architecture, satellite DNA (satDNA) composition, and evolution of the sex chromosomes in *Euschistus* (*E. heros*, *E. crenator*, *E. taurulus*, *E. cornutos* and *E. picticornis*). The data was obtained by molecular cytogenetic analyzes and comparative genomic studies of satDNAs using bioinformatics aproaches. Our results showed a similar number of satDNA repeats for four species (ranging 11 to 15) and only for *E. heros* this number increased to 29 families. Total satellite abundance ranged 0.54% (*E. taurulus*) to 2.32% (*E. cornutos*) and for all five species the abundance on males were higher than in female genomes, with the lowest increase for *E. crenator* in about only 2% and the highest for *E. heros* ranging to 2.75x fold more. The chromosomal mapping for the sex specific/abundant satDNAs confirmed intense signals on sex chromosomes. We also checked the sharing of satDNAs between the species using all satDNA libraries as reference. The numbers of shared satDNAs increased significantly for all species with at least 3x fold higher and reaching 72 to 77 total numbers of families for each species. However, the number of shared satDNAs presented in males in comparison to females was not high with only one additional family for *E. heros*, *E. crenator* and *E. cornutos* and five families for *E. picticornis*. As for *E. taurulus*, the number of shared families was lower for males. Regarding the genome abundance, all species revealed an increase among total satDNA reaching at least 60% higher. In contrast to number of shared satDNAs, the abundance increased in males for all species, with 6% for *E. cornutos*, 10% for *E. crenator*, 38% for *E. picticornis*, 42% for *E. heros* and 42% for *E. taurulus*. Our results suggest possible events for satDNA accumulation and diversification related to sex chromosomes. Moreover, the sharing of many satDNA repeats among *Euschistus* species with significant increase in male genomes in relation to female put forward the library model that explains the conservation of some satDNAs for long periods, by which could be influenced by heteromorphic sex chromosome systems.

Key-words: cytogenomics; pentatomidae; evolution;

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ID - 155

KARYOTYPE NOVELTIES FOR *CYCLORAMPHUS BANDEIRENSIS* HEYER, 1983 (ANURA: CYCLORAMPHIDAE) BY EXPLORING MOLECULAR CYTOGENETICS

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Abstract:

Cycloramphus belongs to Cycloramphidae and has 30 species endemic to the Atlantic Forest. Cytogenetic data for this genus are scarce, since karyotypes of only 11 species were described. Most descriptions are restricted to diploid number and classic cytogenetic techniques. Molecular cytogenetic techniques such as Fluorescent *in situ* Hybridization (FISH) exploring microsatellite markers are useful to understand the karyotypic evolution in frogs, and in *Cycloramphus* it was only studied in *C. bolitoglossus*. Studying the karyotype of other *Cycloramphus* species may enlighten its evolutionary history. *Cycloramphus bandeirensis* is endemic to the open highlands of Parque Nacional do Caparaó, southeastern Brazil, and the mapping of repetitive sequences on its karyotype has never been done. This data is essential to a better comprehension of the chromosomal changes that may have happened during the evolution of its karyotype. Also, since it belongs to the same clade as *C. bolitoglossus,* this description may provide helpful data for comparative cytogenetics. Therefore, the main goal of this work was to characterize the karyotype of *C. bandeirensis* by mapping repetitive sequences such as rDNA 18S, and microsatellites including $(GA)_{15}$ and telomeric probe $(TTAGGG)_n$. For this study, eight specimens of *C. bandeirensis* were collected at PARNA- Caparaó, municipality of Dores do Rio Preto, Espírito Santo state. The animals were treated with 0.1% colchicine for 4 hours before euthanasia. Cell suspensions were obtained from intestinal epithelium. The chromosomes were paired according to centromere position, and morphology was determined after karyomorphometric analysis. 18S rDNA probe was amplified by PCR using stingless bee *Mellipona quinquefasciata* primers with *C. bandeirensis* DNA as template. $(TTAGGG)_n$ probe was also produced by PCR using $(TTAGGG)_5$ and $(CCCTAA)_5$ primers that also served as templates. Both probes were indirectly marked by nick-translation with digoxigenin. $(GA)_{15}$ was already marked with Cyanine-3. All repetitive sequences were mapped through FISH essay. All specimens presented 2n = 26 chromosomes, with fundamental number of 52. *C. bandeirensis* karyotype is composed of ten metacentric (1, 4, 6-13), two submetacentric (2,5) and one subtelocentric (3) pair, presenting a karyotypic formula of 10m + 2sm + 1st. Secondary constriction was observed on the long arm or pair 6. Regarding molecular cytogenetics, for $(GA)_{15}$ and $(TTAGGG)_n$ microsatellites, only terminal hybridization signals were observed, with no interstitial bands. 18S probe showed coincident hybridization at secondary constriction site. Despite having the same diploid number, the different chromosomal morphologies observed for other *Cycloramphus* species show that intrachromosomal rearrangements had an important role in the karyotipic diversification of the group. Differently than what is observed for *C. bolitoglossus, C. bandeirensis* didn't show any centromeric or interstitial microsatellite bands, indicating that the species either lost this condition during evolution or intrachromosomal rearrangements and/or association with transposable elements were responsible for producing these patterns in *C. bolitoglossus*. The new data herein obtained allows a comparison among species of the genus and contributes to better understanding of the evolutionary changes on the karyotype of the group.

Key-words: Repetitive sequences; Microsatellite; Frogs;

ID - 224

MICROSATELLITES DISTRIBUTION IN THE KARYOTYPE OF *DESMODUS ROTUNDUS* (CHIROPTERA, PHYLLOSTOMIDAE)

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Abstract:

Bats from the family Phyllostomidae are the third most specious group within the mammals, with a wide variety of dietary guilds that encompass a vast range of morphological variations. Among them, the subfamily Desmodontinae comprises the only mammals with obligate blood feeding, with three species currently described. Cytogenetically, these species have been extensively studied, however, the repetitive portion of their genome, especially the distribution of microsatellites, hasn't been explored yet. In this sense, we aimed to map the distribution of microsatellite sequences in the karyotype of *Desmodus rotundus* (2n= 28, FN=52). For this, we performed experiments of fluorescence *in situ* hybridization in metaphase chromosomes of D. rotundus, obtained from fibroblast cultures. As probes, we used seven microsatellite sequences: $(CAA)_{10}$, $(CGG)_{10}$, $(CAG)_{10}$, $(GA)_{15}$, $(GAG)_{10}$, $(GC)_{15}$ and $(TA)_{15}$. Overall, the microsatellites were observed in a dispersed distribution in the genome of *D. rotundus*, accumulating preferentially in the tips of the chromosome arms, centromeric regions, and in the negative G-band regions. Additionally, two microsatellites produced signals in two regions of proximal long arms of pairs 6 and 10 which were not hybridized by any of the chromosome paints used before (Phyllostomus hastatus and Carollia brevicauda). In this sense, these regions may correspond to an amplification/accumulation of repetitive DNA sequences specific to this species that were not positive C-band.

Key-words: Microsatellites; Bats; Karyotype;

ID - 109

CYTOGENETIC DESCRIPTION OF A SMALL RED BROCKET DEER FROM NORTHWEST ARGENTINA *MAZAMA RUFA TOBA* (LONNBERG, 1919) AND CHROMOSOMAL DIVERGENCES WITH *MAZAMA RUFA* (ILLIGER, 1815) REVEALS A POTENTIALLY VALID SPECIES WITHIN THE GENUS *MAZAMA*

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Abstract:

Mazama rufa toba is a red brocket deer that was described in Chaco Central of Argentina by its smaller dimension compared with *M. rufa rufa* from Paraguay. As no further analyses have been performed to clarify its genetic identity, we aimed to assess the cytogenetic data from a current topotype kept in captivity at the Reserva Horco Molle, with known origin in the wild from Duque Escaba in Tucuman Province of Argentina. We obtained chromosomal preparations for cytogenetic characterization through fibroblast cell culture and performed conventional staining, Ag-NOR, G and C- banding. Using bovine chromosomal painting probes (WCP) we compared the karyotypes of *M. rufa toba* with the previously characterized karyotype of *M. rufa* from Paraguay (2n = 52 and FN = 56) to detect chromosomal divergences. Classic chromosomal banding revealed a differentiated karyotype with 2n = 50 and FN = 64, formed by 25 pairs of autosomes and a simple sexual system. The Ag-NOR staining showed three nucleolus organizing regions in the telomeric area of the long arms of the two chromosomes of pair 7 and only in one chromosome of pair 8. C-banding showed constitutive heterochromatin blocks in the pericentromeric region of all chromosomes, weak interstitials bands in the long arms of chromosomal pairs 1, 2 and 3, and strong C band in the distal region of pairs 6 and 7. G-banding associated with WCP mapping revealed that *Mazama rufa toba* underwent different chromosomal changes compared to the karyotype of *Mazama rufa.* The species diverged during their karyotypic evolution in at least 10 chromosomal rearrangements including tandem fusions, centric fusions and inversions involving seven pairs of autosome chromosomes. Moreover, *M. rufa toba* possess simple sexual system compared to the multiple sexual system XY_1Y_2 in *M. rufa*. Previous studies have showed that accumulation of chromosomal divergences, such as fusions and inversions, leads to error in chromosome pairing during meiotic segregation. Then, the observed chromosomal differences are a substantially evidence of a reproductive isolation mechanism between *M. rufa toba* and *M. rufa* individuals. This distinctive karyotype of *M. rufa toba* putatively support the recognition as a valid species following the biological species concept.

Key-words: karyotype; Neotropical deer; cytotaxonomy; FISH;

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ID - 126

MANY TRANSPOSABLE ELEMENT FAMILIES ARE TRANSCRIPTIONALLY ACTIVE AND DIFFERENTIALLY EXPRESSED IN *AEDES* AND *ANOPHELES* MOSQUITOES.

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Abstract:

The functional role of transposable elements (TEs) has gained much attention over the last decade. These repetitive DNA sequences can insert themselves at different locations in the genome, often resulting in negative consequences for the host. To suppress their expression and subsequent transposition, TEs are often transcriptionally repressed. Despite this, studies show that some families of TEs display increased mRNA expression after exposure to stress. Evidence also shows that the level of repression differs between somatic and germinal tissues. Much of the evidence, however, derives from model species, excluding many mosquito species of public health importance. In this study, we seek to characterize the expressed mobilome of 12 species of mosquitoes of 3 genera, *Aedes*, *Anopheles* and *Culex*, in addition to comparing the expression of TEs under different conditions. First, TEs were detected by the TEdenovo pipeline. Consensus sequences underwent a semi-automatic curation to eliminate artifacts and duplicate structures and, finally, were submitted to TEannot for annotating the copies. Public RNA-Seq data for all species were extracted from the SRA (NCBI). These were used as input to the TEcount program, responsible for estimating the expressed families. Differential expression analyses of TEs between somatic and germinal tissues of 7 species were conducted with TEtranscripts and DEseq2 software. These same programs were also used to analyze the expression of 6 species against biotic and abiotic stresses. Results indicated that at least 50% of TE families are expressed in all species. The expression is more abundant and ubiquitous among the LTRs (*Gypsy*, *Bel-Pao* and *Copia*), but we can also observe ubiquitous expression of the superfamilies *Jockey*, *L1*, *RTE*, *Tc1-Mariner*, *hAT* and *PiggyBac*. The TE expression, however, is highly variable among tissues, between 12.3% and 62.1% of families are differentially expressed in the ovaries, which, in general, show strong repression of TEs compared to testes or somatic tissues. It was also observed that only a small fraction of families showed expression modulation after exposure to abiotic stress conditions (0% to 15.6%), and in 94% of cases, the number of repressed TE families is higher than the number of induced ones. The biotic stress conditions, tested only on *Ae. aegypti*, produced very few changes on genome-wide TE expression (0% to 4.6% of families), generally with a greater number of repressed than induced TEs. These results contrast with the classic view of the induction of TEs after stress, being in line with recent evidence from *Drosophila melanogaster*. In sum, the study shows that TEs are not transcriptionally static structures in mosquitoes and that their activation or repression depends on the type, intensity, and post-stress interval analyzed.

Key-words: Transposable Elements; Transcriptomics; Differential expression; Mosquitoes;

Acknowledgement

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ID - 120

FIRST CYTOGENETIC DESCRIPTION AND MAPPING OF MICROSATELLITES IN SAGUINUS URSULUS E. GEOFFROY, 1803 (PRIMATES; CALLITRICHIDAE)

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Abstract:

Saguinus ursulus is a recently described species of Neotropical primate, morphologically and phylogenetically close to *Saguinus niger*. Currently, its conservation status is vulnerable to extinction due to the expected population decline of more than 30% based on Global Forest Watch data for the Brazilian Amazon regions of Pará and Maranhão, where the species inhabits and which are losing a large amount of forest cover due to human settlement and advanced environmental degradation. Although there is a great diversity of studies with Neotropical primates, for this species, studies focusing on genetic and cytogenetic aspects of this species are still scarce. Thus, this study aimed to describe the distribution of microsatellite sequences in the karyotype of *S. ursulus*. For this, chromosome preparations were obtained from fibroblast cultures of skin biopsies from two specimens of *S. ursulus*. Chromosomes were subjected to G-banding and fluorescence in situ hybridization (FISH) using four microsatellite probes - $(CA)_{15}$, $(GA)_{15}$, $(CAC)_{10}$, and $(GAG)_{10}$. Our results showed that the species presents 2n=46 corresponding to 15 metacentric/submetacentric pairs and 7 acrocentric pairs; the X chromosome is submetacentric and the Y is acrocentric. The distribution of microsatellite sequences $(CA)_{15}$ and $(CAC)_{10}$ showed a similar pattern, producing signals on the telomeres and centromeres of the chromosomes and a conspicuous accumulation on pair 4. The sequence $(GA)_{15}$ produced signals in the secondary constrictions of pairs 4, 18, 20, and 22 and in the distal portion of pair 5. Finally, the sequence $(GAG)_{15}$ produced signals on telomeres and centromeres, and also on the euchromatic regions of pair 1. Regarding the macrostructure, when compared to other species of *Saguinus*, we noticed that *S. ursulus* exhibits the typical karyotype of the genus with 2n=46. However, considering that repetitive sequences play an important role in the genome organization, studies focusing on the distribution of microsatellites can reveal a hidden variation and hence bring important information concerning karyotypic evolution within this genus.

Key-words: Chromosomal conservatism; Primates; Repetitive sequences;

ID - 221

CHROMOSOME-SPECIFIC PROBES FROM BLACK-HAWK-EAGLE (*SPIZAETUS TYRANNUS*): A NEW TOOL FOR AVIAN GENOME EVOLUTION STUDIES.

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Abstract:

Because of the importance of the chicken (*Gallus g*allus GGA) as a model organism, and similarity between the chromosome complement of this species and the putative avian ancestral karyotype, the chromosome-specific probes of GGA have become the basis for the beginning of chromosomal painting studies in the class Aves. For instance, comparative chromosome painting using whole chromosome probes of the chicken (*Gallus gallus* - GGA) has shown that the relative low diploid numbers found in birds of prey resulted from an intense chromosomal reorganization including several fissions in the larger macrochromosome pairs of the ancestral karyotype, while microchromosomes were involved in fusions. Consequently, many chromosome pairs observed in species of family Accipitridae (2n usually ranging from 54-68) correspond to fragments of the ancestral macrochromosomes. Hence, the sets of probes from birds of prey work as region-specific probes, revealing intrachromosomal rearrangements not observed with the use of GGA paints. In the present study we present the results of a new set of whole-chromosome-specific probes obtained from the Black-Hawk-Eagle (*Spizaetus tyrannus* STY), a neotropical diurnal bird of prey with 2n=68. The karyotype of *S. tyrannus* corresponded to 18 peaks on flow karyotype, in which STY pairs 2, 15, 16 and 31 formed separate peak each. However, pairs 1+3 were found in the same peak as well as pairs 4+5, 8+11, 9+10, 12+W, 13+14, 18+19, 27+28, 29+30 and 32+33, also in some peaks we identified three or four chromosome together, such as 11+14+W, 9+10+12+W, 20+21+22, 23+24+26, this happened because of the similar size of these chromosomes. The results of comparative chromosome painting of STY probes in metaphases of GGA indicates a great similarity of STY paints and the ones of *Leucopternis albicollis*, and this is expected given their phylogenetic proximity, both belonging to the same family (Accipitridae, Accipitriformes) and with very similar diploid numbers. In conclusion, the new probes proved to be able to serve as a new tool to enrich studies focusing on chromosomal evolution in birds.

Key-words: flow citometry; chromosome painting; FISH;

Acknowledgement

PROPESP CNPq UNIVERSIDADE FEDERAL DO PARÁ INSTITUTO EVANDRO CHAGAS

ID - 184

CHARACTERIZATION OF *HAT* DNA TRANSPOSON IN THE GENOME OF NEOTROPICAL FISH *APAREIODON* SP.: ELEMENT DIVERSITY AND GENES CO-OPTION PROPOSAL

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Abstract:
Fish genomes present wide chromosomal and genomic diversity, including a vast diversity of transposable elements (TEs), which have been described to generate genomic evolutionary novelties. Therefore, the present work aimed to obtain a curated TE library from the *Apareiodon* sp. genome, and subsequent selection of hAT superfamily TEs for further use in cytogenetic and evolutionary studies. Initially, the *Apareiodon* sp. genome was screened with the RepeatModeler2 tool, for initial prospection of TE sequences, followed by manual curation of TE libraries, focusing on obtaining a curated hAT library, resulting in a total of 22400 hAT sequences. From this library, sequences were screened for detecting structural characteristics of hAT elements. Through HMMER analysis 1167 sequences were observed to possess protein domains characteristic of hAT transposases, and 9 sequences presented all transposase domains, of which three were on a single ORF. From these 9 conserved sequences relatively conserved TIRs of 10 bp were observed adjacent to the transposase, but no conserved TSD sequences were identified; thus, three possibly autonomous sequences were discovered. As for non-autonomous sequences, MITE-Tracker results revealed 1825 sequences characteristic of MITEs, with relatively conserved TIRs and TSDs. PERF software was used for screening of tandem repeats associated with TEs, revealing the possible origin of microsatellite sequences from degenerated hAT elements, as revealed by the abundance of some microsatellites on the hAT library. Bayesian phylogenetic analysis demonstrated the distribution of hAT elements on the three main hAT groups (Ac, Buster, and Tip), and RepeatMasker results analysis of those sequences showed their division into 6 subgroups: Ac, Charlie, Blackjack, Tip100, hAT6, and hAT5, from most to least numerous, with a significant predominance of Ac elements, including the presence of all 9 most conserved sequences on the Ac group. hAT probes were obtained for fluorescent in situ hybridization on *Apareiodon* sp. chromosomes, where hAT sites were dispersed on the chromosomes and not involved in the differentiation of the heteromorphic region of the W chromosome. At least, further analysis of the protein domains derived from the hAT library revealed two protein domains not characteristic of hAT: General transcription factor II-I repeat domain-containing protein 2-like (GTF2IRD2) and FAM200A-like, which were further investigated. Blastp analysis with the respective *Apareiodon* sp. proteins as query against the NCBI database revealed the presence of similar proteins on several fish and other vertebrate species, and further RepeatMasker analysis detected that the sequences of these two genes presented regions of similarity to hAT-Charlie elements on all evaluated species. Finally, the alignment of amino acid sequences showed high similarity for the GTF2IRD2 and FAM200A-like proteins between *Apareiodon* sp. and other fish species, demonstrating the possible origin of these genes from hAT-Charlie elements and its high conservation between distant species. In conclusion, the genomic analysis of hAT superfamily in *Apareiodon* sp. demonstrated a great diversity of TEs, with a predominance of hAT-Ac elements, besides possible autonomous copies in this group. Furthermore, mechanisms for the differentiation of degenerated TEs into tandem sequences and cooption for gene origin were also identified.
Key-words: Genomic data; Transposable elements; Genome evolution;

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ID - 55

COMPARATIVE CYTOGENOMIC ANALYZES IN *ANCISTRUS* (SILURIFORMES: LORICARIIDAE)

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Abstract:

Satellite DNAs (satDNAs) are the most dynamic repetitive elements in the genomes of eukaryotes. The genus *Ancistrus* Kner, 1852, belongs to the Loricariidae family and from the cytogenetic point of view is a very interesting group. Its representatives have large karyotype variation with individuals with 2n = 34 to 2n = 54 chromosomes, in addition to heteromorphic sexual chromosomal systems of the type ZZ/ZW, XX/XY and multiple systems. The knowledge about the genomic organization in *Ancistrus* is still limited and there is no updated or complete data about satDNAs. In this work, high-throughput cytogenomic analyzes were performed for the characterization of the satellitome of *Ancistrus* sp. A total of 39 satellites were found corresponding to 11.5% of the sequenced genome, being the first in-depth characterization of the of *Ancistrus* sp. satellitome. Furthermore, we verified the presence and sharing aspect of the two most abundant satellites, AnSat1-142 and AnSat2-139, in other species of the genus (*Ancistrus multispinis*, *Ancistrus ranunculus* and *Ancistrus cryptophthalmus*) using the *RepeatExplorer2*, *TAREAN* and *RepeatMasker* platforms. Through *RepeatMasker*, the abundance and divergence (*Kimura-2-parameter* - K2P) analyzes show that AnSat1-142 is practically absent in *A. multispinis*, *A. ranunculus* e *A. cryptophthalmus*, except for *Ancistrus sp*., where it is abundant, suggesting its conservation in this species. On the other hand, the presence of AnSat2-139 was better evidenced in *A. multispinis*, *A. ranunculus* e *A. cryptophthalmus*, besides *Ancistrus sp*., configuring a certain degree of sharing, corroborating with the satellite library hypothesis. Our comparative analyses revealed the genomic organization of satDNAs in *Ancistrus* and contributed to the elucidation of evolutionary mechanisms of repetitive sequences in different fish species.

Key-words: Repetitive DNA; Cytogenetics; Satellitome; Ancistrus; Fish

Acknowledgement

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ID - 195

FIRST CYTOGENETICS FOR *PHEIDOLE OBSCURITHORAX* NAVES, 1985 AND INSIGHTS ABOUT THE 18S RDNA GENES DISTRIBUTION FOR THE *FALLAX*-GROUP WILSON, 2003

Gabriela de Figueiredo Jacintho [1]; Gisele Amaro Teixeira [2]; Eduarda Melo de Abreu Vieira [1]; Luísa Antônia Campos Barros [2]; Denilce Meneses Lopes [1]

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Abstract:

Pheidole is the most abundant genus in the Formicidae family and is distributed worldwide. Given its high radiation, this clade was divided into several species groups that were delimited based on their morphological similarities. Despite the great number of species, *Pheidole* has a conserved chromosome number, with the majority of the species having 2n=20 with all or most metacentric pairs. This pattern was also observed in *fallax*-group representatives, a large assemblage of approximately 100 Neotropical species. An interesting note about the karyotype of that group is that two of its five karyotyped species, *Pheidole midas* and *Pheidole fallax*, showed a heteromorphism in the size of their longest chromosome pair. Molecular cytogenetic techniques such as Fluorescent *in situ* Hybridization (FISH) are useful for understanding the karyotypic evolution of ants. FISH assays using 18S rDNA as a probe revealed clusters of different sizes in the chromosome pair that bears the ribosomal gene of *P. midas.* These findings suggest a potential explanation for the heteromorphism observed in its first metacentric pair. This also led to the hypothesis that the heteromorphism observed in *P. fallax* might be due to the same cause. To investigate whether these patterns were also observed in other representatives of the *fallax-group*, we studied the karyotype of *Pheidole obscurithorax*, describing its number, karyomorphology, and 18S rDNA location for the first time. The colonies were collected in the municipality of Viçosa, MG, Brazil. Mitotic chromosomes were obtained from the cerebral ganglia of larval stages after *meconium* elimination, subjected to hypotonic colchicine solution and fixatives, and stained with Giemsa. Chromosomes were measured, arranged in decreasing order of size, and classified as metacentric (m), submetacentric, subtelocentric (st), or acrocentric (a). 18S rDNA probes were amplified by polymerase chain reaction (PCR), indirectly marked, and mapped by FISH. *Pheidole obscurithorax* presented 2n=20 with a karyotype formula of 18m+2st. Although the diploid number is conserved among the cytogenetically characterized species of the *fallax*-group, differences in the morphology of their chromosome pairs can be noted. This may be due to non-Robertsonian rearrangements because there is no numerical change. 18S rDNA genes were located in the pericentromeric region of the first metacentric pair. Furthermore, *P. obscurithorax* exhibited heteromorphism in the size of 18S rDNA clusters between homologs. This type of variation can be present in other species with rDNA clusters in the pericentromeric region, which is possibly linked to the formation of extrachromosomal circular DNAs. Our results reinforce that the karyotype of the *fallax* species group is highly conserved in number, and some species share a heteromorphism in the bearer chromosome pair of the 18S rDNA, caused by the difference in the 18S rDNA clusters. The new data obtained in this study will contribute to a better understanding of the karyotypes in this group.

Key-words: Formicidae; molecular cytogenetics; ribosomal genes;

ID - 199

COMPARATIVE ANALYSIS AND NOVEL FINDINGS ON THE CYTOGENETICS OF THE *GUNDLACHI*-GROUP OF SPECIES OF *STRUMIGENYS* SMITH (HYMENOPTERA:FORMICIDAE: MYRMICINAE)

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Abstract:

Strumigenys, a highly diverse and globally distributed ant genus is organized taxonomically into groups of similar species, including the *gundlachi*-group. It is further divided into two complexes and includes some common and widespread species, *e.g*., *Strumigenys crassicornis*, *Strumigenys denticulata,* and *Strumigenys subedentata*. Despite its high diversity, the cytogenetics of *Strumigenys* is poorly known. To date, for the Neotropical region, there are five karyotyped species, two of which are members of the *gundlachi*-group (*crassicornis*-complex): *S. crassicornis* (2n=26) and *Strumigenys* aff. *stenotes* (2n=16). To improve the knowledge of *Strumigenys* cytogenetics, the objective was to evaluate the karyotype of two members of the *gundlachi*-complex: *S. denticulata* and *S. subedentata*. Colonies were collected from small logs in two fragments of the Atlantic rainforest in Viçosa, MG, Brazil. Mitotic chromosomes were obtained from the brain of larval stages after *meconium* elimination, submitted to hypotonic colchicine solution, fixatives, and stained with Giemsa. Chromosomes were measured, arranged in decreasing order of size, and classified as metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric (a). 18S rDNA probes were amplified by polymerase chain reaction (PCR), indirectly marked, and mapped by fluorescent *in situ* hybridization (FISH). Microsatellite $(GA)_{15}$ probe was directly labeled and used to map the location of repetitive DNA by FISH. The chromosome number of *S. denticulata* was 2n=18 (2n=16m+2sm) and for *S. subedentata* (2n=18m). Both species showed 18S rDNA pericentromeric blocks on the short chromosome arm in the second metacentric pair of *S. denticulata*, and in the third metacentric pair of *S. subedentata*. Both species presented dispersed distributions of $(GA)_{15}$, except for the centromeric region. However, *Strumigenys crassicornis* showed $(GA)_{15}$ clusters in the first and second submetacentric pairs, and *Strumigenys subedentata* lacks $(GA)_{15}$ signals in the 18S rDNA pericentromeric site and in its whole 9th metacentric pair. The karyological variation of *gundlachi*-group species here analyzed and previously studied (2n=16, 18, and 26) may be explained by pericentromeric inversion and centric fissions or fusions, which are common processes in the evolution of ants chromosomes. Additionally, the studied *Strumigenys* have a single 18S rDNA labeled pair, a common pattern in ants. The species studied showed intrachromosomal location of rDNA which should influence its restriction to a single chromosome pair. However, the location of rDNA sites in chromosome pairs varies among species. The *crassicornis*-complex show them on their long arm, whereas *gundlachi*-complex on their short arms. This finding supports the hypothesis of pericentromeric inversion. The repetitive $(GA)_{15}$ sequence is prevalent in euchromatic regions of ants analyzed so far, suggesting that the studied species may exhibit similar distribution patterns despite the lack of C-banding data. The presence of $(GA)_{15}$ clusters in *S. crassicornis* and *S. subedentata* may be due to polymerase slip, DNA recombination, or transposition. However, it is unclear whether the absence of this microsatellite in the last metacentric pair of *S. subedentata* is due to similar mechanisms, or simply because it is a completely heterochromatic pair. The study reveals cytogenetics as a tool to distinguish closely related Strumigenys and highlights the potential for further research on this genus.

Key-words: molecular cytogenetics; Attini; karyotype;

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ID - 44

KARYOTYPIC CHARACTERIZATION OF THE FUNGUS-FARMING ANT *MYRMICOCRYPTA* SP. FROM THE AMAZON REGION, GUIANA SHIELD, SHOWS CYTOGENETIC VARIABILITY IN THE GENUS

Gisele Amaro Teixeira [1]; Luísa Antônia Campos Barros [1]; Denilce Meneses Lopes [2]; Hilton Jeferson Alves Cardoso de Aguiar [1]

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Abstract:

The fungus-farming ant genus *Myrmicocrypta* Smith (Formicidae, Myrmicinae, Attina) includes 27 species and 4 subspecies distributed from Mexico to northern Argentina. *Myrmicocrypta* is monophyletic with origin approximately 27 m.y.a. included in the basal clade Paleoattina and shows basal features within fungus-farming ants such as small colonies with few hundred individuals, one queen per colony mated by a single male, and monomorphic workers. Nesting is usually on the ground, however, at least two taxa are known to nest in rotten logs, which is considered a derived characteristic within the genus. *Myrmicocrypta* is one of the fungus-farming ant genera with scarce biological knowledge available, which includes cytogenetic data with only two unspecified taxa studied from French Guiana. One of them was collected in rotten logs. In this study, we characterize the karyotype of the one colony of the fungus-farming ant *Myrmicocrypta* sp. collected from rotten logs in Petit Saut - French Guiana, in the Guiana shield on the Amazon rainforest in order to enrich the chromosomal information about *Myrmicocrypta*. Mitotic metaphases were obtained from cerebral ganglia of larvae after meconium elimination and submitted the hypotonic solution of colchicine and fixatives. Chromosome number and morphology of metaphases were analyzed using conventional 4% Giemsa staining. Chromosomes were measured and classified according to the ratio of the chromosome arm lengths in metacentric (m), submetacentric (sm) and subtelocentric (st). The chromosome number and karyotypic formula observed in female workers were 2n=32 and 2n=20m+6sm+6st, respectively. This karyotype differs from the other two previously studied taxa of *Myrmicocrypta*, which presented 2n=30 chromosomes and similar chromosome morphology, with 2n=22m+2sm+6st. Comparing the karyotype of this study with 2n=32 and the previously studied 2n=30, it is possible to hypothesize a greater role for Robertsonian rearrangements generating variation in the chromosome number between taxa. Possibly two submetacentric chromosome pairs of medium size may originate a larger metacentric chromosome by chromosomal fusion, or vice versa through fission. Considering the chromosomal variation observed between taxa, it is also possible to suggest that *Myrmicocrypta* sp. of this study may be a different taxon from the others previously studied, which would increase the number of known species that nest in rotten logs. This study expanded the cytogenetic knowledge for *Myrmicocrypta* revealing chromosomal variability among taxa and the increase of species studied through classical and molecular cytogenetics will be important to understand more deeply the karyotypic evolution of the genus.

Key-words: Classical cytogenetics; Chromosomes; Biodiversity; Rearrangements;

Acknowledgement

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ID - 28

CHARACTERIZATION AND ANALYSIS OF THE SATELITOME OF THE ASIAN AROWANA (*SCLEROPAGES FORMOSUS*): A COMPARATIVE STUDY OF SATELLITES DNAS IN *SCLEROPAGES*

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Abstract:

A collection of satellite DNAs (satDNAs) in a genome is known as the satellitome. These repetitive DNAs are made up of tandem repetitions of a single DNA sequence and are frequently found in heterochromatin and pericentromeric areas. Recent research has linked these DNAs to crucial processes including the creation of heterochromatin and centromeres, control of gene transcription, and a vital part in chromosomal pairing, recombination, and segregation. The highly diverse repeat landscapes and occasional long-conserved species-specific satDNAs in fish genomes make them particularly useful models for studying the structure and function of the genomes and its repetitive elements. In this study, we characterized the satellitome of *Scleropages formosus* variety Highback Golden (Osteoglossidae, Osteoglossiformes), an species with a dated phylogenetic tree and previously examined by our research team, and we compared its chromosomal patterns with those of its sister groups (*S. jardinii* and *S. leichardti*). We employed the BGISEQ-500 platform (BGI Shenzhen Corporation, China) for the sequencing of our samples and the pipeline TAREAN for the isolation of our satDNA library. Following this, we generated specific primers for these SatDNAs, amplified them using PCR, and carried out Fluorescence in situ Hybridization (FISH) investigations in the metaphases of *S. formosus*, *S. jardinii*, and *S. leichardti*. We successfully recovered 25 satDNA families in total, of which 17 could be amplified by PCR. Our FISH experiments revealed two major outcomes: The investigations in *S. jardinii* and *S. leichardti* demonstrated negative FISH signal for all probes; in contrast, the hybridization on the source species (*S. formosus*) showed positive signals for all 17 probes, primarily in pericentromeric and terminal chromosomal areas. These findings suggest that the satellite DNAs obtained from *S. formosus* might actually be species-specific or that, even if shared, the congeneric species' copy numbers would vary, as suggested by the library hypotheses. Our next step is to sequence and characterize the satelitomes of *S. jardinii* and *S. leichardti*, and compare its catalogs with the one recovered for *S. formosus*.

Key-words: Repetitive DNAs; SatDNAs; Cytogenomics; Chromosomes; Asian Arowana

Acknowledgement

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ID - 187

CYTOGENETIC ANALYSIS OF A NEW POPULATION OF THE SPECIES COMPLEX *PHYSALAEMUS CUVIERI - PHYSALAEMUS EPHIPPIFER* (ANURA, LEPTODACTYLIDAE): EVIDENCE OF INTROGRESSION BETWEEN TWO LINEAGES WITH DISTINCT KARYOTYPES

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Abstract:

Previous phylogenetic studies have recognized that at least three independent evolutionary lineages, referred to as Lineages 1-3 (L1-L3), are currently under the name *Physalaemus cuvieri*. Furthermore, the paraphyly of *P. cuvieri* with respect to *Physalaemus ephippifer* and *Physalaemus* sp. (a group composed of one or two undescribed species) is also known, as these two latter lineages are placed as sisters to, respectively, L1 and the clade composed of L1 and *P. ephippifer*. Secondary contact zones and introgression between some lineages of this species complex were previously inferred, with Balsas-MA showing evidence of introgression from L3 to L1, and the population included in the region of São Pedro de Água Branca-MA, Vila Nova dos Martírios-MA, Trecho Seco-MA, and Imperatriz-MA (referred to as SPAB-VNM-TS/Imp) being derived from secondary contact between L1 and *P. ephippifer*. However, the extension of the putative secondary contact zones is still unknown. In this context, we aimed to investigate if there is evidence of hybridization or introgression between different lineages in Riachão-MA, a municipality located between Balsas and SPAB-VNM-TS/Imp. Since each of the above-mentioned lineages has karyotypical signatures, with *P. ephippifer* and the population from the SPAB-VNM-TS/Imp region being noticeable for having heteromorphic sex chromosomes, and L3 for having numerous and polymorphic nucleolar organizer regions (NORs), we used cytogenetic analysis in our study. Cell suspensions were dropped into slides and stained with Giemsa for the analysis of the diploid number and chromosome morphology, and posteriorly, submitted to C-banding and silver impregnation to detect heterochromatic bands and NORs, respectively. All specimens analyzed showed a diploid number of 22 chromosomes with meta/submetacentric morphology. C-bands were distributed in the peri/centromeric region of all chromosomes and in the interstitial region of the short arm of chromosome 5, and the long arm of chromosomes 8 and 9. One of the males showed terminal C-bands in chromosome pair 2. Furthermore, pairs 8 and 9 of all individuals had NORs in the long arm. One of the females showed an additional NOR in the short arm of one homolog of pair 5, which is coincident with a secondary constriction and a heterochromatic band. This additional NOR-bearing chromosome 5 is not found in L1 and is very similar to that commonly found in specimens of L3. In contrast, the NOR-bearing chromosomes 8 and 9 present in all analyzed specimens differ from those found in L3 and are similar to those of L1. Moreover, considering the absence of NORs in pair 7, the analyzed karyotypes differ from that of specimens from SPAB-VNM-TS/Imp. Therefore, the current data suggest that Riachão is included in the region with evidence of introgression from L3 into L1.

Key-words: NOR polymorphism; Leiuperinae; C-band;

Acknowledgement

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ID - 39

ABUNDANCE AND PROFILE OF THE *GALILEO* TRANSPOSON, A CHROMOSOMAL REARRANGEMENT INDUCER, IN THE GENOMES OF THE *WILLISTONI* GROUP OF *DROSOPHILA* (DIPTERA, DROSOPHILIDAE)

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Abstract:

Galileo belongs to the *P* superfamily -class II transposable element- which was detected due to its association with chromosomal inversions in *Drosophila buzzatii*, and later found in genomes across several species of *Drosophila*. To date, it is one of the few transposable elements known to induce chromosomal rearrangements in natural populations. This transposon was also found to be very abundant in the genome of *D. willistoni*, in which more than 190 copies were characterized. Furthermore, this neotropical species has high levels of chromosomal polymorphism, in which fifty chromosomal rearrangements have been recorded so far. Here, we describe the relative abundance and the profile of this transposon in the *willistoni* group of *Drosophila*, in intra- and interspecific frameworks, using the RepeatProfiler pipeline. Whole genome sequences of six *D. willistoni* strains were sequenced using short-reads and paired-end approach with a ~10X coverage. In addition short-reads of whole genome sequencing data were downloaded from the SRA repository of NCBI for species belonging to the *willistoni* group of *Drosophila* (*D. sucinea*, *D. nebulosa*, *D. equinoxialis*, *D. tropicalis*, *D. insularis*, five strains of *D. willistoni*, and the six subspecies of *D. paulistorum*). Complete copies of *Galileo*, deposited in GenBank and characterized in the genomes of *D. ananassae* (*Dana\Galileo*), *D. mojavensis* (*Dmoj\Galileo*) and *D. willistoni* (*Dwil\Galileo*), served as queries. The software BUSCO was run in the representative of the genome of *D. willistoni* with the Diptera database to obtain nucleotide sequences of single copy orthologous genes. Two genes from each chromosome arm of *D. willistoni* were randomly selected to normalize read abundance across the samples. Data from the three queries revealed that only *Dwil\Galileo* showed positive results. The terminal inverted repeats (TIRs) are the regions with higher coverage in all genomes, while the transposase (TPase) showed shallow and even none coverage. Interestingly, only *D. nebulosa* presented levels of ~10X coverage along the entire *Dwil\Galileo* sequence. The profile shapes within *D. willistoni* were similar across strains, however, TIRs coverage varied from ~25X in the French Guyanese to ~200X in the Nicaraguan strains. The pattern of degeneration seen in these results are congruent with the life cycle described for DNA transposons, which increase in copy number after invading a genome and eventually become inactive due to insertions or deletions, remaining as "fossils". The low or none coverage of the TPase suggests that the copies of *Galileo* in these genomes are no longer capable of autonomous transposition. In this scenario, *Galileo* may be transposed through mechanisms similar to non-autonomous transposons, such as Miniature Inverted-repeat Transposable Elements (MITEs). Additionally, the TIRs may be acting as sites for ectopic recombination, which potentially leads to chromosomal rearrangements similar to those seen in *D. willistoni*.

Key-words: chromosomal inversion; ectopic recombination; Galileo; RepeatProfiler; transposable elements

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ID – 124

KARYOTYPE CHARACTERIZATION OF WHITE-COLLARED SWIFT (STREPTOPROCNE ZONARIS) AND DISTRIBUTION OF TELOMERIC SEQUENCE AND RIBOSOMAL GENE 5S AND 18S

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Abstract:

Birds present an extraordinary diversity of species (10.857) and a range of ecological, morphological, behavior and genetics characteristics, that for years have inspired researchers from different areas. In cytogenetics, it is observed that most bird species preserve a karyotype compound by macrochromosomes and microchromosomes and diploid number (2n) close to 80. However, in some species, a reduction or increase in the number of chromosomes is seen. White-collared Swift (*Streptoprocne zonaris*) belong to the family Apodidae which include 113 species. In the cytogenetic view, Apodidae is one of the least studied groups of birds. The purpose of this work is to analyze the karyotype of S. zonaris. The analyzed karyotype of three females collected in Parque Estadual de Vila Velha/Ponta Grossa/Paraná, Brazil. The obtention of specimens were realized in accordance with the license IAT nº 23.18, SISBIO n° 61047-2 and CEUA-UNIPAMPA 010/2018. The mitotic chromosomes were obtained from fibroblast culture and the 2n were determined through analysis of 40 cells. For the analysis, C-band and FISH with telomeric probe, and rDNA 18S and 5S, were used. *S. zonaris* has 2n=68 chromosomes. In the karyotype was observed two pairs of submetacentric chromosomes (pairs 1 and 4). The pairs 2, and 3, and 5 to 33 are all telocentric. The Z chromosome has submetacentric and W chromosome is acrocentric. W chromosome is entire heterochromatic in the C-band analysis. The 18S rDNA was observed one pair of microchromosomes, and 5S rDNA in four pairs. The telomeric probe was seen in the centromeric region of the microchromosomes, and 7 pairs of macrochromosomes showed an unexpected block in the centromeric region marked by this probe. The results indicate that *S. zonaris*, has a reduced chromosome number, when compared to other species of the Apodidae family, possibly originated by the fusion of microchromosomes with macrochromosomes. These fusions possibly explain the atypical pattern observed with the telomeric probe. However, studies with chromosome painting may better elucidate these events.

Key-words: Birds; Apodidae; Karyotype;

Acknowledgement

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ID - 135

PHYSICAL CHROMOSOME MAPPING OF REPETITIVE SEQUENCES IN THREE SPECIES OF FAMILY ESTRILDIDAE (AVES, PASSERIFORMES).

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Abstract:

Passeriformes includes more than half of the total species of birds. Like other orders of Aves, there is a small number of reports focusing on their chromosome complement, and usually only with classical cytogenetics. Hence, several aspects of its genomic organization are still unknown, such as the distribution of repetitive sequences, among which we highlight 18SrDNA clusters and microsatellites. The latter correspond to in tandem repeats, with two to six nucleotides in length, being among the most polymorphic loci in genomes. Few bird species have microsatellite mapping data in their karyotypes. Thus, the present study aimed to map the distribution of 18SrDNA genes and microsatellite sequences in three species of family Estrildidae: *Amadina fasciata*, *Poephila personata* and *Taeniopygia guttata*, contributing to the knowledge about the genomic organization of this group. Metaphase chromosomes were obtained from cell culture using feather pulp. Diploid number and chromosome morphology were analyzed using conventional Giemsa staining. Experiments of fluorescence in situ hybridization (FISH) were performed using 18SrDNA and 12 microsatellite sequences. Our results showed that all the species showed 2n=78. The distribution of 18SrDNA was restricted to a pair of microchromosomes, a trait considered ancestral to Aves. Great similarities were observed in the distribution of five sequences of microsatellites along their microchromosomes and in telomeric and centromeric regions of macrochromosomes, but with some peculiarities. In *P. personata*, there was an accumulation of six sequences in the terminal and pericentromeric regions, mainly in the microchromosomes. In *A. fasciata*, three sequences were concentrated at the ends of the arms of microchromosomes and some macrochromosomes. In *T. guttata*, four sequences accumulated in the telomeric regions and some macrochromosomes showed a concentration in the centromere zone. In conclusion, the species maintained the standard karyotype of the class and a single pair of microchromosomes with 18SrDNA cluster. The distribution of microsatellites denoted a greater similarity between the species *A. fasciata* and *T. guttata*, from different subfamilies, while *P. personata* showed a more distinct pattern.

Key-words: Estrildidae; microsatellites; Oscines;

Acknowledgement

PROPESP/UFPA CNPq Instituto Evandro Chagas

ID -163

HETEROGENEITY IN THE HETEROCHROMATIN COMPOSITION OF THE GENUS *MELIPONA* (APIDAE) REVEALED BY CYTOGENOMICS

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Abstract:

Heterochromatin, an important region of chromosomes, is mainly made up of repetitive DNA sequences. Among these sequences is the satellite DNA (satDNA), which is characterized by arrangements of sequences in tandem. These sequences are highly dynamic so that they can undergo changes in copy number, nucleotide sequence, and this can contribute to heterochromatin and genome modification over time. In stingless bees of the genus *Melipona*, the evolution of heterochromatin has been studied due to a peculiarity in relation to variations in the distribution and quantity of this region in the chromosomes of these species. Based on this characteristic, species of this genus were artificially divided into two groups: Group I, species with a low amount of heterochromatin, occupying the pericentromeric region of the chromosomes, and Group II, with species with a high amount of heterochromatin, located in almost the entire length of the chromosomes. Therefore, our objective was to investigate the composition of heterochromatin and the mechanisms that contributed to the differentiation of this region in *Melipona*. Thus, we combined low-pass genome sequencing and chromosomal analysis for satellite DNA in representatives of four subgenera of this group: *Michmelia*, *Melikerria*, *Melipona* and *Eomelipona.* Our results revealed 11 new satDNA families to genus *Melipona*, most of which were shared between the genomes of the studied species. Despite this, we noticed a great variation in relation to abundance between species of different subgenera. The abundance of satDNA in the genome was related to the amount of heterochromatin in each species. Thus in *Michmelia*, with high heterochromatin content, MeliponaSat01-395 represented 22.64% and 24.03% of the genome of *Melipona scutellaris* and *Melipona mondury*, respectively. In *Melikerria*, which also presents species with high heterochromatin content, MeliponaSat02-196 presented as the most abundant satDNA in the genome, with 20.80% in *Melipona interrupta* and 5.35% in *Melipona fasciculata*. On the other hand, in subgenera with species with low heterochromatin content, the entire satDNA portion of the genome characterized was 1.24% in *Melipona bicolor* and 3.05% in *Melipona quadrifasciata*. Based on these results, we show that the heterochromatin composition is different between the two subgenera with a high proportion of heterochromatin, which highlights that the amplification of this region occurred independently between *Michmelia* and *Melikerria*. This process was due to the massive amplification of specific satDNA families. Finally, we note that heterochromatin is also evolving independently between subgenus with low heterochromatin content, *Melipona* and *Eomelipona.*

Key-words: Meliponini; satellite DNA; genome;

Acknowledgement

Financial support for this study was provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo ao Estado de Minas Gerais (FAPEMIG) APQ-01369-22 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

ID - 76

PCP190 SATELLITE DNA IN THE TUNGARA FROG *ENGYSTOMOPS PUSTULOSUS* (ANURA, LEPTODACTYLIDAE): CHARACTERIZATION AND EVIDENCE OF TRANSCRIPTION

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Abstract:

The genome of eukaryotes is largely made of repetitive DNAs, which can be found scattered throughout the genome or in tandem arrays. Satellite DNAs (satDNA) are tandemly repeated sequences that have several functions in the genome of many species, such as centromere organization and regulation of gene expression. However, the exact mechanism of action of most satDNA remains undetermined. Studies of repetitive sequences from anurans led to the discovery of a satDNA family called PcP190, present in several species of the superfamily Hyloidea and likely derived from 5S rDNA. The PcP190 monomer has approximately 190 bp and is characterized by a 120 bp conserved region (CR), which is highly similar among species, and a hypervariable region (HR) that varies in size and nucleotide composition. Preliminary evidence supporting the hypothesis of transcription of the PcP190 satDNA was recently found in the bufonid *Rhinella marina*. Here, we searched for evidence of transcription of the PcP190 satDNA in the leptodactylid species *Engystomops pustulosus*. First, we extracted PcP190 sequences from the genome assembly of *E. pustulosus* available in the NCBI (GCA_019512145.1) using the CR of a PcP190 sequence of *Physalaemus cuvieri* (JF281109.1) as a query in BLASTn searches. Multiple repeats of this satDNA were found in contigs assigned to chromosomes 1-3 and 6 of *E. pustulosus*, in addition to 10 different unmapped contigs. A total of 490 PcP190 sequences were analyzed, which sized from 171bp to 194bp, corresponding to 12 haplotypes. Their overall similarity in CR was 86,63% and two types of HR were found: the type 1 PcP190 HR, which is present in several species of Hyloidea, and a new type of HR, which differs from all the HR described to date. Next, we searched for sequences similar to PcP190 in 16 RNA-seq libraries of *E. pustulosus* available in the NCBI (PRJNA578590/PRJNA626021). The RNA-seq reads were trimmed using the *Trimmomatic* tool and later mapped to a PcP190 sequence of *E. pustulosus* using the *BWA* software. Mapping and coverage statistics were obtained using *Samtools* and the results were visualized in the *Tablet* software. RNA-seq reads from all libraries mapped to the CR of the PcP190 sequence, and 11 libraries had reads that were mapped to the HR. The RNA-seq libraries contained less than 4 million reads, and the egg transcriptome had the highest number of reads mapped to the PcP190 satDNA (94 out of 2,061,646 reads). This study indicates that the PcP190 satDNA is transcribed in *E. pustulosus*, as well as in *R. marina*, with both species transcribing not only CR but HR too. These findings suggest that the PcP190 satDNA transcription is not a sporadic event since it was evidenced to occur in distantly related families of anurans, consisting of relevant information for further studies on the functional role of this satDNA.

Key-words: Hyloidea; Bioinformatics; Repetitive DNA;

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ID - 107

HIGH-THROUGHPUT SATELLITE ANALYSIS ILLUMINATES THE DIFFERENTIATION AMONG GIANT XY SEX CHROMOSOMES OF *OMOPHOITA OCTOGUTTATA* (COLEOPTERA, CHRYSOMELIDAE, ALTICINAE)

Jhon Alex Dziechciarz Vidal [1]; Caio Augusto Gomes Goes [3]; Ricardo Utsunomia [3]; Renata Luiza Rosa de Moraes [1]; Roberto Ferreira Artoni [2]; Marcelo de Bello Cioffi [1]; Mara Cristina de Almeida [2]

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Abstract:

For many species of vertebrate and invertebrate species, the set of satellite DNAs (satellitome) has been characterized; however, there is little data about these elements in the Insecta class, particularly among of Coleoptera species. This study described, for the first time, the whole satDNA library of the one species of Chrysomelidae family and perform the first comparison among the male and female satellitome of Coleoptera, using as a model *Omophoita octoguttata* (Coleoptera, Chrysomelidae, Alticinae, Oedionychini, Oedionychina), which exhibit unusual giant XY sex chromosomes. DNA from males and females of *O. octoguttata* were extracted and sent for sequencing to the company DNBseq on the BGI platform (BGI Group, Shenzhen Corporation, Shenzhen, China). Then, for *in silico* analysis of male and female satellites, were used the RepeatExplorer2 and Tandem Repeat Analyzer (TAREN) tools present on the Galaxy platform and the SatMiner protocol. RepeatMasker software was used to analyze the abundance of satellite DNA in male and female genomes. The most abundant satellites in the genome of *O. octoguttata* (male and female) and the most abundant in the male genome were chosen to perform fluorescent *in situ* hybridization. The mapping of target DNA satellites was performed in meiotic cells of the male genome of *O. octoguttata*. 49 satDNAs were discovered by *in silico* analysis, being three of them more abundant in the male genome (OocSat-15, OocSat-21 and OocSat-35). OocSat-1 had a signal only in the autosomes, while other satDNAs were hybridized only on the Y-chromosome. These preliminary findings show that *O. octoguttata's* genome is made up of a variety of satDNAs, some of which are male-specific ones. The genome of *O. octoguttata* comprises several repetitive DNA sequences, as shown in earlier research, which may have a crucial role in the formation of such giant sex chromosomes.

Key-words: Satellitome; Insects; Repetitive DNA; FISH; Oedionychina

Acknowledgement

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ID - 110

DIVERGENCE IN GENOMIC AND CHROMOSOME STRUCTURE BETWEEN *OMOPHOITA* SPECIES (COLEOPTERA, CHRYSOMELIDAE, ALTICINAE)

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Abstract:
Sex chromosomes have evolved independently in a variety of species. Most vertebrates and invertebrates have the heterogametic male (XY) sex chromosomal system. The Coleoptera have gotten little attention in terms of refined analysis of sex chromosome evolution despite possessing a vast variety of sex chromosomes. The sex chromosome system of the Coleoptera subtribe Oedionychina (Chrysomelidae, Alticinae, Oedionychini) is unusual and is characterized by giant sex chromosomes that are substantially larger than autosomes. The origin and evolution of these huge sex chromosomes are little known. Their genomes have a significant percentage of repetitive sequences, especially on the giant sex chromosomes, according to cytogenetic investigations employing C0t-1 DNA and transposable elements. This study examined the similarity of X and Y sex chromosomes in several *Omophoita* species (named: *O. octoguttata, O. sexnotata, O. magniguttis,* and *O. personata*) and compared genomic differentiation among these species in order to better understand the evolutionary process and the formation of giant sex chromosomes. The X and Y chromosomes of *O. octoguttata* were utilized as probes for this purpose, and interspecific (among different species) and intraspecific (among sexes) hybridization were carried out using the whole chromosome painting (WCP) technique. Using the comparative genomic hybridization (CGH) method, probes produced from the *O. octoguttata* genome hybridized to the genomes of related species. *O. octoguttata* has few genetic differences between the sexes, according to intraspecific CGH, which also showed overlapping hybridization in other chromosomal areas and sex-specific restriction to a distal region of the Y short arm. Interspecific comparisons of *O. octoguttata, O. personata,* and *O. sexnotata,* however, showed that these species have a high level of genomic divergence and are hybridized with few areas in common. In contrast to *O. personata, O. octoguttata* possessed homeology on the Y chromosome and in four autosomal pairings. In contrast to *O. sexnotata*, only two autosomal pairs and the X pericentromeric region showed evidence of hybridization. Contrarily, chromosome painting showed that the X and Y sex chromosomes of *O. octoguttata* have a high level of intra- and interspecific genetic similarity, and all species sex chromosomes and autosomes exhibit signals of hybridization. The findings showed that the group's sex chromosomes have minimal molecular divergence, which may be related to their higher amount of repetitive DNA, as previously shown. The shared origin of the sex chromosomes, which likely have not yet had enough time for significant genomic divergence, is likely the cause of the similarities between X and Y. The sex chromosomes are similar, but the genomes of the different species show significant divergence and contain a number of species-specific regions.
Key-words: Sex chromosome; CGH; WCP; Insects; Oedionychina

Acknowledgement

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ID - 52

IDENTIFICATION OF FEMALE HETEROGAMETY IN A FROG WITH UNDIFFERENTIATED SEX CHROMOSOMES USING 3RAD MARKERS

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Abstract:

The *P. cuvieri-P. ephippifer* species complex is composed of *P. ephippifer* and at least four other lineages genetically related to *P. cuvieri. Physalaemus ephippifer* has heteromorphic sex chromosomes Z and W, while its sister species (lineage L1) does not show differentiated sex chromosomes. Chromosomal markers suggest the homeology between L1 chromosomes 9 and *P. ephippifer* sex chromosomes, but the sex chromosome system (SCS) in L1 remains unknown. Restriction site-associated DNA sequencing (RAD-seq) has been increasingly used to identify male or female heterogamy, especially in cases with undifferentiated sex chromosomes, as L1. Thus, to infer whether L1 has a XX/XY or a ZZ/ZW SCS, sex-linked markers were identified from comparative analyses of 3RAD libraries constructed from 7 males and 3 females of L1. For comparative purposes, we also analyzed 3RAD libraries from 5 males and 4 females of *P. ephippifer*. An average number of 9 and 6 million reads were analysed for *P. ephippifer* and L1 individuals, respectively. A comparative analysis using the RADtools pipeline, was performed to recover candidate sex associated loci for these species. First, tags for each individual were generated from forward reads through the RADtags module (cluster distance = 10, quality threshold = 20, read threshold = 5). The loci from each individual were compared across all individuals by RADmarkers module (mismatches = 2, tag count threshold = 4). The final output was filtered to eliminate false positives, allowing the identification of sex-specific markers. An abundance of female-specific markers was found in both cases, suggesting the presence of ZZ/ZW sex-determination systems. A total of 39 female-specific markers and 6 male-specific markers were recognized for *P. ephippifer*, and 49 female-specific markers and 1 male-specific marker were found for L1. The 3RAD analysis corroborates the *P. ephippifer* SCS and determine a similar system in L1, highlighting the occurrence of two sister species with sex chromosomes in different stages of differentiation.

Key-words: RAD-seq; Sex markers; Frogs;

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ID – 121

A PUTATIVE SATELLITE DNA EMERGING FROM HAT TRANSPOSABLE ELEMENT IN PIPA CARVALHOI (ANURA, PIPIDAE) GENOME REVEALED BY CYTOGENOMIC APPROACHES

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Abstract:

Transposable elements (TEs) are ubiquitous genomic components and a significant portion of the eukaryotic chromosomes. TEs are an essential substrate for genomic remodeling and the emergence of new repetitive sequences, which generate a library of short repeat arrays that will subsequently be dispersed through the genome to become novel tandem repeats eventually. We demonstrate a new repetitive element derivated from the DNA transposable present in the *Pipa carvalhoi*, an endemic frog species from Atlantic Forest in Brazil. This new repetitive element was captured completely at random using heterologous primers of the retrotransposable REX 3 elements. The sequences isolated by PCR showed approximately 300 bp, which differs from expected amplicons (~500 bp) to REX 3 elements. The cloned and sequenced inserts resulted in a consensus sequence of 260 bp. Local tBLASTn against the *P. carvalhoi* genome, retrieved an abundance of contigs with high identity (91-100%) with the sequence cloned. Homology searches in the REPBASE database recovered similarity (66% - 73%) with copies of the hAT element, present in the *Xenopus tropicalis* (hAT-9_XT, hAT-10_XT), a species phylogenetically close that diverged around 136 mya. The molecular structure of this sequence showed small ORFs without any conserved transposase domains. Conserved terminal inverted repeats (TIRS) with 19 bp and target site duplication (TSDs) with 8 bp were recognized in the clones, both features of hAT elements. The lack of coding capacity for transposase, small size, and high copy number in the genome, besides of presence of TIRs and TSDs, could suggest that this new repetitive element is a miniature inverted-repeat transposable element (MITE). However, MITEs are often found close to or within genes and are involved in their regulation. We seek to know if the *hAT-derived* sequences were sufficiently clustered in the *P. carvalhoi* chromosome to be observed by FISH experiments and to evaluate their chromosomal distribution in euchromatin and heterochromatin. The *P.carvalhoi* karyotype (2n=6M+4SM+6ST+4T). The FISH assays using the *hAT-derived* probe revealed hybridization signals in the pericentromeric region of 2p and 4p, besides the subtle markings on pairs 5, 6, and 7. An accumulation of probes was detected in the pericentromeric region of 1q, 7q, 8q, and the subterminal region of 9q, coincident with NOR sites. While by conception MITEs elements are often located within euchromatin regions, and the association with active genes is expected, our chromosomal data argue that these new repetitive copies are a non-MITE transposable element. We believe this sequences tandem array represents a novel tandem repetitive DNA, putatively a satellite DNA that emerges from transposable elements *hAT* (autonomous or non-autonomous). Future assays will be designed to validate this hypothesis and allow a better comprehension of one of the evolutionary mechanisms involved in the emergence of new satDNA in Anura chromosomes.

Key-words: Transposable element; Repetitive DNA; Anura; Evolution;

ID – 123

CHROMOSOMAL ORGANIZATION OF THE OLFACTORY RECEPTOR GENE REPERTOIRE IN ANURAN SPECIES CORROBORATE WITH CONSERVED SYNTENIC ELEMENTS IN FROG CHROMOSOMES

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Abstract:

Olfaction plays a crucial role in animal survival and is expressed through olfactory receptors in the nasal cavity. The olfactory receptors (ORs) are encoded by a high-diversity multigene family composed of numerous OR genes. Gene duplication events create this high diversity of ORa, and the evolutionary pathway follows a Birth-and-Death model. Consequently, genomes exhibit intact, pseudogenes, or partial OR gene majority clustered within a genomic region. Our study analyzed OR genes' diversity and chromosomal distribution in six anuran species (*Engystomops pustulosus, Bufo bufo, Hymenochirus boettgeri, Xenopus laevis, X. tropicalis and Xenopus borealis*) to evaluate the role of this multigene family in chromosomal evolution. Bioinformatic scripts identified genes through homology searches and phylogenetic approaches. Our results show a high diversity of OR genes. 2076 OR genes were recovered in *E. pustulosus*, 917 in *B. bufo*, 1194 in *H. boettgeri, 1053 in X. laevis, 1241 in X. tropicalis and 842 in X. borealis*. Six monophyletic OR groups (named α - alpha, β - beta, γ - gamma, δ - delta, ε - epsilon, and η - eta) were recognized in an evolutionary gene tree. Second, we seek to evaluate the chromosomal organization of OR groups on a genomic scale. We observed on the subtelomeric region of chromosome 3 of *B. bufo* and chromosome 2 *of H. boettgeri, X. tropicalis, X. laevis, X. borealis* a genomic arrangement that hold clusters from each OR group. Previous studies based on ortholog alignment suggest chromosome 2 as homologous in *X. tropicalis, X. laevis, X. borealis, H. boettgeri*, and *E. pustulosus* homologous from chromosome 3 from *B. gargarizans* and *B. bufo* karyotypes. Our data of these OR gene arrangement corroborate with this homology hypothesis and confirm that large segments of ancestral chromosome have been preserved throughout the evolutionary history of Anura, around 200Ma. We also reported the case of *E. pustulosus* as an exception among Anura lineages studies until now. This genome has a synteny break on the subterminal region of chromosome 2 in comparison with other Anura species in which OR genes chromosome distribution is known. Interestingly, OR genes groups are spreading in different genomic regions: alpha is located on the chromosome 1 and 3, while delta is located on chromosome pairs 2, 4, 5, and 9, a singleton from epsilon is exclusively observed on chromosome pair 11 while a cluster from eta is located on the chromosome 2. As expected, to the Birth-and-Death model, we observed that chromosomal OR clusters are a mixture of divergent groups of genes and highly homologous genes within groups plus a substantial number of pseudogenes. The chromosome distribution of gamma genes, the most abundant OR group in Anura, shows a widely dispersive pattern among chromosomes from genomes analyzed. The most unusual case was observed in *the E. pustulosus* genome, where we attended the pan-genomic distribution of clusters of functional gamma genes. Our data revealed the evolutionary model of OR genes in six Anura lineages and opened new perspectives about the role of this multigene family on the chromosomal evolution of this group.

Key-words: Olfactory receptors; odorant; bioinformatics; Anurans; Amphibians

Acknowledgement

We thank Dr. Yoshihito Niimura from the University of Miyazaki, Japan, for helping in the elucidation and elaboration of the bioinformatic scripts, analyses and suggestions.

ID - 48

CHROMOSOME MAPPING AND MOLECULAR CHARACTERIZATION OF A TC1-LIKE TRANSPOSON IN *BOANA* SPECIES (ANURA, HYLIDAE)

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Abstract:

DNA transposons play an important role in determining the size and structure of eukaryotic genomes. Represented by several families, the *Tc1/Mariner* transposon is widely distributed in animal and plant genomes, with its structure has been well studied. The hylid genus *Boana* comprises a diverse genus of Neotropical treefrogs, and despite the frequent 2n=24 chromosomes found in its representatives, the karyotypic organization of the species cannot be considered conserved, given the scarcity of studies focusing on chromosomal mapping of repetitive DNA sequences. Here, the *Tc1/Mariner* element was isolated, characterized, and mapped on chromosomes of three *Boana* species in order to examine their sequence and their potential role in shaping the karyotypes of hylid anurans. The physical mapping revealed dispersed signals in euchromatins with small accumulations in some heterochromatic regions of all species analyzed. All *Tc1*-like transposons isolated in this study presented high sequence integrity and exhibited all functional domains, suggesting that these transposons had a recent invasion phase and are active in the host genomes of these anuran species. *B. albopuntata* and *B. faber* presented a DD36E motif, identical in structure to that of the DD36E/*Incomer* family, while *B. prasina* showed a DD37E motif, but with evidence that its origin is also from the DD36E/*Incomer* family. These findings improve our understanding of the diversity of *Tc1*/*mariner* transposons and their participation in the karyotype evolution within hylid anurans.

Key-words: Amphibian; Karyotype; Transposable Element;

Acknowledgement

CAPES, Fundação Araucária

ID - 130

IDENTIFICATION OF A NEW EVOLUTIONARY LINEAGE FOR THE GENUS *MESOMYS* (RODENTIA, ECHIMYIDAE) IN THE BRAZILIAN AMAZON.

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Abstract:

The genus *Mesomys* (Rodentia, Echimyidae) is constituted by *M. hispidus*, *M. stimulax*, *M. occultus* and *M. leniceps*, and is widely distributed in the Amazon with sympatry occurring among species. Different authors have described high rates of genetic divergence in the genus, suggesting to an unknown diversity and the possibility of species complexes in the group. In this sense, the present work aimed to investigate the genetic and karyotypic diversity of *Mesomys* samples not yet analyzed. The cytogenetic analysis included a male, from the municipality of Itaituba, state of Pará, Brazil. The chromosome preparation was submitted to the G-banding and FISH with telomeric and 18S rDNA probes. This specimen showed 2n=60/FNa=110, presented 29 autosomal pairs, ranging from large to small, with 26 two-armed autosomal pairs and 3 one-armed pairs, the large submetacentric sexual X and the small metacentric Y. FISH with telomeric probes showed only distal signals and those with 18S rDNA probes marked in the interstitial region of an autosomal pair. Molecular analysis included 16 tissue samples (from 14 localities) for sequencing the mitochondrial genes Cytochrome b (Cytb) and Cytochrome Oxidase - Subunit I (COI), complemented with sequences available on GenBank. The maximum likelihood analysis recovered the monophyly of the genus *Mesomys*, and a new lineage for the sample from Itaituba with 97% of support (bootstrap), treated here as *Mesomys* sp. nov. Our data show a new lineage for the genus *Mesomys*, suggesting that it is a new species not yet described, occurring in sympatry with *M. hispidus* and *M. stimulax*. The comparative analysis of the karyotypes currently described for the genus shows that *Mesomys* sp. n. exhibits a similar karyotype described for *M. stimulax* (2n = 60/FNa=110), but differs in the karyotypic formula, with *M. stimulax* exhibiting 21 metacentric + 5 submetacentric + 3 acrocentric (20m + 5 sm + 3a), while *Mesomys* sp. n. shows 18 metacentric + 8 submetacentric + 1 subtelocentric + 2 acrocentric (18m + 8sm + 1st + 2a). In the present study, we describe a new lineage and a new cytotype, corresponding to the sample from Itaituba, unrelated to taxa currently recognized for the genus. In addition, we corroborate the underestimated diversity of the group and the need for an integrative multidisciplinary approach in *Mesomys*.

Key-words: Rodent; Echimyidae; Cytogenetics; Phylogeny; Mitochondrial DNA

ID - 5

CYTOGENETIC CHARACTERIZATION OF THREE SPECIES OF THE CHARACIDAE FAMILY USING CELL CULTURE

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Abstract:

The Characidae family is the largest family of Neotropical fish, comprising 1126 species, divided into four lineages, subfamily Spintherobolinae, Stevardiinae, Clade B and Stethaprioninae. Cytogenetic information for some of these lineages is non-existent, such as Spintherobolinae, and for others, they demonstrate a wide variation in karyotypic structure with the modal diploid number ranging from 2n=50-52. However, what draws attention to the Stethaprioninae is the presence of a large metacentric pair not found in other lineages. Thus, the objective of this work was to use cell culture to obtain chromosomes and cytogenetic characterization of at least one representative of each lineage. Cell culture of *Mimagoniates microlepis*, *Hollandichthys multifasciatus* and *Roeboides descalvadensis*, representatives of the Stevardiinae, Stethaprioninae and Clade B lineages, respectively, had fin fragments removed and isolated to establish cell lines maintained at 28ºC and 5%CO2. When these cells grew, we treated them with hypotonic 0.0016% colchicine and fixative for chromosome preparation and, later, we submitted them to staining techniques (Giemsa), C-Band, Ag-RON and fluorescent in situ hybridization (FISH) with probes obtained via PCR of 18S and 5S rDNA and telomeric labeled with biotin and digoxigenin, respectively. It was not yet possible to carry out the chromosome preparation of a representative of the Sphinterobolinae lineage. All chromosomes obtained showed clear and defined morphology with a large number of metaphases per slide. The diploid number of *H. multifasciatus* and *R. descalvadensis* was 50 chromosomes, and *M. microlepi*s 52, the karyotypic formula 8m+10sm+32st, 4m+ 8Sm+ 34st, 6m+32Sm+14st, reciprocally, and only the first one presents the large metacentric pair, data evidenced in the literature. For FISH, 5S rDNA are located in pericentromeric regions for *H. multifasciatus* and *M. microlepis* in one and two pairs, whereas for *R. descalvadensis*, in terminal regions of two pairs. However, 18S rDNA is present in the large metacentric pair and in another pair in the pericentromeric regions of *H. multifasciatus*, whereas in *M. microlepis* and *R. descalvadensis* we observed six pairs and in one pair, respectively, in the terminal regions. Band C is in the large metacentric pair in the terminal region of both arms and in the pericentromeric region of other chromosomes for *H. multifasciatus* and in terminal regions in all chromosomes, and in the metacentric chromosome pair it was possible to observe only one block in the pericentromeric region for *R. descavaldensis*. The nucleolus organizer regions match the 18S rDNA tags for both species. Telomeric sequences were present in all chromosomal pairs of the terminal portions. Therefore, the results presented may help in understanding the evolution of these chromosomes depending on the regions of the markings: was there a reversal of the large metacentric pairs in the other groups? Could this represent a unique character of Stethaprioninae? Therefore, additional information from Spintherobolinae is needed to answer these questions, as well as the sequencing of the large metacentric pair followed by chromosome painting in other species that do not have it to understand the sharing of sequences between species of the family.

Key-words: cytogenetics; FISH; karyotype;

Acknowledgement

UNESP, FAPESP

ID – 212

CLASSICAL CYTOGENETICS AND CHROMOSOMAL MAPPING OF RIBOSOMAL GENES OF THE ANT *CREMATOGASTER TENUICULA* FOREL, 1904 FROM AMAZON REGION (FORMICIDAE: MYRMICINAE)

Linda Inês Silveira [1]; Gisele Amaro Teixeira [1]; Luísa Antônia Campos Barros [1]; Paulo Castro Ferreira [1]; Rodrigo Batista Lod [1]; Hilton Jeferson Alves Cardoso de Aguiar [1]

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Abstract:

The genus *Crematogaster* is monophyletic and includes a wide diversity of taxa, 521 valid species and 260 subspecies, which are distributed worldwide. Despite this richness of taxa, only a few cytogenetic studies have been performed on *Crematogaster* and most of them concentrated in the Old World. Only one species, *C. longispina*, collected in French Guiana was cytogenetically characterized using classical and molecular methods. Biogeographic inferences about *Crematogaster* suggest that dispersal to the Neotropics occurred in more than one event from different lineages, which may suggest the existence of chromosomal variability between species. Thus, in this study we characterized the karyotype of the ant *Crematogaster tenuicula* from Oiapoque, state of Amapá, Brazil, in an Amazon forest region, through classical cytogenetics and 18S ribosomal genes mapping. Mitotic metaphases were obtained from cerebral ganglia of larvae after meconium elimination. Chromosome number and morphology of metaphases were analyzed using conventional 4% Giemsa staining. Physical mapping of ribosomal genes was performed using fluorescence *in situ* hybridization (FISH). Ribosomal 18S gene probes were obtained via PCR and indirectly labeled with digoxigenin-11-dUTP. Fluorescence signals of probes were obtained with anti-digoxigenin-rhodamine. Females of *C. tenuicula* had 2n=38 chromosomes, with karyotypic formula 2n=22m+14sm+2st. This karyotype notably contrasts with previously studied *C. longispina* which has 2n=24 chromosomes and this variability suggests the occurrence of Robertsonian rearrangements (fissions or fusions) throughout the evolution of the genus. Concerning the ribosomal gene distribution, *C. tenuicula* showed these genes located in the interstitial region, occupying almost the entire long chromosome arm of the fifth metacentric pair. *C. longispina* also had a single rDNA site, as well as most ant species studied so far, a trait considered to be the ancestral character in Formicidae. However, the chromosome pair that bear the rDNA genes is not the same, which suggests chromosomal rearrangements involving this genic region. This is the first record of the occurrence of *C. tenuicula* in the state of Amapá which is a region where myrmecological data are scarce. In this study we show a wide chromosomal variability in Neotropical *Crematogaster* and the increase of species studied through classical and molecular cytogenetics will continue to contribute to the understanding of the evolutionary history in the genus.

Key-words: ants; karyotype; Amapá; Biodiverstiy; Ribosomal genes

Acknowledgement

We are grateful to Dr. Jacques Delabie for species identification. This study was supported by Fundação de Amparo ao Estado do Amapá (FAPEAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Programa de Auxílio ao Pesquisador PAPESQ/UNIFAP and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

ID - 56

KARYOTYPE DIVERSITY AMONG MICROTEIID LIZARDS OF THE ECPLEOPODINI TRIBE (SQUAMATA: GYMNOPHTHALMIDAE)

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Abstract:

The Gymnophthalmidae lizards occur in the subtropical and tropical forests of South and Central America and the Antilles. They are small body-sized with a great diversity of morphological and ecological adaptations, including some parthenogenetic species, and presence of several cryptic and undescribed species complexes. So far, out of the 284 known species, only 48 (~17%) were karyotyped after conventional staining. In this study, we aimed to characterize the karyotypes of the Ecpleopodini tribe based on number and morphology of chromosomes and NORs pattern, in order contribute to the understanding of its chromosomal evolution. We sampled: *Anotosaura vanzolinia* (Cabaceiras/PB, 1 embryo), *Colobosauroides cearensis* (Pacoti/CE, 3 males), *Ecpleopus gaudichaudii* (Jequitinhonha/MG, 3 males), *Leposoma annectans* (Una/BA, 2 males) and *Arthrosaura kockii* (Melgaço/AP, 1 male). Chromosomal preparations were obtained from fibroblasts culture, intestine, liver and testicles, and analyzed after Giemsa and Ag-NOR staining following routine protocols. The karyotype of *A. vanzolinia* has 2N=46 (22M+24m) formed by six pairs of submetacentric, three metacentric and two acrocentric macrochromosomes and, at least, 12 biarmed microchromosome pairs. *C. cearensis* presented a 2N=44 (20M+24m) karyotype with meta/submetacentric macrochromosomes and some biarmed microchromosomes. Both species have a microchromosome pair bearing the Ag-NOR on the telomeric region of the long arm. *A. kockii* has 2N=46 (16M+30m) with eight pairs of submeta/metacentric and acrocentric macrochromosomes. *E. gaudichaudii* showed a different karyotype with 2N=48 and chromosomes varying gradually in size, being the pair 1 a large acrocentric, pair 2 a submetacentric and the remaining pairs acrocentrics. *L. annectans* exhibited a peculiar karyotype of 2N=42, comprised of 4 macrochromosomes pairs, 4 chromosome pairs of an intermediated size and 26 microchromosomes, with meiotic cells confirming this structure. The cytogenetic data of Ecpleopodini compiled from our research group on the view of a recent molecular phylogeny suggest that the genus *Leposoma* from the Atlantic forest is more diverse in diploid number and karyotype structure (2N=42 for *L. annectans* to 2N=52 for *L. scincoides*), when compared to the closely related genus *Loxopholis* which is restricted to the Amazon rainforest (*L. guianense*, *L. percarinatum* 2N and *L. osvaldoi*, all with 2N=44, 20M+24m). In contrast to the *Anotosaura collaris* (2n=44, 20M+24m) karyotype, *A. vanzolinia* studied herein has an additional macrochromosomes pair. Among all species described here for the first time, *Ecpleopus gaudichaudii* exhibited the most distinctive karyotype without a clear cut between macro- and microchromosomes as that of *Leposoma scincoides* (2N=52), but they have differences in morphology and number of chromosomes. The tribe Ecpleopodini is the second most diverse of the family Gymnophthalmidae (2N=42-52), behind only to the tribe Gymnophthalmini (2N=34 in *Gymnophthalmus pleii* to 2N=64 in *Nothobachia ablephara)*, which has sex-determining systems and supernumerary chromosomes. The position of the Ag-NOR in Ecpleopodini usually appears to be located on the telomeric region of the long arm of a microchromosome or small pair, except for *Loxopholis osvaldoi*, in which a macrochromosome pair bears de Ag-NOR. Unlike some groups that have a more conserved karyotype, the Ecpleopodini exhibit extensive chromosomal variation which may have important phylogenetic implications.

Key-words: Phylogeny; *Leposoma*; Ag-NOR; chromosomal evolution; rainforest

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ID – 111

A NEW POWERFUL TOOL FOR THE DETECTION OF B CHROMOSOMES IN THREE *PSALIDODON* FISH SPECIES

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Abstract:
At least eight species of the fish genus *Psalidodon*, popularly known as "lambaris", harbor additional genetic elements called B chromosomes. Despite decades of investigation, several aspects of the B chromosome biology are still a mystery. Traditionally, the detection and characterization of B chromosomes in *Psalidodon* samples relied on conventional cytogenetic techniques, which involded sacrificing the samples followed by an extensive protocol. However, to maintain samples alive for directed crosses and several other biological experiments, a fast genotyping method is desirable. To this end, we developed a PCR-based protocol to genotype samples with and without B chromosomes in different species of *Psalidodon* genus. The protocol was based on the presence of two retroinserted pseudogenes (sbno2-B and simc1-B) in four B chromosome variants of three *Psalidodon* species. The presence and structure of these pseudogenes were described using short reads (BGIseq) and long reads (PacBio) and confirmed by PCR using primers designed at exon-exon junctions. Based on this, we developed primer sets containing one forward primer inside an exon and two reverse primers, one inside an exon and the other on the exon-exon junction for both sbno2-B and simc1-B genes. After independent and multiple reactions, results were analyzed on agarose gels, which showed that 0B samples exhibited a single band, while B-bearing specimens exhibited two bands, as expected. This protocol was successfully applied for B chromosome variants found in several *Psalidodon* species (*P. paranae*, *P. bockmanni* and two populations of *P. fasciatus*), with high precision and efficacy. Thus, the entire protocol took only three and a half hours. In summary, we present a fast and efficient method for the detection of B chromosomes in three *Psalidodon* species. This method will open interesting opportunities to detect B chromosomes in live samples that cannot be sacrificed, as well as samples deposited in collections and/or museums that were collected in specific locations.
Key-words: B chromosomes; Pseudogenes; *Genotyping*; *Psalidodon*; *Astyanax*

Acknowledgement

CAPES, FAPESP, CNPq

ID – 65

CHROMOSOME MAPPING OF U2 SNRNA GENE CLUSTERS IN THE FROG SPECIES COMPLEX *PHYSALAEMUS CUVIERI - PHYSALAEMUS EPHIPPIFER* (ANURA, LEPTODACTYLIDAE)

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Abstract:

Repetitive DNA sequences organized *in tandem* could be useful in chromosome mapping and comparative cytogenetics. The tandemly repeated multigenic family of U snRNAs has been used as chromosome markers in numerous taxa, but anurans studies using these sequences are still scarce. The frog species complex *Physalaemus cuvieri - Physalaemus ephippifer* encompasses at least five well-defined genetic lineages, namely lineage 1 (L1), lineage 2 (L2), lineage 3 (L3) of "*P. cuvieri*", *Physalaemus* sp. and *P. ephippifer*. A great chromosomal diversity is present in this species complex, including a high polymorphism of nucleolar organizer regions (NORs) in L3 and the presence of heteromorphic sex chromosomes in *P. ephippifer*. Here, we map the U2 snRNA gene sequence in the karyotypes of specimens from all major clades of the species complex *P. cuvieri - P. ephippifer* aiming to better compare them and aid in the evolutionary study of this group. We analyzed specimens from Urbano Santos (MA)/Araruna (PB), Campinas (SP), Porto Nacional (TO), Santa Bárbara (PA) and Óbidos (PA)/Viruá (RR), which belong to L1, L2, L3, *P. ephippifer* and *Physalaemus* sp., respectively. Specific primers for the PCR-amplification of the U2 snRNA gene sequence were designed from a *Xenopus laevis* sequence and were used to isolate this sequence from the *P. ephippifer* genome. The resulting fragments were sequenced to confirm their nucleotide sequence, PCR-labeled with digoxigenin-dUTP or biotin-dUTP and used as a probe in Fluorescent *in situ* Hybridization (FISH) assays. The U2 snRNA gene probe revealed a strong signal terminally in the short arm of chromosomes 6 in all the analyzed specimens. Additional strong probe signals were detected pericentromerically in the long arm of one small submetacentric chromosome of specimens from L3 and distally in the short arm of one homolog of pair 9 of one of the eight analyzed specimens from L1. Furthermore, variable number and position of weak probe signals were found in L2, *P. ephippifer* (including a terminal signal in the short arm of the Z chromosome) and especially in L3. The conserved cluster of U2 snRNA gene sequence present in chromosome pair 6 was useful to distinguish it from chromosome pair 5 in all the analyzed karyotypes, since chromosomes 5 and 6 are very similar in morphology and size in this species complex. Similarly, in a previous study, a U2 snRNA gene cluster was detected in the short arm of chromosome 6 species of *Leptodactylus*, another genus of Leptodactylidae. Taken together, these results suggest that chromosome 6 of these *Leptodactylus* and *Physalaemus* species might be homeologous.

Key-words: Repetitive DNA; Comparative cytogenetics; Fluorescent *in situ* Hybridization;

Acknowledgement

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ID – 62

CYTOGENETIC CHARACTERIZATION OF *NANNOPTERUM BRASILIANUM* AND *FREGATA MAGNIFISCENS* (SULIFORMES)

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Abstract:

There has been few cytogenetic research in the avian taxon; only 9.9% of species have been karyotyped. In the Suliformes order, only 6 of the 61 species have had their karyotypes examined. This work sought to fill this gap by giving the cytogenetic characterization by both conventional and molecular analysis of two waterbird species native to Brazil: *Nannopterum brasilianum* (Phalacrocoracidae) and *Fregata magnifiscens* (Fregatidae). Cell cultures from feather pulp were stablished from male and female individuals of both species to obtain metaphase chromosome spreads. The protocol to obtain chromosome preparations involved treatment with colchicine (0.05%) for 3h, followed by hypotonic solution (0.075 M KCl, 9 min) and fixation with 3:1 methanol/acetic acid. The C-banding was performed in females chromosomes of both species using barium hydroxide (5%). The distribution of 18S rDNA and telomeric sequences was detected by fluorescence *in situ* hybridization (FISH). *N. brasilianum* was previous described with a diploid number of 2n=74, which has been confirmed in this study. The first, second, third, and fifth pairs are submetacentric, the sixth and eighth are metacentric, and the remaining autosomal pairs are acrocentric. *F. magnificens* has a diploid number of 2n=76. The first, second, fourth, sixth, and twelfth pairs are submetacentric, the third is acrocentric, the fifth, seventh, ninth, tenth, and eleventh are submetacentric, and the remaining autosomal pairs are telocentric. For both species, the Z sex chromosome is submetacentric, and the W sex chromosome is acrocentric. The C-banding for *F. magnificens* was observed in the centromeres of all chromosomes except for the Z sex chromosome, while *N. brasilianus* was not observed in any chromatin region. 18S rDNA probes hybridized onto 4 pairs of chromosomes in *N. brasilianum* and one pair in *F. magnificens*. Telomeres probes showed stronger signals in the microchromosomes than the macrochromosomes for *F. magnificens* and similar signals in all chromosomes for *N. brasilianum*. While belonging to the same order, they display different cytogenetic features. The absence of heterochromatin in *N. brasilianus* could be an indication that repetitive regions have been lost. Compared to *N. brasilianum*, the karyotype of *F. magnificens* is more similar to the hypothesized avian ancestral karyotype. First, although *N. brasilianum* has a diploid number of 2n=74, *F. magnificens* has a diploid number of 2n=76, and that is closer to the putative avian ancestral karyotype (2n=80).The second reason is that *N. brasilianum* has 4 chromosome pairs with ribosomal clusters, and *F. magnificens* has only one. Overall, these results shed light on important aspects of the karyotype of Suliformes members and contribute to a better understanding of chromosomal evolution in this group.

Key-words: Suliformes; Avian cytogenetics; Evolution; C-band; FISH

ID – 58

OVERABUNDANCE OF PBOSAT01-176 SATELLITE DNA IN THE FROG *PROCERATOPHRYS BOIEI* REVEALED BY CYTOGENOMICS

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Abstract:
Satellite DNAs (satDNAs) are one of the most abundant elements in genomes. Characterized as tandemly organized sequences that can be amplified into multiple copies, mainly in heterochromatic regions. The frog *Proceratophrys boiei* (2n = 22, ZZ?/ZW?) is found in the Brazilian Atlantic forest and has an atypical pattern of heterochromatin distribution when compared to other anuran amphibians, with large pericentromeric blocks on all chromosomes. In addition, females of *P. boiei* have a metacentric sex chromosome W showing heterochromatin in all chromosomal extension. Here we advance our understanding of the evolution of satDNA in a frog species with defined sex chromosomes, by fully characterizing the superabundant satDNA PboSat01-176 in the genome of *Proceratophrys boiei*, integrating genomic and chromosomal analysis. After all the analyses, it is remarkable that the satellitome of *P. boiei* is composed of a high number of satDNA families (226 in total), making *P. boiei* the frog species with the highest number of satellites described so far. Interesting, a single satDNA, PboSat01-176, comprises 93.62% of the total amount of satDNAs families, being the most abundant satDNA in the genome of *P. boiei*, comprising 10.49% of genomic abundance. The second most abundant satDNA corresponds only to 0.97% of total satDNA content and all other satDNAs are in very low abundances. For the highly abundant PboSat01-176 satellite, the successful mapping by FISH highlights the presence of certain satDNAs in strategic regions of the chromosomes of *P. boiei* (e.g. centromere and pericentromeric regions), which leads to their supposedly part in crucial processes for genomic organization and maintenance in this species. In amphibians, abundant repeats of satDNAs seem not to be dispersed in the karyotype, and even in specific chromosomes, but rather clustered in peri/centromeric regions, and possibly playing a role in the organization, regulation and maintenance of these chromosomal regions.
Key-words: repetitive DNA; satellitome; cytogenetics; evolution; Amphibia

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ID - 205

BAC-FISH ANALYSIS UNVEILS REMARKABLY CONSERVED MICROCHROMOSOMAL ORGANIZATION AMONG PASSERINE BIRDS

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Abstract:

Passerine birds (Passeriformes) are usually recognized for their extraordinary diversity, with more than 5,700 species. Like all birds, they have a bimodal karyotype which includes macrochromosomes and microchromosomes. This karyotype was fixed before the divergence of birds and turtles, and was present in the theropod lineage between 100 and 250 million years ago. In this study, we sought to understand the extent to which karyotypic organization is conserved in this group using Bacterial Artificial Chromosomes by Fluorescence In Situ Hybridization (BAC-FISH) with microchromosome probes from chicken (*Gallus gallus*) or zebra finch (*Taeniopygia guttata*) per microchromosomes (GGA10-28, except GGA16). We have investigated the chromosome complement of four passerine species - *Myiodynastes maculatus*, *Molothrus bonariensis*, *Troglodytes aedon*, and *Sporophila caerulescens*. Our findings indicate a strong conservation of microchromosome organization within the studied species, as the results showed no signs of interchromosomal rearrangements involving microchromosomes homologous to GGA. Besides that, regarding the diploind numbers, we confirmed previous studies for *M. maculatus* (2n=80), *M. bonariensis* (2n=80), and *S. caerulescens* (2n=78), but we discovered a new diploid number for *T. aedon* (2n=76), which underscores the importance of carefully examining a large number of good quality chromosome preparations to accurately determine diploid number in birds. Our BAC-FISH experiments support the literature's early karyotypic descriptions on the high degree of conservation of these structures in this group. Passeriformes karyotypes have mainly evolved through intrachromosomal rearrangements, which seem to be much more common, maintaining the organizational arrangement of microchromosomes and the 2n highly preserved in these species. The absence of observable interchromosomal rearrangements among microchromosomes may have contributed to the evolutionary success of this feature in Passeriformes, collaborating to make them the most diverse tetrapod group on the planet.

Key-words: Aves; Karyotype organization; Molecular cytogenetic;

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ID – 64

CHROMOSOMIC ANALYSIS IN *AKODON MONTENSIS* THOMAS, 1913, FROM PALMAS (PR), WITH SUPERNUMERARY CHROMOSOME PRESENCE IN ONE INDIVIDUAL

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Abstract:
In small non-flying mammal populations, as in the Rodentia order, the morphological differentiation for particular species is not precise due to the considerable similarity of the individuals, as it happens in the Akodontini tribe. Hence, the present study aimed to analyze and characterize cytogenetically small rodents of the *Akodon montensis* species, from Palmas in Paraná state, Brazil. The individuals of the *A. montensis* have a standard karyotype 2n=24 and NA=42; however, the appearance of the B chromosome is typical. In the analysis, we observed the specimen karyotype, the chromosome arms count (NA), and the morphological difference between the chromosomes of different samples. For the slide preparation, we used the bone marrow tissue of the rodents, using the mitotic preparation following the method of Ford & Hamerton (1956), with modifications (SBALQUEIRO & NASCIMENTO, 1996), stained with Giemsa. We observed and analyzed the metaphases in the microscope, with the 10x objective to search for the metaphases and the 100x objective to observe them individually; when a good metaphase was found, we drew it and counted its chromosomes. We analyzed six specimens, of which five had the karyotype described as 2n=24 and chromosome arms count (NA) equal to 42, with two pairs of acrocentric chromosomes, ten pairs of submetacentric and metacentric chromosomes, and the sex chromosomes were acrocentric, with the X chromosome being a bigger acrocentric than the Y chromosome. Furthermore, in one of the specimens analyzed, we found a supernumerary submetacentric chromosome, chromosome B, characterizing the specimen as 2n=25 and NA=44. The chromosome B presence in *A. montensis* has been described before; this evidences de species dynamic about chromosomal variation, showing that genetic material could have accumulated, leading to chromosome B formation. Therefore, with the other specimens analyzed (who have the standard karyotype 2n=24), questions about the chromosomic variation of the *A. montensis* species could be elucidated once the individual with 2n=25 was sympatric with the 2n=24 individuals, and for some reason had the formation of the chromosome B. Finally, we understood that with new analyses of the collected samples, it would be possible to know better the chromosomic variation of the *Akodon montensis* from Palmas region.
Key-words: Rodentia; Chromosome B; Akodontini;

ID – 127

CYTOGENETIC CHARACTERIZATION OF THREE CAPTIVE RED BROCKET MALES *(MAZAMA AMERICANA*) WITH KNOWN ORIGIN IN ECUADOR

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Abstract:

The *Mazama americana* cryptic species complex has a wide geographic distribution in central and south America. Cytogenetic studies have shown the karyotypical variation among individuals from different localities in Brazil despite a great morphological similarity. The recently integrative analysis of a neotype for the species revealed a differentiated karyotype compared to the five described cytotype for Brazil, and became the main reference for cytogenetic studies of *M. americana* individuals from different countries in the Neotropical region as for example, Ecuador. Therefore, there is a lack of karyotypical information of any red brocket deer inhabiting Ecuador. The aim of this study is to describe cytogenetically three *M. americana* individuals kept in captivity in Ecuador with known origin in the wild. All individuals were adult males, and received and identification number in the Deer Research and Conservation Center (NUPECCE) as follow: T456, male from Esmeraldas province in the coast region; T460, male from Morona Santiago Province in the Amazon Forest; and T461, male from Orellana Province in the northern Amazon region. We collected a skin fragment of the inguinal region of each individual to perform the cell culture and obtain chromosomal preparations. After that, slides were stained with conventional Giemsa coloration to analyzed the diploid and fundamental number of each metaphase, and also, presence or absence of B chromosomes. The results evidenced that male T456 from coastal region of Ecuador has a diploid number (2n) =58 and a fundamental number (FN) = 76. Male T460 from the south of the Ecuadorian amazon has a 2n = 58, FN = 60 and male T461 from the northern amazon has a 2n = 58 and FN of 60. B chromosomes were observed in the metaphases of all individuals. These karyotypes differ from the *M. americana* neotype (2n = 45 + 3Bs, FN = 51) and the Brazilian cytotypes reported (2n= 42-51 + 3-6 Bs, FN = 46-56). Thus, evidenced that the Ecuadorian red brocket karyotypes do not correspond to the cytogenetic characterization from the previously described *M. americana* individuals. Since the great karyotypical divergences commonly involve chromosomal rearrangement that may be considered as a reproductive barrier, we hypothesized that the Ecuadorian red brocket males do not belong to *M. americana* species. Future research must focus in developing an integrative analysis to clarify the taxonomic identity of *Mazama* individuals inhabiting Ecuador.

Key-words: captive animals; karyotype; neotropical deer;

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ID - 38

INTEGRATING CYTOGENETICS AND GENOMICS IN THE STUDY OF DIFFERENTIATION OF THE ZZ/ZW SEX CHROMOSOME SYSTEM. THE FISH GENUS TRIPORTHEUS (TELEOSTEI: CHARACIFORMES) AS A MODEL

Mariannah Pravatti Barcellos de Oliveira [1]; Gustavo Akira Toma [1]; Caio Augusto Gomes Goes [2]; Rafael Kretschmer [3]; Ricardo Utsunomia [2]; Fábio Porto-foresti [2]; Marcelo de Bello Cioffi [1]

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Abstract:

Sex chromosomes are important elements of the genome, responsible for signaling the gene pathways that leads to genetic sex determination (GSD). In general, their arise from an autosomal pair that acquires a putative sex determining gene linked to DNA sequences that play a sexual antagonistic role, favoring the recruitment of repetitive DNAs and suppressing the recombination between homologous chromosomes. Approximately 5% of the studied Neotropical fish species have differentiated sex chromosomes. Fishes of the Triportheidae family (Characiformes, Teleostei) stand out as an interesting model in this regard because nearly all species contain similar ZZ/ZW sex chromosomes, with variations in their W chromosomes' size and form. Although many studies focus on the evolution of sex chromosomes, only lately have researchers begun to gain a deeper insight into the repetitive DNA found in these chromosomes and their function in the mechanisms that lead to their differentiation. Here, we present the results of an integrated genomic and chromosomal investigation of the satellitome previously isolated from Triportheus auritus (TauSat) (with an emphasis on the very prevalent and sex-biased satDNAs) by mapping them by fish in the chromosomes of the congeneric species, T. trifurcatus. Only four of the 19 satDNA sequences were found in the ZZ/ZW chromosomes of T. trifurcatus, two of which were W-specific, one Z-specific, and one found on both the Z and W chromosomes. These findings show that, although being conserved, the sex chromosomes in Triportheus underwent significant changes in their DNA content, most likely as a consequence of gene loss and W chromosome degeneration.

Key-words: Cytogenomics; Sex Chromosomes; Satellite DNA; FISH;

ID – 208

ANALYSIS OF FULL GENE CONTENT IN *PSALIDODON* B CHROMOSOMES REVEALS COMMON ORIGIN

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Abstract:

B chromosomes are additional genetic elements occurring frequently in eukaryotes. Recent studies showed they can persist for millions of years enduring speciation processes, despite not being essential for host survival. Regarding their content, several active protein-coding genes were found in B chromosomes of different organisms in the last decade. The fish genus *Psalidodon*, has at least eight species bearing B chromosomes, being one of the most well-studied group within this topic. Previous high-throughput DNAseq analyses followed by qPCR validation identified 20 genes in the metacentric B chromosome of *P. scabripinnis* species complex (*P. scabripinnis* and *P. paranae*). In addition, using the same set of qPCR primers, the authors verified overabundance of some of these genes in individuals of *P. fasciatus* and *P. bockmanni* with B chromosomes as well, which suggests a common origin at approximately 4 million years ago. However, the full gene content of these B chromosomes has remained unexplored until now. Here, we aimed to identify the gene content of two metacentric B chromosome variants (BfMa and BfMb) of *P. fasciatus* and one metacentric variant (BbM) of *P. bockmanni*, to test their possible common origin and identify their evolutionary pathways. Animals with each B-variant (BfMa, BfMb, BbM) were collected from different populations in São Paulo state, Brazil. DNA samples from three 0B and three 1B individuals from each population were sequenced with high throughput short-read BGIseq technology to decrease the intraindividual variation interference in the analysis. After quality check and adapter-trimming, we performed read mapping of each library against the *P. scabripinnis* transcriptome, available from a previous study, with SSAHA2 software. Then, we used the pipeline whatGene (https://github.com/fjruizruano/whatGene) to count mapped reads and estimate average copy number for each contig. After that, we eliminated repetitive sequences or poorly mapped reads, thus selecting only the most probable contigs as candidate genes on the B chromosomes. These contigs were annotated using Blast2GO. Our analyses revealed the number of genes in each variant is BfMa =392, BfMb = 97, and BbM = 62. Comparing our data with previous findings in literature, all the analyzed B chromosomes in *Psalidodon* genus share at least 6 genes. This underpins the hypothesis that these variants share a common origin and suggest these genes might play or had played a crucial role in evolutionary success of these B chromosomes, mainly considering that half of them is involved in cell cycle mechanisms. Additionally, the BfMa variant showed the highest number of genes using *P. scabripinnis* transcriptome as reference, which is not expected, given their higher phylogenetic distance compared to the other analyzed species. Thus, we hypothesize BfMa variant could have been originated by a hybridization event with *P. scabripinnis*. Further bioinformatic analysis will allow us to test this hypothesis. Our findings revealed the gene content of three new variants of B chromosomes in *Psalidodon* genus, adding high value information to the investigation area. This will allow us to decipher their evolutionary history and investigate processes of gene acquisition by B chromosomes.

Key-words: Astyanax; Genomics; Evolution;

Acknowledgement

CAPES, CNPQ, FAPESP

ID – 171

SATELLITOME ANALYSIS IN *APAREIODON* SP. (TELEOSTEI: CHARACIFORMES) AND KARYOTYPE DIVERSIFICATION IN PARODONTIDAE SPECIES

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Abstract:

The satellitome corresponds to the satellite DNAs (satDNA) library of a genome, and it is represented by repetitive DNA sequences with varied lengths, number of copies, and genomic organization. In some cases, they may be involved in the differentiation of sex chromosomes. Parodontidae is a Neotropical fish family with a female heterogametic sex chromosome system in some species. The repetitive genomic characterization in *Apareiodon* sp. (ZZ/ZW) showed that 1.7% of its repetitive fraction corresponds to satDNAs. This study aimed to characterize the satellitome of *Apareiodon* sp., integrating genomic and chromosomal data and investigating the distributions of six most abundant satDNAs in five Parodontidae species. The genomic data (Illumina 150 paired-end reads) available for a male (ZZ) and a female (ZW) *Apareiodon* sp. were pre-processed, and four random subsets with 1,000,000 reads each (2 - ZZ and 2 - ZW) were used in the TAREAN software for the satDNAs identification. Primers for the satDNAs AspSat01, 03, 04, 05, 06, and 07 were designed in Primer3plus, and used in PCR for probe labeling using *Apareiodon* sp. genomic DNA, and digoxigenin-11dUTP or biotin-16dUTP. Fluorescence *in situ* hybridization (FISH) was performed on *Apareiodon* sp., *A. affinis*, *A. piracicabae*, *Parodon hilarii*, and *P. nasus*. 92 satDNAs were identified in the *Apareiodon* sp. genome, ranging from 15 to 1,703 bp. 27 satDNAs were uniquely identified in the female genome. FISH with satDNAs showed different patterns among the species, but all satellites hybridized at the centromeric/pericentromeric region of chromosomes. AspSat01 signals were observed in all chromosomes in *Apareiodon* sp., in 21 chromosome pairs plus on Z chromosome in *A. affinis*, in 21 chromosome pairs in *A. piracicabae*, and in 2 chromosome pairs in *P. hilarii* and *P. nasus*. Signals for the AspSat03 were observed in 7, 4, and 12 chromosome pairs in *Apareiodon* sp., *A. affinis*, and *A. piracicabae*, respectively. The AspSat04 was mapped in 6, 10, 12, 4, and 3 chromosome pairs in *Apareiodon* sp., *A. affinis*, *A. piracicabae*, *P. hilarii*, and *P. nasus*, respectively. Aspsat05 only showed signals in 8 chromosome pairs in *Apareiodon* sp. The AspSat06 showed signals on 4 chromosome pairs in *Apareiodon* sp., 10 in *A. affinis*, and 2 in *A. piracicabae.* AspSat07 was mapped in 2 chromosome pairs in *Apareiodon* sp. and one in *A. piracicabae*. Some satDNAs presented colocalized signals. No signals were observed at the specific regions of the W chromosomes of *Apareiodon* sp., *A. affinis*, and *P. hilarii*. The obtained data demonstrated the occurrence of satellites with higher abundance in the female than in the male *Apareiodon* sp. genome. The *in situ* localization of the most abundant satellites of *Apareiodon* sp. showed predominately shared satDNA units on the *Apareiodon* and *Parodon* karyotypes, besides an extensive variation in loci number. The variation in loci number indicated distinct satDNA units diversification and dispersion on the Parodontidae karyotypes.

Key-words: Satellite DNA; repetitive DNA; FISH; chromosomes;

Acknowledgement

CAPES, FAPESP and CNPq.

ID – 141

CHARACTERIZATION AND CHROMOSOMAL MAPPING OF SATELLITE DNAS IN THREE SPECIES OF *THOROPA* (AMPHIBIA, ANURA) THROUGH CYTOGENOMICS

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Abstract:

Satellite DNAs (satDNAs) are non-coding sequences of variable length, organized in tandem-repeat fashion and are generally associated with heterochromatic regions of chromosomes. According to the library hypothesis, related species may share a common library of satellite DNAs which can be amplified differently, resulting in differences between closely related species. The emergence of bioinformatics tools enabled the identification and characterization of large collections of satellite DNAs and further enabled their comparison between species. In the present work, we carried out a complete characterization of satellite DNAs of the anuran species *Thoropa miliaris* performed through bioinformatics analyses. Later, some of these sequences were physically mapped for comparative purposes on the chromosomes of two populations of this species and also on two more species of the genus, *T. taophora* and *T. saxatilis*. *T. miliaris* genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, WI, USA) and using Illumina® HiSeqTM 4000 (2x101bp paired-end) by Macrogen Inc. (Seoul, Republic of Korea). The search for repetitive clusters was carried out using the RepeatExplorer and RepeatExplorer2 platforms, in which the latter detected clusters that represented potential satellite DNAs using the TAREAN tool. The abundance and divergence for the satellite DNAs identified in *T. miliaris* were estimated using the RepeatMasker script generating a graphic landscape in R Studio. Chromosome mapping was performed using fluorescent *in situ* hybridization (FISH) technique. Thirty-eight satellite DNAs for *T. miliaris* were isolated and named following their order of abundance. Six of these were succesfully mapped by the FISH technique with visible bands, and are present in the chromosomes of the three species tested but with differences in location and distribution. Location and distribution of these satellite DNAs was also different between the two populations of *T. miliaris* - Santa Teresa/ES and Paraty/RJ -, reinforcing the idea that *T. miliaris* is a complex of species. Thus, in addition to presenting the *T. miliaris* satellite content, this research contributes to a better understanding of the evolution of satellite DNAs in anuran amphibians, adding information that may help in solving taxonomic problems and corroborating the library hypothesis.

Key-words: Bioinformatics; Repetitive DNA; Evolution; Library Hypothesis; Chromosomes

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ID – 8

COMPARATIVE CYTOGENETICS OF THREE SPECIES OF FAMILY FELIDAE (CARNIVORA)

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Abstract:
The Felidae family is composed of species of wild cats, which are morphologically similar animals, despite having the widest range of body sizes compared to other living families of carnivores. Most feline species have a diploid number that varies from 2n=36 to 2n=38, and their fundamental number (NF) presents the same variation. The aim of this work was to cytogenetically analyze three species of South American cats present in the province of Misiones (Argentina): Panthera onca (PON), Puma concolor (PCO) and Leopardus wiedii (LWI). Blood samples were obtained from animals confiscated in the rehabilitation process at Parque Ecológico El Puma. To obtain metaphases, long-term lymphocyte cultures were performed in L. wiedii (01?), P. concolor (01?) and P. onca (02?, 03?). The chromosomes were classified morphologically according to the Conference of San Juan, Costa Rica, with their respective groups: "A, B, C, D, E, F, X and Y". Comparisons between the three species were based on the ancestral feline karyotype of the cat Felis catus (FCA) composed of 2n=38 and NF=72. P. onca has 2n=38, with 34 bibranched chromosomes and 4 telocentric chromosomes. P. concolor also presented 2n=38, with 36 bibrachial and 2 telocentric chromosomes. In L. wiedii, a different karyotype was revealed, with 2n=36 and NF=72, in which all chromosomes that make up the autosomal and sexual complement are bibranched. The analyzes obtained by NOR banding in P. onca and P. concolor allowed us to observe that the secondary constrictions of the E1 pair coincide with the places where nucleolus organizer regions were revealed. P. onca and P. concolor showed the same chromosome complement, but differed in their NF. The difference in chromosome number of PON and PCO in relation to LWI is based on the absence of telocentric chromosomes from group F. PON is the closest species to FCA, as it differs from it only by the morphological alteration of one chromosome from group A, suggesting that the pair of FCA chromosomes B4 underwent a pericentric inversion, becoming part of group A in PON. In group F, PCO has only one pair of telocentric chromosomes. The presence of this small fourth metacentric pair in group E, together with the concomitant deletion of the telocentric pair in group F, suggests pericentric inversion. The chromosomes of groups A, B, D and E of LWI are morphologically the same as those of FCA, the fundamental difference being the absence of chromosomes in group F, due to a fusion of two telocentric pairs, and an extra chromosome pair in group D. Analyzes performed by silver staining showed that the nucleolus organizer regions in PON and PCO are located on the short arm of the E1 chromosome pair, in the secondary constriction. Thus, chromosome banding techniques helped clarify the origin of extra chromosome pairs, such as the C3 pair in the genus Leopardus, the E4 pairs in the genus Puma, and the absence of acrocentric pairs in these two genera.
Key-words: Chromosome; Felids; Cytogenetic;

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ID – 74

COMPARATIVE GENOMIC ANALYSES REVEAL SATELLITE DNA CONTENT IN THE FROG *PROCERATOPHRYS MELANOPOGON*

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Abstract:

Satellite DNA (satDNA) are characterized as tandemly organized repetitive DNA sequences, often present in large quantities in the heterochromatin of many organisms. In this study, we analysed the satDNA content in the genome of the *Proceratophrys melanopogon* frog, a species from mountainous regions of north-central and southern Rio de Janeiro state, eastern São Paulo state, and southern and southestern Minas Gerais state, Brazil. Regarding cytogenetic data, *P. melanopogon* was recently karyotyped with a diploid number 2n=22 chromosomes, nucleolus organized regions located on pair 4 and discrete blocks of heterochromatin at the centromeres, besides the absence of evident sexual chromosomes. Through analyses of *P. melanopogon* genomic data using bioinformatic tools, we characterized the entire satDNA content of this species which is composed of 137 satellite families. Furthermore, 41% of the genome corresponds to repetitive sequences, of whom 7,6% are composed of satDNA. A comparative analysis was performed between the sexes in the search for the most abundant sequences in male and female, and it was revealed a major expressivity of satDNA in the female genome. Among the satellites found, 102 are more abundant in female, with Pmesat22-31 and PmeSat113-52 being predominantly female-biased. This disproportionate occurrence of satDNAs between the sexes may be an indicator of heterochromatinization with a probable influence on a process of sex chromosome differentiation which might contribute to comparative analyses from an evolutionary point of view. Therefore, it is still early to state that this phenomenon is happening in the genome of *P. melanopogon*, and more detailed studies of genomic content in this frog species are of utmost importance. Our study reviews for the first time the *P. melanopogon* satellitome and reinforces that although still lacking in major genomic analyses amphibians might show diverse genomes and important evolutionary aspects, moreover adds genomic data and evolutionary association of repetitive sequences for an amphibian species.

Key-words: Cytogenetic; Amphibia; Repetitive DNA; Satellitoma; Evolution

Acknowledgement

I would like to thank the members of the Laboratory of Animal Cytogenetics for all the teaching and help in my first research, my family for the emotional support, UNESP - Universidade Estadual Paulista - Rio Claro and CNPq for all the support offered.

ID – 43

COMPARATIVE CHROMOSOME PAINTING AND MOLECULAR ANALYSES OF *MYCTERIA AMERICANA* (CICONIIFORMES, CICONIIDAE): INSIGHTS ON THE PHYLOGENY OF THE STORKS

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Abstract:

The wood stork (*Mycteria americana*) is the only representative of this genus found in the all American continent. The current numbers of *M. americana* listed globally are not of concern, but in regions such as Mexico and USA, the populations have been reduced due to habitat loss. Thus, the wood stork is listed as a species in "special protection" in Mexico, while in the USA it is listed as threatened. *M. americana* has been the focus of several molecular studies aiming to provide data to support not only its conservation, but also wetland areas monitoring. However, the relationships of this species with other storks are still unclear. Although genetic information has been well described, data concerning the karyotypical organization of the wood stork are still scarce. Thus, considering that comparative cytogenetics using chromosome painting has substantially increased knowledge about karyotype evolution and phylogenetic relationships in birds, we aimed to analyze the chromosomal organization and diversification of *M. americana*, in addition to providing evolutionary insights based on phylogenetic data of Ciconiidae. For this, we applied both classical and molecular cytogenetic techniques in metaphase chromosomes obtained using fibroblast cell culture from a sample of feather pulp collected from a male individual of *M. americana*. C-banding was performed in order to define the pattern of distribution of heterochromatic blocks. Homologies between *M. americana* and *Gallus g*allus (GGA) were established by experiments of fluorescent *in situ* hybridization with whole chromosome paints corresponding to pairs 1-10 and the Z chromosome from *Gallus gallus*. Maximum likelihood analyses and Bayesian inferences (*COI* and *Cytb* genes) were used to determine their phylogenetic relationship with other storks. As a result, we confirmed the diploid number of 2n=72, and the heterochromatin distribution pattern which was restricted to centromeric regions of the chromosomes. Chromosome painting experiments showed fusions involving GGA8/GGA9 and GGA6/microchromosome, two rearrangements also found in two other species of storks, possibly corresponding to synapomorphies for the group. Phylogenetic analyses resulted in a tree that recovered only Ciconinii as a monophyletic group, while Mycteriini and Leptoptlini tribes were configured as paraphyletic clades. In addition, the association between phylogenetic and cytogenetic data corroborates the hypothesis of a reduction in the diploid number throughout the evolution of Ciconiidae.

Key-words: Wood stork; Ciconiiformes; *Cytochrome Oxidase I*; *Cytochrome b*; Chromosome painting

Acknowledgement

Parque Mangal das Garças CNPq

ID – 213

FIRST KARYOTYPIC DESCRIPTION OF EGRETTA THULA (ARDEIDAE) AND REPETITIVE SEQUENCE ANALYSIS

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Abstract:

The class Aves includes about 10,900 species divided into 40 orders and is the largest group of tetrapods today. The order Pelecaniformes is a group of birds consisting of five families of medium to large individuals that live in regions with abundant water such as swamps. Individuals of the family Ardeidae (herons and bitterns) live in flocks and feed primarily on fish. Approximately 20 species in this family have been analyzed cytogenetically and diploid numbers range from 60 to 72 chromosomes. Some variation in this family involves the morphology of pairs 4, 5, 6 and 10 among species, suggesting that rearrangements have occurred, changing the centromere position during the evolution of karyotypes in this group. Despite their importance and diversity, there have been few genomic studies of the members of this family. Therefore, this work aims to describe the chromosome complement of the species Egretta thula (Ardeidae) as well as to analyze the distribution pattern of constitutive heterochromatin regions, reporting the Nucleolus Organizing Regions (NOR) and the organization pattern of two simple repetitive sequences (SSRs) (GA)15 and (TA)15 by fluorescent in situ hybridization (FISH). The female specimen is an accidental animal collected in São Gabriel - Rio Grande do Sul, Brazil. To obtain mitotic chromosomes, the direct bone marrow culture method of short duration was used. Slides were then stained with Giemsa 5% and the best 30 metaphases were photographed and used for karyotype assembly. Constitutive heterochromatin was analyzed by C-banding with Barium hydroxide. The repetitive sequences (TA)15 and (GA)15 were labeled with Cy-3 throughout the synthesis. The Egretta thula diplid number is described for the first time with 62 chromosomes. In the karyotype of this species, the first, second, third, fourth, and eighth pairs are submetacentric; the fifth and tenth pairs are metacentric; the sixth, ninth, and all microchromosomes are telocentric or have punctiform morphology. Z and W sex chromosomes have submetacentric morphology. The C-band showed that the W chromosome is completely heterochromatic and some macrochromosomes have markings at the centromeres. For the Nucleus Organizing Regions it showed markings in one macrochromosome pair at the telomeric region and in one pair of microchromosomes. The repetitive sequence (GA)15 showed signals in only one pair of microchromosomes, which was also observed in other species. The sequence (TA)15 was present in centromeric regions of some macrochromosomes. The second pair had strong markings in the telomeric and centromeric regions. The diploid number was found to be comparable to other species of the family, as well as morphological differences in the submetacentric W chromosome, since a telocentric W was identified in the nearby species (Egretta garzetta), suggesting a possible rearrangement. The two repetitive DNA sequences were present in both species and showed accumulation in regions already observed in other orders of the class Aves. Thus, we can speculate that a possible accumulation of sequences (GA)15 in the macrochromosomes occurred during the evolutionary history of this species.

Key-words: Ardeidae; FISH; NOR; karyotype;

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ID – 200

GENOMIC SCREENING OF SNRNA U2 CLUSTER REVEALED AN INTERPLAY BETWEEN DISTICTIVE REPETITIVE ELEMENTS UNDER CONCERTED-EVOLUTION MODEL IN *XENOPUS TROPICALIS* GENOME

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Abstract:

LINE retrotransposons elements are estimated to be as old as eukaryotes, with each of the main clades dating back to at least 600 mya. Previous studies evidenced that the clade TX-1 showed a high diversity of young copies in *Xenopus tropicalis*, an aquatic species of Anura from Africa. Here we designed a bioinformatic pipeline to evaluate the element Keno-XT, from the TX-1 clade, in the genome of this species. For each query, the sequences with greater significance (E-value scores less than 10-6) were retrieved. For each hit, the detection of ORFs was performed by the ORFinder tool, and protein domains were predicted using the NCBI CD-Search. During the contigs inspection, we found a frequent association between L1-elements with U2 small nuclear RNA gene (snRNA U2). These genes are in tandem repeat in vertebrates genomes, and in *X. tropicalis*, we recovered the association between U2 snRNA and L1-copies clusterized in a ~4 Mb region in chromosome 8. Although this association between the U2 snRNA gene and Keno-XT copies is not entirely a novelty in tetrapods genomes, at the fine scale, we observed an interesting chromosomal organization of this 450 Kb: in the peripheric region of arrays (around 137 Kb), upstream of central arrays, are composed by an interspersed pattern of the Keno-XT sequences and snRNA U2 genes in which Keno copies are potentially functional and exhibits the majority of protein domains. Microsatellite motifs (perfect or imperfect) separated Keno-XT from snRNA U2. We observed an increase in truncated or incomplete Keno copies from the peripheric to the central cluster. In both marginal arrays, we detected snRNA U2 copies exhibit higher nucleotide divergence than the central cluster, and in ML tree was assembled in the same branch. The central cluster comprises arrays that exhibit copies of the snRNA U2 copies virtually identical. The snRNA U2 copies are equidistantly separated and the few Keno-XT copies recovered in this region showed structural loss (truncated). Our results pointed to strong repeats homogenization which we observed low nucleotide variability and clustering patterns of repeats on ML trees. This evidence suggests a strong homogenization ratio of repeats occurring at the chromosomal level and validates the occurrence of Concerted Evolution in this region mediated by unequal-crossing over and/or gene conversion. The few repeats of U2 snRNA were mapped in different chromosomes and always were reported in association with Keno-XT retrotransposon, suggesting recent dispersion by retrotransposition events from the chromosome 8 cluster. We provide a genomic view of the concert evolution model operated at chromosomal-level and the interplay between different repetitive DNA classes to chromosomal evolution.

Key-words: Transposable elements; snRNA U2; Keno-XT;

ID -106
INVESTIGATING THE MOST ABUNDANT SATELLITE DNAS IN TWO MYRMECOPHAGIDAE SPECIES (SUPERORDER XENARTHRA)

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Abstract:
The superorder Xenarthra has species with some of the largest mammalian genomes. Nevertheless, the repetitive DNA content, which makes up a substantial portion of the eukaryotic genome (>50%), is still poorly studied in this group. Satellite DNAs (satDNAs), one of the most abundant classes of repetitive DNAs, are highly repetitive sequences, tandemly organized in large arrays that may extend up to hundreds of Mb. They are the main component of telomeres and centromeres and tend to evolve very rapidly compared with other genomic sequences. In this work, we used *in silico* and cytogenetic analyses to identify and characterize the most abundant satDNAs present in the Myrmecophagidae family. First, we ran the TAREAN pipeline using the sequenced genomes of *Myrmecophaga tridactyla* and *Tamandua tetradactyla* available on GenBank. TAREAN identified four putative satDNA families in *M. tridactyla* (named MTR_156, MTR_696, MTR_1550, and MTR_3029) and two in *T. tetradactyla* (named TTE_35 and TTE_745) genomes. These satDNAs comprise about ~1% and ~0,21 of the *M. tridactyla* and *T. tetradactyla* genomes, respectively. Although TAREAN did not identify MTR_156 on the *T. tetradactyla* genome, we found MTR_156 repeats forming long arrays (>32Kb) in the assembled genome of this species. We used the six identified satDNAs as queries against the Repbase and GenBank databases. The MTR_696 and TTE_35 satDNAs did not reveal significant sequence identity with any previously described DNA sequence. The MTR_156 showed sequence identity with a Penelope-like retroelement. This retroelement is an ancient eukaryotic class of repetitive DNA that shares a common ancestor with the telomerase reverse transcriptase. MTR_156 is composed of ~156 bp long repeats and has a higher-order organization (HORs) comprised of twenty-six fragments of six bp each (~68% of pairwise identity). MTR_156 also shares some pairwise identity with other MTR satDNAs (~51% with MTR_696, ~57% with MTR_1550, and ~64% with MTR_3029) and with the vertebrate conserved telomeric sequence (TTAGGG)n (~71%). The MTR_156 copies retrieved from the assembled genomes of *M. tridactyla* and *T. tetradactyla* presented ~0.4% of nucleotide variability. The Neighbor-Joining tree constructed did not show repeats clustering into species-specific branches. To perform the satDNAs characterization, we used genomic DNA (gDNA) and chromosome spreads from a male specimen of *M. tridactyla* (2n=60, FN=104) and a female of *T. tetradactyla* (2n=54, FN=104). The MTR_156, MTR_696, MTR_1550, and MTR_3029 sequences were PCR-amplified from gDNA of *M. tridactyla* and will be used as probes in FISH experiments. MTR_156 was also amplified from *T. tetradactyla* gDNA. Initial FISH results were obtained for *M. tridactyla*, in which MTR_156 mapped in the subtelomeric regions of all chromosomes and in the pericentromeric region of two small metacentric pairs. To further characterize this satDNA, we are carrying out dual probe-FISH on *M. tridactyla* chromosomes (MTR_156 x telomeric probes "$TTAGGG_7$", MTR_156 x MTR_696, and MTR_696 x telomeric probes "$TTAGGG_7$". The next steps will include chromosome mapping of the other satDNAs (MTR_1550, MTR_3029, TTE_35, and TTE_745) in *M. tridactyla* and *T. tetradactyla*. We expect that these results will give insights into how satDNAs are related to Myrmecophagidae genome evolution.
Key-words: Heterochromatin; Satellite DNAs; RepeatExplorer; TAREAN; FISH

Acknowledgement

CAPES; CNPQ; FAPEMIG

ID – 160

INVESTIGATING THE CHROMOSOME ORGANIZATION OF *PIAYA CAYANA* (AVES: CUCULIFORMES)

Rafael Kretschmer [1]; Marcelo Santos de Souza [2]; Ricardo José Gunski [2]; Analía Del Valle Garnero [2]; Thales Renato Ochotorena de Freitas [3]; Gustavo Akira Toma [4]; Marcelo de Bello Cioffi [4]; Edivaldo Herculano Corrêa de Oliveira [5]

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Abstract:
Cuckoos, belonging to the Cuculiformes order, are a diverse group of birds consisting of about 150 species that inhabit various environments such as forests, savannas, and deserts. These birds are highly valued for their unique and distinct calls that have cultural significance in many traditions and cultures worldwide. The diploid number (2n) of most cuckoo species is around 78, although some species have a higher or lower 2n. This study focused on the chromosome organization of the squirrel cuckoo (*Piaya cayana*, 2n=90). To conduct the cytogenetic analyses, we collected skin biopsies from three individuals and performed fibroblast cultures to obtain chromosome preparations. We mapped the distribution of constitutive heterochromatin through C-banding. Furthermore, we used bacterial artificial chromosome (BAC) probes from chicken (*Gallus gallus*) and zebra finch (*Taeniopygia guttata*) to investigate chromosome homologies between chicken and *P. cayana* for macrochromosome pairs 1-10, Z and W sex chromosomes, and for microchromosome pairs 11-28 (except 16). Overall, our results confirmed the fissions previously found in *P. cayana* by chromosome painting and revealed a total of five intrachromosomal rearrangements in the first five macrochromosomes and in the Z. No evidence of interchromosomal rearrangements was found in the microchromosomes. In addition, a conspicuous polymorphism involving a heterochromatic block was found in pair 20 of *P. cayana*. Taken together, our cytogenetic analyses demonstrated that the mechanisms of chromosomal evolution in *P. cayana* involved fissions, fusions, inversions, and accumulation of repetitive sequences, which gave rise to unusual rearrangements, such as a huge heterochromatic polymorphism.
Key-words: Birds; Karyotype; Bacterial artificial chromosome; Intrachromosomal rearrangements; Polymorphism

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ID – 96

B CHROMOSOMES ARE MITOTICALLY STABLE IN SOMATIC TISSUES OF PSALIDODON PARANAE (CHARACIFORMES, CHARACIDAE)

Raquel da Costa Machado [1]; Mateus Rossetto Vidal [1]; Duilio Mazzoni Zerbinato Andrade Silva [1]; Lucas Fortino Lasmar [1]; Leticia Batista Soares [1]; Pamela Cristina Ferreira de Nadai [1]; Claudio Olveira [1]; Fausto Foresti [1]

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Abstract:

B chromosomes are extra elements not essential for the survival of the hosts. Fish genus Psalidodon has at least 8 species with B chromosomes, and the P. paranae has the most studied B chromosome within them. This species has been highlighted as a potential study model for Bs in fish, and several aspects such as gene content, transmission and meiotic behavior have already been elucidated. B chromosomes of P. paranae were shown to be mitotically stable on a few tissues, such as kidneys and gonads. However, recent studies have shown that B chromosomes can undergo deletion in specific tissues during the embryonic period. Thus, it is not known whether these chromosomes are, in fact, present in all tissues of the P. paranae. This is important to know, keeping in mind that different tissues can be used for genomic and transcriptomic studies. Thus, the aim of the present work was to test if the B chromosome of P. paranae is mitotically stable in tissues from different embryonic leaflets, such as brain, heart and intestine. For this purpose, specimens were collected from Ribeirão Cascatinha stream, Botucatu, São Paulo. After euthanasia, we prepared interphase nuclei by fixing the brain, heart and intestine in methanol acetic. Subsequently, the tissues were dissociated, and the cell suspensions were subjected to the FISH technique, using a microdissected B chromosome probe already available in our laboratory. The probe revealed the presence of this chromosome in all analyzed tissues. This presence was confirmed due to a very conspicuous signal in animals with the B chromosome, while animals without B chromosome show scattered and weak signals throughout the nuclei. So far, the deletion of B chromosomes has been observed in literature only during the embryonic stage. Thus, the presence of B chromosomes in tissues originating from all three embryonic leaflets suggests that most likely these chromosomes are present in all tissues of P. paranae in a stable manner. As an example, the elimination of B chromosomes in roots of the plant Aegilops speltoides was hypothesized as a mechanism to avoid detrimental expression and imbalance of regulatory networks in root tissues. Previous studies have revealed overexpression of nusap1, nobox, and msh4 genes in ovaries with B chromosomes. Thus, as future perspective, gene expression analysis in other tissues will reveal whether these chromosomes lead to gene overexpression in other tissues, which will help to understand the effects of B chromosomes in very important somatic tissues as the brain.

Key-words: Supernumerary chromosome; Astyanax; Cytogenomics;

Acknowledgement

FAPESP, CAPES, CNPQ

ID – 142

MOLECULAR CYTOGENETICS OF INDIVIDUALS OF THE SPECIES *MAZAMA NEMORIVAGA* FROM THE BRAZILIAN AMAZON RAINFOREST

Raquel Muhlbeier Bonato [1]; Maria Dolores Astudillo Vallejo [1]; Agda Maria Bernegossi [1]; José Maurício Barbanti Duarte [1]

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Abstract:

Mazama nemorivaga is a gray brocket deer that lives in the Amazon rainforest, being characterized by medium-sized animals, weighing between 14 and 15 kg, with simple antlers and a gray coat. Like other species of the genus *Mazama*, it stands out for its expressive chromosomal polymorphism, studied so far only through classical cytogenetics, showing variations of diploid number (2n) from 67 to 70, fundamental arm number (FN) from 69 to 72, multiple B chromosomes, in addition to a simple chromosomal sexual system XX/XY in some individuals, and multiple sexual chromosomes XX/XY_1Y_2 in others. Then, this study aims to describe the occurrence of inter and intrachromosomal variations for the species using molecular cytogenetics technique. For that, we compared the karyotype of seven individuals of *M. nemorivaga*, three males and four females, from different localities of Brazil's Amazon, with the topotype of the species, collected in French Guiana and characterized with 2n = 69 and FN = 72, carrying a centric fusion in heterozygosis, involving chromosomes 7 and 27, and simple sexual system. Chromosomal preparations were evaluated using the methodology of Fluorescence *in situ* hybridization with bacterial artificial chromosome clones derived from the cattle genome *(Bos taurus)* as markers. The results showed five animals presenting simple sexual system and with centric fusions also involving the chromosomes 7 and 27, three of them with this centric fusion in heterozygous (one male, two female), and two in homozygous (one male and one female). Also, one male and one female showed a multiple sexual system. The sexual system was derived due to an X-autosomal fusion with chromosome 15 of the topotype. The male had one small metacentric Y_1 chromosome and one small acrocentric Y_2 chromosome, and in addition, a homozygous centric fusion between chromosomes 7 and 27 (2n = 67, NF = 70), and the female had no other rearrangement besides de X-autosomal fusion (2n = 68, NF = 70). These polymorphic karyotypes could indicate some degree of population differentiation and lead to a decrease in reproductive efficiency. Furthermore, the species was divided into three distinct clades with geographic correlation in the latest phylogenetic analyzes carried out from mitochondrial DNA sequences. Then, our results will be used to relate the existence of a correspondence between the phylogenetic structure and the chromosomal differences and test the hypothesis that *M. nemorivaga* constitutes a cryptic species complex.

Key-words: Molecular cytogenetics; chromosomal polymorphism; Cervidae;

ID – 140

ANALYSIS OF REPETITIVE DNAS ON SPOTTED-WING *DROSOPHILA* PEST SPECIES REVEAL INTERPLAY BETWEEN SATDNAS AND MULTIGENE FAMILIES

Rhavenna Thais Alves Gomes da Silva [1]; Ana Beatriz Stein Machado Ferretti [1]; Emiliano Martí [1]; Frederico Hickmann [2]; Larine de Paiva Mendonça [2]; Diogo Cavalcanti Cabral-de-mello [1]

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Abstract:

The spotted-wing *Drosophila* (*Drosophila suzukii*) is a pest species with great economic importance due to its worldwide distribution and its oviposition habit that allows the attack of a wide variety of fruits. Chromosomal and genomic studies are important to advance on the genome assembly of species, especially for pest species and for repetitive DNAs that are in general neglected on genomic projects. Here we advance on the analysis of repetitive DNAs on *D. suzukii*, focused on the understanding of multigene family chromosomal organization and evolution. For this we characterized the chromosomes of *D. suzukii* using classical cytogenetic tools, as conventional staining and C-banding and prospected on sequenced genome and mapped at chromosomal level the sequences for 18S and 5S ribosomal DNAs (rDNAs) and H4 histone gene. As in other reports *D. suzukii* presented karyotype 2n = 8, XX (female)/XY (male). The heterochromatin is present on pericentromere of all chromosomes, but the dot chromosome (pair IV). Specially the chromosome II has high amount of heterochromatin, covering 2/3 of its extension, revealing putative intense amplification of repeats on this chromosome, an uncommon pattern on other *Dorsophila*. Additionally, on the Y chromosome the heterochromatin covered its whole extension, pointing extensive molecular differentiation to the X chromosome. As in all other *Drosophila* species the 18S rDNA showed discrete signals on the centromeric regions of X and Y chromosome. Unexpectedly, the H4 histone and 5S rDNA showed scattered signals in all chromosomes, except in the dot chromosome, with most accumulations in the regions rich in heterochromatin. The same probes labeled only a discrete region of chromosome II of *D. melanogaster*, that is the expected region for the position of these genes by genomic data. This supports that the signals observed in *D. suzukii* are caused by the presence of sequences from these multigene families in multiple positions. Complementary analyzes revealed that Histone H4 (H4-satDNA) and 5S rDNA (5S-satDNA) have homology with satellite DNAs identified by us in this species. Finally, the mapping of a heterologous probe of H4 gene (from *D. melanogaster*), revealed a discrete cluster on *D. suzukki* chromosome II, but not coincident with the H4-satDNA. These data reveal a clear highly dynamism for repeats on *D. suzukki* genome involving the multigene families and its relationship with satDNA evolution.

Key-words: Satellite DNA; Repetitive DNA; Multigene families;

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ID – 158

IN SITU LOCALIZATION OF SATELLITE DNA IN *RINELORICARIA LATIROSTRIS* (SILURIFORMES: LORICARIIDAE)

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Abstract:

Loricariidae (armored catfishes) comprises a family of fish considered interesting from the cytogenetic and evolutionary point of view due to its extensive karyotypic variation. The involvement of different classes of repetitive DNAs (rDNAs, transposable elements, and microsatellites) were suggested to trigger chromosomal breaks and rearrangements in the *Rineloricaria* species, a genus of loricariids. Satellite DNAs (satDNAs) are tandemly repetitive DNAs presenting variations in the size unit, number of copies, and chromosomal organization. The availability of the whole genome sequencing allowed to describe and characterize the sequences of satDNA in several species and, further studies, showed its distribution in centromeric, telomeric, euchromatine and/or heterochromatin regions of chromosomes, which may be accumulated in block or dispersed. Recently, the DNA library of *Rineloricaria latirostris* (2n = 46, karyotype formula 10m+4sm+32st/a) from Laranjinha river (Cinzas basin, Ventania, Paraná state, Brazil) was sequenced on the Illumina Hiseq platform, which allowed us to investigate the enriched clusters with repetitive DNAs. So, in order to understand the possible involvement of repetitive DNAs in break-prone chromosomal sites, we aimed to screen satDNA fragments from *R. latirostris* genome through the TAREAN tool (Tandem Repeats Analyzer). Considering the most representative satDNA copies in the genome, we designed specific oligonucleotides for them to isolate and characterize these repeat DNA regions. Using *R. latirostris* total DNA as template, we amplified the cluster 105 (1,095 bp) by Polymerase Chain Reaction (PCR), and submitted it to agarose gel electrophoresis. PCR products were labeled using digoxigenin-11dUTP and used as probes for fluorescence in situ hybridization (FISH) on slides with mitotic chromosomes of *R. latirostris*. Signals were detected using anti-digoxigenin rhodamine Fab fragments. In situ localization of the satDNA showed one accumulated block on the pericentromeric region of the acrocentric pair 20. Our data confirm that cluster 105 is a satDNA from *R. latirostris*, named *Rlat* 1. Based on previous cytogenetic data, it is assumed that some metacentric pairs in *R. latirostris* (2n = 46) had origin by Rb fusion of subtelo and acrocentric chromosomes. In common, the fused chromosomes of *R. latirostris* have extensive repetitive DNA accumulation surrounding the fusion point. The *Rlat* 1 obtained here showed no evidence of chromosome rearrangement involvement. A comparative chromosome mapping of *Rlat* 1 among *Rineloricaria* species could permit a better scenario of its conservation or dispersion into karyotypes.

Key-words: Chromosomal rearrangements; FISH; satDNA;

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ID - 77

COMPARATIVE ANALYSIS OF MICROSATELLITE DISTRIBUTION IN TWO TETRA FISH SPECIES *ASTYANAX BIMACULATUS* AND *PSALIDODON SCABRIPINNIS* (CHARACIFORMES; CHARACIDAE)

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Abstract:

Fishes belonging to family Characidae present a wide range of karyotypic diversity, including the occurrence of supernumerary chromosomes, making them excellent models for cytogenetic and evolutionary studies. Hence, considering that repetitive DNA sequences play an important role in chromosomal rearrangements occurring during the karyotypical evolution in many groups of organisms, and that cytogenetic studies involving these sequences are still scarce among Characidae, we aimed to analyze comparatively the distribution of microsatellites sequences in *Astyanax bimaculatus* and *Psalidodon scabripinnis* in order to better understand the dynamics and distribution pattern of these sequences in the genome of these species. For this, chromosome preparations were obtained from cell cultures using fin biopsies and fluorescence *in situ* hybridization (FISH) experiments were performed using probes corresponding to 6 microsatellite sequences- $(CA)_{15}$; $(GC)_{15}$; $(GA)_{15}$; $(CAT)_{10}$; $(CAC)_{10}$, and $(CAG)_{10}$. As results, both species had the same diploid number 2n=50, with differences in chromosomal formula and fundamental number (FN). In *A. bimaculatus* the chromosome formula was 6m + 28sm + 8st + 8a, and FN = 92, while the karyotype of *P. scabripinnis* was composed of 2m + 22sm + 12st + 14a, and FN = 86. Regarding FISH experiments in the karyotype of *A. bimaculatus*, the probes produced signals mainly in centromeric regions. However, the probes of $(GC)_{10}$, $(CAT)_{10}$, $(GAG)_{10}$, and $(GA)_{15}$ showed hybridization signals in euchromatic regions and scattered along the chromosome arms. In addition, $(CA)_{15}$ and $(GC)_{15}$ probes produced conspicuous signals were produced on the B chromosome. In *P. scabripinnis*, the hybridization was mainly in telomeric regions, in addition to a large accumulation of microsatellites on its B chromosome, and the probe of $(GC)_{15}$ hybridized also on the terminal portion of 5 pair of chromosomes. Overall, the distribution patterns of microsatellites observe in Characidae are consistent with previous reports, in which microsatellite sequences were more abundant in regions of low recombination rate, such as the centromere and telomeres. However, these genomic differences observed between these species suggests that the microsatellite distribution profile could correspond to a potential cytotaxonomic marker for the group. In addition, the differences in the distribution of microsatellites on the autosomal and supernumerary chromosomes of the two species represent excellent data on chromosomal rearrangements, since such sequences are widely associated with breakpoints, which are evolutionary hotspots. The results of the present work expand the knowledge about the distribution and evolution of microsatellites in Characidae, generating data that help in understanding the karyotypic evolution in this family.

Key-words: B Chromosome; Chromosomal evolution; Chromosomal rearrangements; Genetic diversity; Repetitive sequences.

Acknowledgement

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ID 137

CYTOGENOMIC ANALYSIS OF THREE SPECIES OF THE FAMILY TEIIDAE (REPTILIA, SQUAMATA)

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Abstract:
The Teiidae family includes two subfamilies of lizards (Teiinae and Tupinambinae), which have a wide distribution in Brazilian biomes. Karyotypic studies using cytogenomic markers are still poorly addressed in this family. Thus, the present work aimed to analyze the karyotype of three species of the Teiidae family: *Crocodilurus amazonicus* and *Tupinambis teguixin* (Tupinambinae) and *Kentropyx calcarata* (Teiinae), by means of classical (Giemsa staining and C-band) and molecular cytogenetic techniques (microsatellite, telomere and 18S rDNA probes), in order to better understand the genomic organization and karyotypic evolution of this group. The specimens were collected in Abaetetuba, Pará. Chromosomes were obtained from bone marrow. The species *C. amazonicus* presented 2n=34 with 12 macrochromosomes and 22 microchromosomes, *T. teguixin* 2n=36, with 12 macrochromosomes and 24 microchromosomes and in *K. calcarata* 2n=50 without clear distinction between macros and microchromosomes. C-banding revealed constitutive heterochromatic blocks in the centromeric regions and tenuous marks in the terminal regions of the chromosomes of *T. teguixin* and *C. amazonicus* and conspicuous blocks in telomeric region for *K. calcarata*, corroborating previous data and demonstrating a conserved karyotypic pattern along the geographical distributions of these species. The 18S rDNA probes hybridized on pair 2 for Tupinambinae species and pair 1 for Teiinae, coinciding with silver staining. Telomeric DNA probes were observed in the distal regions of the chromosomes, however, *C. amazonicus* and *T. teguixin* showed interstitial telomeric sequences (ITS) in two and five pairs, respectively. These ITS found in these species could be explained by the hypothesis of telomeric heterochromatin, since their karyotypes are more similar to the ancestral Squamata karyotype, while *K cararata* has the most derived one. Experiments using five microsatellite sequences (CAG, GAA, GAG, CGG and TA) revealed a prevalence of hybridization signals in the centromeric and telomeric regions of chromosomes, coinciding with positive C-Band, as well as in the microchromosomes. In addition, some of the microsatellite sequences produced signals on the NOR region. Overall, the distribution of these sequences has shown both distinct and shared characteristics in the species analyzed herein, evidencing an active participation of microsatellites in the karyotype evolution of the family.
Key-words: Teiidae; Chromosomes; Repetitive sequences;

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ID – 40

"WHO LET THE LAMBARIS OUT?" - A SIMILAR AND COMPLEMENTARY POLYMORPHISM OF 5S AND 18S RDNA SITES BETWEEN NATIVE AND FISH-FARMED *ASTYANAX LACUSTRIS* SPECIMENS.

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Abstract:

The *Astyanax bimaculatus* species complex, also known as the "yellow-tailed lambaris", is a group of Neotropical species easily found in the Paraná River Basin III, municipality of Santa Helena (PR, Brazil). Fish farmers are encouraged to breed them in an effort to capitalize on their economic potential due to their value as snacks and, more importantly, their use as living fishbaits by both professional and recreational anglers. *Astyanax lacustris* Lütken 1875 and *Astyanax abramis* Jenyns 1842 are the only two valid species of the *Astyanax bimaculatus* group with occurrence in the Paraná River Basin III, but it is unknown which species are maintained in fish farms. The only information the proprietors of the fish farms have is that the species are procured from outside the municipality, this way, the fish-farms can create more than one species of the *Astyanax bimaculatus* group. Due to the risk of species introduction, we aimed to identify the species from two fish-farm of Santa Helena municipality using DNA Barcode (Cytochrome Oxidase I - COI) and classic and molecular cytogenetics. All the specimens from both fish-farms were identified as *Astyanax lacustris* via DNA Barcode, with no population differentiation. The classic cytogenetic data reveal no differences between the fish-farms populations, showing 2n=50, karyotype formulae of 10m+24sm+6st+10a, NF=90, absence of sexual dimorphism, and single AgNORs marks in the short arm of subtelocentric pair 21 for both samples. The chromosome pair 2 presented 5S rDNA marks in the centromeric region in all exemplars, although some exemplars presented another 5S rDNA mark in the centromeric region of one acrocentric chromosome from pair 25. The hybridization using 18S rDNA probe detected an extensive polymorphism in both populations, being constant only the 18S rDNA marks in the short arm of the subtelocentric pair 21. Looking to the classic cytogenetic and the molecular identification with DNA Barcode, the fish-farm presented no population distinction when compared to the native ones already described. The 5S and 18S rDNA probes, which exhibit identical or complimentary cytotypes in a coincidental polymorphism, highlight the similarities between fish farm and native populations. This study evidenced a possible gene-flow between fish-farmed and native specimens of *Astyanax lacustris*. When dams break, fish from fish farms can escape into the wild, or when these fish are used as live bait in unsuccessful fishing attempts, they may be released into the environment. Genetic diversity selected over the years can be lost in natural populations due the gene flow between different *Astyanax lacustris* lineages, leaving these populations with an uncertain future.

Key-words: Fluorescence *in situ* Hybridization (FISH); Specimens Introduction; Chromosome Markers; DNA Barcode;

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ID – 41

TYMPANOPLEURA ATRONASUS REANALYZED AFTER 31 YEARS: NEW INFORMATION ABOUT *TYMPANOPLEURA* AND *AGENEIOSUS*

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Abstract:
Auchenipteridae includes fishes without scales, commonly named as driftwood catfish, and his evolutionary and taxonomy history is complicated and unresolved. Ageneiosini, one of the Auchenipteridae tribes, includes numerous species redescriptions and even genus revalidations. Currently, *Ageneiosus*, *Tympanopleura*, and *Tetranematichthys* are the three recognized Ageneiosini genera. Despite advances in classical and molecular cytogenetic studies, only *Tympanopleura atronasus* and *Ageneiosus inermis* have known karyotypes. However, the only cytogenetic data for *T. atronasus* dates 1992 from only one population, leaving 31 years without further cytogenetic analyses. The aim of this study is to raise more data from two Ageneiosini species, *A. inermis* and *T. atronasus*, using classic cytogenetic approaches (giemsa staining, C-Banding and AgNOR). Thirteen *T. atronasus* individuals were gathered using cast nets in Catalão Lake, near Manaus (AM), while 9 *A. inermis* specimens were captured using fishing rods in the Purus River, Abufarí Biological Reserve (AM). The cell suspension obtaining was performed *in vitro* using RPMI 1640, colchicine 0,0250%, KCl 0,2N and fixation with Methanol + Acetic Acid (Glacial) 3:1. The chromosomes were classified using its arms proportion as metacentric (1.00 - 1.70), submetacentric (1.71 - 3.00), subtelocentric (3.01 - 7.00), and acrocentric (>7.00). *T. atronausus* has 2n=54, karyotype formulae of 30m+14sm+8st+2a, FN=106, no sexual chromosomes, and C-bands primarily pericentromeric and terminal, but also centromeric bands. AgNORs are found in the pericentromeric region of submetacentric pair 16, just below the heterochromatic region. *Ageneiosus inermis* has 2n=56 with 32m+18sm+4st+2a, FN=110, no sexual chromosomes, and C-Bands preferentially pericentromeric and terminal, but also in the centromeric region. AgNORs were found in the pericentromeric region of submetacentric pair 24, just below the heterochromatic region. The first karyotype description for *T. atronasus* (identified as *Ageneiosus atronases* in 1992) found the same AgNOR pattern, but the diploid number and karyotype formulae have been different with 2n=56 and 16m+16sm+12st+12a (FN=100). Due to large differences in karyotype formulae and even diploid number, the only cytogenetic record of *T. atronasus* until now may be from another *Ageneiosus* or *Tympanopleura* species, which must have been probably mistakenly identified at the time. Despite the minor karyotype differences found between the previously published cytogenetic data of *A. inermis* (2n=56, with 32m+16sm+4st+4a, FN=108) and compared the current study, we can attribute them to differences in chromosome condensations or even measurements. An intriguing difference between the karyotypes of *A. inermis* and *T. atronasus* is the AgNORs-bearing chromosomes, which may have been generated by a fusion event and could explain the reduced diploid number in *T. atronasus*. Could this be a feature of *Tympanopleura*? New studies characterizing other *Tympanopleura* species, in addition to the use of other chromosomal markers, are needed to confirm the centric fusion hypothesis, with more revealing information to be discovered for both Ageneiosini genera. Moreover, our data show it is important to reanalyze previously studied species to update our knowledge, which in this study is helping us clarify this intriguing part of the Auchenipteridae.
Key-words: AgNORs; Auchenipteridae; Reanalyzing Species; Heterochromatin;

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ID 85

KARYOTYPIC DESCRIPTION OF *COPEOGLOSSUM NIGROPUNCTATUM* (SQUAMATA, SCINCIDAE) THROUGH CLASSICAL AND MOLECULAR CYTOGENETICS.

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Abstract:
The Scincidae family includes the most specious group of lizards being found all over the planet. The chromosomal data is available for a few species, limiting the knowledge about the karyoevolutionary process in these animals. Thus, the present work aimed to descript the karyotypic information of *Copeoglossum nigropunctatum* using data from classical and molecular cytogenetics. Three specimens of *C. nigropunctatum* were captured using pitfall traps and hand hunt in the municipality of Abaetetuba-PA. Chromosome preparations were obtained from bone marrow and spleen. Fluorescent in situ hybridization (FISH) was made using 18S rDNA and telomeric probes. Our results showed that *C. nigropunctatum* has 2n=30, with eight pairs of bi-armed macrochromosomes and 14 pairs of microchromosomes. The fundamental number (FN) was difficult to obtain due to the size and morphology of the microchromosomes. Probes of 18S rDNA probes produced signals in the long arm of pair 2, coinciding with a secondary constriction. The single NOR is very common in this group, although its chromosome location may vary depending on the species. FISH with telomere probes revealed signals at distal regions of chromosomes without interstitial telomeric site (ITS), which could suggest the occurrence of recent rearrangements. The data obtained here corroborate with previous studies on the stasis of chromosomal evolution in lizards, but there is still a great need for studies in other species of the group, with other cytogenetic tools, for a better understanding of the scenario of chromosomal evolution in Scincidae.
Key-words: Lizards; Scincidae; Cytogenomics;

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ID 133

CYTOGENETIC CHARACTERIZATION OF THE LEAFCUTTER BEE *MEGACHILE SUSURRANS* HALIDAY, 1836 (HYMENOPTERA, APIDAE) USING CLASSICAL AND MOLECULAR TECHNIQUES

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Abstract:
Representatives of the genus *Megachile* are popularly known as leaf-cutting bees due to the use of leaves to build their nests. These bees constitute a cosmopolitan group of solitary bees, consisting of about 1320 species, which play the role of pollinators of plants of economic interest. Despite this biological importance, only four species of the genus *Megachile* collected in Japan have been karyotyped. The aim of this study was to describe the karyotype and location of 18S ribosomal gene sites from *Megachile susurrans* in order to expand the cytogenetic information about the genus. Larvae of the species were collected in Viçosa, Minas Gerais, through trap-nests constructed with bamboo. Metaphase chromosomes were obtained from the brain ganglia of larvae after elimination of meconium, according to the methodology proposed by Imai et al. (1988). For analysis of the chromosome number, staining with 4% Giemsa was performed. Chromosomal mapping of 18S rDNA clusters was performed using fluorescent *in situ* hybridization. The analyzed females presented 2n=32 chromosomes, the same chromosome number described for the other *Megachile* species, from the old world, previously studied. This suggests a certain stability of the karyotype in the genus. The analysis of the number and distribution of ribosomal clusters, in turn, showed that this species has, at least ,8 markings of 18S rDNA genes in the terminal regions of the chromosomes. The location of these markings may have influenced the dispersion of these genes, since terminal rDNA clusters can more easily associate with other sequences of non-homologous chromosomes. This would facilitate the occurrence of ectopic recombination between non-homologous chromosomes leading to dispersal of rDNA genes to other chromosomes. This is the first cytogenetic study that characterizes a species of the genus *Megachile* from the neotropical region and thus contributes to expanding the biological information available on the genus. Future comparative studies may help to better understand the karyotypic evolution of the genus.
Key-words: solitary bee; karyotype; ribosomal genes;

ID – 122

COMPARATIVE CHROMOSOME PAINTING CLARIFIES THE INTRASPECIFIC CHROMOSOMAL VARIATION IN TWO *CTENOMYS* SPECIES (RODENTIA: CTENOMYIDAE)

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Abstract:

Ctenomys is the current mammal with the greatest interspecific (2n=10 to 70) and intraspecific chromosomal variation. *Ctenomys minutus* (CMI) has the highest number of cytotypes described so far. *Ctenomys lami* (CLA) is the species with the greatest karyotypic variation in a smaller geographic area. Both species are distributed in the coastal plain of southern Brazil, *C. minutus* lives in the dunes and sandy fields from Santa Catarina to Rio Grande do Sul State, Brazil, while *C. lami* lives in a restricted Rio Grande do Sul State area known as "Coxilha das Lombas". Here, we performed whole chromosome painting with *C. flamarioni* (CFL) probes to explore the chromosomal evolution in *C. minutus* and *C. lami*. These two closely related species do not have external morphological differences. These experiments allowed us to construct chromosomal homology maps that revealed the rearrangements that differentiate the cytotypes of each species and these species. Comparative chromosome maps show that *C. minutus* and *C. lami* karyotypes have undergone multiple rearrangements compared to *C. flamarioni*. We identified that CMI (CMI7, CMI9, CMI12, and CMI21) and CLA (CLA6, CLA7, CLA10, and CLA17) have four completely conserved chromosomes between *C. flamarioni* (CFL6, CFL9, CFL12, and CFL17), these chromosomes are conserved in all cytotypes of *C. minutus*, and all cytotypes of *C. lami*. On the other hand, seven large chromosomes of *C. flamarioni* (CFL1, CFL2, CFL3, CFL4, CFL13, CFL14, and CFL16) are fissioned in two or three pairs in *C. minutus* and *C. lami*. However, some entire chromosomes and large chromosome segments remain conserved between *C. minutus* and *C. lami*. We observed that 16 autosomes pairs of CMI (CMI5, CMI6, CMI7, CMI8, CMI9, CMI10, CMI11, CMI12, CMI13, CMI14, CMI15, CMI16, CMI18, CMI21, CMI22, CMI24) showed complete homology (1:1) with CLA (CLA2, CLA4, CLA6, CLA5, CLA7, CLA8, CLA9, CLA10, CLA11, CLA12, CLA14, CLA20, CLA15, CLA17, CLA21, CLA26). Our data indicate that chromosomal rearrangements played an important role in *Ctenomys* speciation and demonstrated that the common rearrangements between species could relate to the recent speciation process between these two species, which is based on the role of the rearrangements. Furthermore, we demonstrated that the karyotype of *C. minutus* and *C. lami* showed shared rearrangements, which may be exclusive to the *torquatus* group, indicating that these rearrangements represent a cytogenetic signature for the group.

Key-words: rearrangements; Ctenomyidae; Rodentia; chromosome paiting; chromosomal evolution

Acknowledgement

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ID – 193

CHROMOSOMAL MAPPING OF THE HISTONE CLUSTER IN BIRDS: A EVOLUTION NOT-SO-PARSIMONIOUS

Tiago Marafiga Degrandi [1]; Iasmin Ieteka [1]; Ricardo José Gunski [2]; Analía Del Valle Garnero [2]; Edivaldo Herculano Corrêa de Oliveira [3]; Roberto Ferreira Artoni [1]

[1]. Universidade Estadual de Ponta Grossa- Laboratório de Genética Evolutiva; [2]. Universidade Federal do Pampa- Laboratório de Diversidade Genética Animal; [3]. Universidade Federal do Pará. Instituto Evandro Chagas- Laboratório de Cultura de Tecidos e Citogenética.

Abstract:

The histone gene cluster (H1, H2A, H2B, H3, H4, and variants) can be considered one of the main elements of the eukaryotic genome, since the proteins encoded by these genes constitute the basic compacting unit of DNA (nucleosome), in addition to playing an important role in the regulation of transcription, replication, DNA repair, and maintenance of chromosomal stability. Recent advances in the knowledge of avian genomics have contributed to the understanding of the architecture and chromosomal distribution of these important elements. Furthermore, birds exhibit relatively conserved karyotypes, as confirmed by comparative chromosome painting (CCP) with *Gallus gallus* (GGA) probes, a technique which allows the establishment of homologies between distantly related species. Thus, the objective of this work was to analyze the chromosomal distribution of the histone cluster and identify the patterns of evolution of these elements in the karyotype of different bird species. For this, *in silico* mapping of the histone cluster was performed in 68 species from 22 orders with genome assembled at the chromosome level, available at NCBI. To find the cluster in each genome, the BLASTn tool and the histone H4 sequence (NM_001037845.2) from GGA were used. The chromosome pair, the position, and the number of sites occupied by the cluster were analyzed. The results were plotted on a phylogenetic tree and confronted with CCP data available in the Bird Chromosome Database v.3/2022. It was seen that 25 (37.31%) of the species preserved a single histone cluster located on chromosome pair 1, and 42 (62.69%) species had the cluster occupying different chromosomes that varied from the 2nd to the 34th chromosome pair. No duplications were observed in any of the analyzed species. CCP data showed that the cluster occupies a highly conserved region and that it follows the evolutionary karyotype history of the syntenic group corresponding to Putative Ancestral Avian Karyotype PAK1 (homologous to the GGA1 chromosome). From this, it was assumed that the hypothesis that the ancestral condition present in PAK is the location of the cluster on chromosome 1 in the terminal region. The variations observed can be attributed to rearrangements such as inversions, observed in species belonging to the orders Galliformes, Anseriformes, Phoenicopteriformes, Caprimulgiformes (Trochilidae, Apodidae), Gruiformes, Charadriiformes (Laridae and Alcidae). And PAK1 fissions were found in species of Pterocliformes, Musophagiformes, Caprimulgiformes (Caprimulgidae), Pelecaniformes, Accipitriformes (Accipitridae), Bucerotiformes, Piciformes, Piciformes, Coraciiformes, Cariamiformes, Falconiformes, Psittaciformes, Passeriformes. The results of this work also demonstrated that chromosomal rearrangements were recurrent within the groups and constitute important phylogenetic characteristic for birds.

Key-words: Chromosome mapping; Genome assembly; Neognathae ; Paleognathae ; Evolution

Acknowledgement

Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq/Brazil.

ID – 144

DESCRIPTION OF THREE KARYOTYPE VARIATIONS IN THE ENDANGERED SPECIES *MAZAMA NANA* (ARTIODACTYLA: CERVIDAE)

Valdir Nogueira Neto [1]; Agda Maria Bernegossi [1]; Raquel Muhlbeier Bonato [1]; Maria Dolores Astudillo Vallejo [1]; José Mauricio Barbanti Duarte [1]

[1]. Via de Acesso Professor Paulo Donato Castelane Castellane S/N - Vila Industrial. Deer Research Deer Research and Conservation Center (NUPECCE), School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP).

Abstract:

The Brazilian dwarf brocket (*Mazama nana*) is a small-sized cervid species endemic to the Atlantic Forest that inhabits the south of Brazil, Argentina, and Paraguay. The species is classified as "Vulnerable" by IUCN red list and is endangered mainly by habitat loss, hunting, and predation by dogs. It is one of the *Mazama* genus species diagnosed by classical cytogenetics presenting a chromosomal variation of its diploid number varying from 2n = 36 to 2n = 39, plus supernumerary chromosomes. However, these findings were limited to classical cytogenetic staining and it is still unknown the types of rearrangements associated with the chromosomal variation. Due to these limitations, we employed molecular cytogenetics technique, the Fluorescence *in situ* hybridization, to describe the origin of chromosomal changes in *M. nana*. We hybridized Bacterial Artificial Chromosome probes derived from *Bos taurus* genome, previously used in other Neotropical deer species, in three pygmy brocket individuals' metaphases: T221 and T353 with known locality, from Cascavel and Pitanga, respectively, localized in the state of Paraná - Brazil, and T242 from captivity with unknown origin. We observed that T221 and T242 possessed similar karyotypes. The both have 2n = 36, and presented ten pairs of submetacentric chromosomes, six pairs of acrocentric, a submetacentric X and a small submetacentric Y chromosome. Their karyotypes differ only by two centric fusions in heterozygosis evolving two chromosomes of the same pair corresponding to the q-arm, but two different chromosomes corresponding to the p-arm which resulted in two acrocentric matchless. Meanwhile, the chromosome homologous to one of the p-arm were acrocentric in homozygous in T221 and the other is fused in homozygous with the homologous to the q-arm. On other hand, the individual T353 from Pitanga presented a curious and divergent karyotype with 2n = 39, being nine pairs of submetacentric, one submetacentric chromosome in heterozygosis, nine pairs of acrocentric chromosomes plus sexual chromosomes. From the nine two-armed chromosomes only six were completely correspondent to one two-armed chromosome in T221 and T242 and the other three two-armed in addition to one large acrocentric were partially homologous to submetacentric chromosomes of the other two individuals. In summary, the chromosomal divergence between this individual and the other two corresponded to the presence of six centric fusions besides two tandem fusions. Even with all variations between these karyotypes, the fundamental number does not change. Surprisingly, wildlife individuals came from locations approximately 175 km straight line distance, being possibly the hypothetical match in nature. However, the costs of chromosomal rearrangements to reproduction or hybridization between these two divergent karyotypes are still unknown. These findings are of great importance considering the anthropic threats suffered by the species in an area of native forest that has been historically damaged.

Key-words: Cervid; Chromosomal variation; Fusions; Intraspecific polymorphisms; Rearrangements

Acknowledgement

We are grateful for funding from the National Council for Scientific and Technological Development - CNPq under process number 162942/2021-4.

ID – 169

PHYLOGEOGRAPHIC DISTRIBUTION PATTERN OF B CHROMOSOMES IN NATURAL POPULATIONS OF PARTAMONA HELLERI (HYMENOPTERA, APIDAE) IN THE BRAZILIAN ATLANTIC FOREST.

Vander Calmon Tosta [1]; Zulemara Boldrini Vignati Manhago [2]; Diana de Paula Machado [1]; Jamilly Carminati Scandian [1]; Julia de Souza Rocha [1]; Denilce Meneses Lopes [2]; Lucio Antonio de Oliveira Campos [2]; Samuel Resende Paiva [3]; Juan Pedro Martinez Camacho [4]

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Abstract:

This study was conducted in order to assess the patterns of distribution of B chromosomes in natural populations of bee *Partamona helleri* from Brazilian Atlantic Forest. To do the study were collected larvae, adults and entire colonies of *P. helleri* in four cities: Jaguaré, Governador Lindenberg, Alfredo Chaves and Venda Nova do Imigrante. To achieve the goal were performed cytogenetic analysis, SCAR marker analysis and Mitochondrial DNA analysis. Cytogenetic revealed the existence of seven types of B chromosomes in *P. helleri* populations from Atlantic Forest in Espirito Santo; of them two types are new in the literature. These new B chromosomes were found in Governador Lindemberg and Jaguaré populations which are located in northern of Espírito Santo. These populations also showed higher prevalence, load and frequency of B chromosomes comparing with populations in the southern state. Analysis using SCAR marker also detected higher prevalence values to populations in northern. Phylogenetic Analysis using *cyt B* clearly showed the separation between northern populations (Governador Lyndenberg and Jaguaré) from the south (Alfredo Chaves and Venda Nova do Imigrante) of Espirito Santo in different clades. These data are congruent with the theory from Del Lama et al. (2022) that show a phylogeographical origin of *P. helleri* in the state of Bahia 86000 year ago and the divergence of lineages at 56000 year ago. Probably, in this first central of dispersal area b chromosomes have arisen and diversified. Following a main north to south route this chromosomes loss diversity as expected in the model of Carnaval & Moritz (2008) from Pleistocene forest refuges in the central corridor of the Atlantic Forest. Probably the B chromosomes of *P. helleri* typical of southeastern Brazil (B1 and B2 - Brito 1998) were the only ones that remained before the isolation from some *P. helleri* populations in the central corridor of the Atlantic since they are present in the both populations of northern and southern.

Key-words: *Partamona helleri*; B Chromosomes; SCAR marker; Cyt B;

ID 179
ANÁLISE CITOGENÉTICA DO GÊNERO OXYMYCTERUS (CRICETIDAE: SIGMODONTINAE) DO MUNICÍPIO DE PALMAS (PR).

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Abstract:
A análise citogenética é fundamental para discernir as espécies de roedores, que devido a sua grande semelhança morfológica, necessitam da correta determinação cariotípica para caracterizar a espécie. Este estudo tem como objetivo a análise e caracterização citogenética de pequenos roedores coletados no estado do Paraná, Brasil, no município de Palmas. Na análise, buscou-se observar o cariótipo das amostras, com contagem do número total de cromossomos (2n), bem como número de braços dos cromossomos autossômicos (NA). Os animais estudados pertencem à tribo Akodontini, gênero Oxymycterus. Para o preparo de lâminas foi utilizando o material de medula óssea dos roedores, com preparação mitótica seguindo o método de Ford & Hamerton (1956), com modificações (SBALQUEIRO & NASCIMENTO, 1996), coradas com Giemsa. Foram analisadas no mínimo 10 metáfases ao microscópio, utilizando a objetiva de 10x para procurá-las e a objetiva de 100x para observá-las individualmente, realizando desenhos e contagens dos cromossomos. Também foi empregada a técnica de Bandeamento C, segundo protocolo proposto por SUMNER (1972). A análise dos protocolos LMT 579 e 580, coletados no Refúgio de Vida Silvestre dos Campos de Palmas, apresentaram o cariótipo 2n= 54 e NA= 64, sendo ambos machos (XY). As marcações de Banda C mostraram uma variação na marcação no braço curto do cromossomo X (submetacêntrico), de heterocromatina pericentromérica a heterocromatização total. Estes achados corroboram os descritos na literatura, e ampliam o registro de ocorrência do gênero Oxymycterus na região de Palmas, reforçando a manutenção desta área de preservação.
Key-words: Oxymycterus; citogenética; heterocromatina;

ID - 148

MICROCHROMOSOME ORGANIZATION IN WOODPECKER SPECIES USING BAC-FISH

Victoria Tura [1]; Suziane Alves Barcellos [1]; Marcelo Santos de Souza [1]; Fabiano Pimental Torres [2]; Rafael Kretschmer [3]; Ricardo José Gunski [2]; Analía Del Valle Garnero [2]

[1]. Rua Aluízio Barros Macedo, BR 290 - Km 423. São Gabriel, RS. Universidade Federal do Pampa; [2]. Rua Aluízio Barros Macedo, BR 290 - Km 423. São Gabriel, RS. Universidade Federal do Pampa; [3]. Rua Luís de Camões, 625. Pelotas, RS. Universidade Federal de Pelotas

Abstract:

The class Aves is the most diverse lineage of tetrapod vertebrates in existence, comprising approximately 10.900 species, divided into 40 orders. Piciformes represents an order composed of eight families with small to medium-sized individuals that inhabit arboreal areas. The family Picidae (woodpeckers) has peculiar cytogenetic characteristics when compared to other species of the class, showing the highest proportion of repetitive DNA among the bird genome. In addition, they show an unusual accumulation of repetitive sequences on the Z sex chromosome, and also a large variation in diploid number, ranging from 2n=64 to 2n=110. Despite its importance and diversity, genomic studies of the members of this family are scarce. Therefore, the objective of this work was to describe the patterns of microchromosome organization of two species of this family by fluorescence *in situ* hybridization (FISH) with bacterial artificial chromosome probes (BACs) of Gallus gallus microchromosomes. Two species belonging to the Picidae family were analyzed: *Veniliornis spilogaster* (1 female) and P*icumnus nebulosus* (1 male). The individuals were collected using mist nets in the municipalities of Porto Vera Cruz and São Gabriel - Rio Grande do Sul, Brazil. To obtain mitotic chromosomes, two different methods were applied: fibroblast culture and short term direct bone marrow culture. Subsequently, the slides were stained with Giemsa 5% and the 30 best metaphases were photographed and used to assemble the karyotypes. For chromosomal homologies, FISH analyses were performed using 36 clones of BACs for GGA10-28 chromosomes (except chromosome 16) and the slides were photographed using an epifluorescence microscope with an attached camera (Olympus BX61). The diploid number obtained was 80 chromosomes in *Veniliornis spilogaster* and 110 chromosomes in *Picumnus nebulosus*. Regarding the BACs probes, *V. spilogaster* showed 4 fusions between macro- and microchromosomes. The interchromosomal rearrangements occurred between GGA12 and the 2nd pair of macrochromosomes in this species, and after the fusion, an inversion occurred. GGA13 is fused to the 1st pair of macrochromosomes and GGA19 fused to a pair of macrochromosomes, which could not be identified due to the uniform karyotype of *V. spilogaster*. In addition to fusions between macro- and microchromosomes, this species also showed a fusion between GGA23 and a pair of microchromosomes. The results showed that, although the conservation of microchromosomes is observed in several bird lineages, this species showed interchromosomal rearrangements involving these elements. The species *V. spilogaster* showed a unique rearrangement never observed in birds by BAC-FISH, being a fusion between an ancestral microchromosome (GGA12) and a macrochromosome (VSP2), followed by an inversion. *P. nebulosus* did not show any type of rearrangement, suggesting that fission of ancestral macrochromosomes may have occurred due to the high number of microchromosomes. Thus, the results contributed to the expansion of studies in the area of avian cytogenomics, revealing that microchromosomes, despite being considered conserved, may be involved in chromosome rearrangements in different orders of birds.

Key-words: Rearrangements; BAC-FISH; Cytogenetic;

Acknowledgement

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ID - 125

CHARACTERIZATION AND DISTRIBUTION OF REPETITIVE SEQUENCES IN FURNARIIDAE SPECIES (AVES, PASSERIFORMES)

Vitor Oliveira de Rosso [1]; Victoria Tura [1]; Diego Madruga Saraiva [1]; Nairo Farias de Farias [1]; Marcelo de Bello Cioffi [2]; Analía Del Valle Garnero [1]

[1]. BR-290, 423 - Universitário, São Gabriel - RS, 97300-000. Universidade Federal do Pampa; [2]. Rod. Washington Luiz - Monjolinho, São Carlos - SP, 13565-905. Universidade Federal de São Carlos

Abstract:

The Furnariidae family (Ovenbirds) is represented by species of small birds that are abundant in tropical forests - from Mexico to South America - and known for their enormous variation in nest construction. However, few works have investigated these species under a chromosomal point of view. The majority of bird species that have been researched so far have 80 chromosomes, along with a few macrochromosomes and a large number of indistinguishable microchromosomes. Compared to other vertebrates, most birds have less repetitive DNAs and a compact genome that developed mostly as a result of lineage-specific erosion of repetitive elements, large segmental deletions, and gene loss. Therefore, the objective of this work was to describe the karyotypes and the distribution patterns of microsatellites and constitutive heterochromatin in three Furnaridae species, *Cranioleuca obsoleta*, *Syndactyla rufosuperciliata* and *Synallaxis frontalis*. The species *S. rufosuperciliata* and *C. obsoleta*, which were here reported for the first time, as well as *S. frontalis*, all possessed 82 chromosomes. Pairs 2, 3, 4 and 5 were submetacentric in all species, while pairs 1, 6, and 7 were acrocentric ones. In *S. rufosuperciliata* and *C. obsoleta*, the Z chromosome was submetacentric and acrocentric, respectively. C-positive heterochromatin regions were detected in all three ovenbird species' microchromosomes, and the W chromosome almost entirely heterochromatic. Microsatellite sequences accumulated mainly in microchromosomes, resulting in a scattered pattern overall. The probes (CAG)10, (CGG)10, (GA)15 and (CA)15, hybridized in all species. Whereas (GAG)10 produced scattered signals on the macros' telomeric regions, and in the centromeric region of the W chromosomes, (GAA)10 produced strong signals on just one pair of micros in *C. obsoleta*. The centromere and telomere regions of various macrochromosomes showed accumulations of microsatellites despite the presence of interstitial blocks of repetitive DNAs. A general conservation of the described karyotypes was demonstrated. A significant differentiation among these sequences was found via SSR chromosomal mapping. The fact that (GAG)10 and (GAA)10 were only mapped in *C. obsoleta* genome indicates to us that it probably represents the most derived species among the three Furnarids under study.

Key-words: Cytogenetic; Microsatellite; FISH;

Acknowledgement

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Conselho Nacional de Desenvolvimento Científico e Tecnológico (407285/2021-0) e Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação da Universidade Federal do Pampa (Apoio a Grupos de Pesquisa).

ID – 47

POPULATION CLASSICAL CYTOGENETIC ANALYSIS IN *PHYLLOMEDUSA BAHIANA* (ANURA, PHYLLOMEDUSIDAE)

Yuri Thomas Almeida Costa [1]; Tássia Nery Silva [1]; Juliana Zina Pereira Ramos [1]; Caroline Garcia [1]
[1]. Avenida José Moreira Sobrinho, s/n, Jequiezinho, Jequié-BA. CEP: 45208-091. Universidade Estadual do Sudoeste da Bahia

Abstract:
The genus *Phyllomedusa* Wagler, 1830, endemic to the Neotropical region, includes anurans also known as "leaf frogs" or "foliage tree frogs", and, together with seven other genera, make up the Phyllomedusidae family. Marked by great taxonomic complexity and biogeographical patterns, the genus has been subject of many studies of molecular systematics, however, little is known about the patterns of chromosomal evolution for these animals. The available data demonstrate a tendency towards maintenance of the diploid number (2n=26), occurrence of polyploidy and great variation in relation to the karyotypic formula and location of the NORs. In the present study, we carried out a classic population cytogenetic analysis (Giemsa, banda-C e Ag-RONs) in *P. bahiana* from eight municipalities of Bahia/BR (Brumado, Caetité, Castro Alves, Ilhéus, Jequié, Maracás and Valença). All specimens shown 2n=26 chromosomes, with a fundamental number of 52 and the same karyotypic formula (pairs 1, 4, 8, 11 and 13 metacentric; 2, 3, 5, 6, 10 and 12 submetacentric and pairs 7 and 9 subtelocentric) . NORs were located pericentrically in the long arms of pair 9 for all populations. C-banding revealed a small amount of heterochromatin located pericentrically in most of the complement chromosomes. The preliminary data we obtained differ from those described in the literature for the species, demonstrating that, in principle, there are two patterns of chromosomal organization for *P. bahiana* and that the differences between them are possibly due to pericentric inversions, emphasizing the importance of non-robertsonian rearrangements in group evolution.
Key-words: chromosomal evolution; karyotipic conservation; leaf-frogs; non-robertosonian rearrangements; pericentric inversions

Acknowledgement

We are greatful to UESB for the finacial support and Deivisson Fraga for helping during field work.

ID – 162

VARIATIONS ON MORPHOLOGY AND DISTRIBUTION OF B CHROMOSOMES IN PARTAMONA HELLERI (HYMENOPTERA, APIDAE) POPULATIONS FROM ESPÍRITO SANTO, STATE, BRAZIL.

Zulemara Boldrini Vignati Manhago [2]; Jamilly Carminati Scandian [1]; Julia de Souza Rocha [1]; Denilce Meneses Lopes [2]; Lucio Antonio de Oliveira Campos [2]; Vander Calmon Tosta [1]

[1]. São Mateus, ES, Brasil. Centro Universitário Norte do Espírito Santo, Universidade Federal do Espírito Santo; [2]. Viçosa, MG, Brazil. Universidade Federal de Viçosa

Abstract:

This work aimed to evaluate B chromosomes morphology and B chromosomes distribution in bee *Partamona helleri* populations from Espirito Santo state. To conduct the study were collected larvae, adults and entire colonies of *P. helleri* in four cities in the state: Jaguaré, Governador Lindenberg, Alfredo Chaves and Venda Nova do Imigrante. To achieve the goal were performed cytogenetic analyzes and SCAR marker analyzes. Cytogenetic analyzes revealed the existence of seven types of B chromosomes in P. helleri populations from Espirito Santo. Of the seven kinds found, two types of B chromosomes are new in the literature and were designated B13 and B14 according to the proposed encoding these chromosomes by MARTINS et al 2014. These new B chromosomes were found in Governador Lindemberg and Jaguaré populations which are located in northern of Espirito Santo. These populations also showed higher prevalence, load and frequency of B chromosomes comparing with populations in the southern state. Analyses using SCAR marker also detected higher prevalence values to populations in northern. Data of this study suggest a possible *P. helleri* B chromosomes origin center on populations from Bahia, where diversity and distribution of these chromosomes is greater. From Bahia populations, probably, these chromosomes have spread to populations of Espirito Santo and Minas Gerais where your number and distribution were gradually decrease. This study corroborate datas from Del Lama et al. 2022.

Key-words: *Partamona helleri*; Cytogenetic; B Chromosomes; SCAR marker;

Citogenética Humana

ID – 98

CYTOGENOMIC DELINEATION OF NOVEL REARRANGEMENT 7P AND 15P

Amanda da Silva Januário [1]; Beatriz Martins Wolff [1]; Alexandre Torchio Dias [2]; Lucas Liro Vieira [1]; Gleyson Francisco da Silva Carvalho [1]; Leslie Domenici Kulikowski [1]

[1]. . Hospital das Clínicas da Universidade de São Paulo; [2]. . Citogem - Cito Genética Molecular

Abstract:

Partial 7p duplication associated to deletion of another chromosome is a rare cytogenetic rearrangement making it difficult to define the precise contribution of the different specific genomic segments to the clinical phenotype. We report a cytogenomic study of a female patient with hypotonia, strabismus with microphthalmia, bifid uvula, high-arched palate, bilateral pre auricular pit, facial asymmetry, skeletal anomalies, delayed fontanelle closure and intellectual disability. G banding showed 46,XX,add(15p) results for the patient and normal karyotype for parents. Cytogenomic techniques were performed to investigate the rearrangement. Array analysis using BeadChip 850K (Illumina, CA) showed a gain of ~26,40 Mb in 7p (chr7:44,935-26,403,574) and MLPA confirmed the duplication of *TWIST1*(x3) and *TWISTNB*(x3) genes in 7p21.1. FISH elucidated the rearrangement by showing the additional material at 15p. Cytogenomic combined tools allows to unequivocally delineate the genomic rearrangement and leads to accurate genotype-phenotype correlation.

Key-words: 7p duplication; *TWIST* gene; delay of closure of fontanels;

ID – 100

INVESTIGATION OF THE GENOMIC STATUS OF METHYLATION IN COVID-19 USING ARRAYS

Lucas Liro Vieira [1]; Amanda da Silva Januário [1]; Gleyson Francisco da Silva Carvalho [1]; Beatriz Martins Wolff [1]; Leslie Domenici Kulikowski [1]

[1] Hospital das Clínicas da Universidade de São Paulo

Abstract:

Introduction: COVID-19 (Coronavirus Disease-2019) is a respiratory disease caused by (SARS-CoV-2) a novel single-stranded RNA virus of the Coronaviridae family. Although caused by the same virus, the disease can be milder or be associated with severe respiratory manifestations leading to acute respiratory distress syndrome and death. Additionally, elderly individuals and patients with pre-existing chronic illnesses are at an increased risk for COVID-19. Thus, the susceptibility and clinical variability observed in patients with this disease may not be limited only to variations in the genomic structure of individuals, it being possible that other mechanisms related to the activation or inactivation of promoters and/or exons of actively transcribed genes, such as DNA methylation, which occurs mainly in "CpG islands", are involved. Therefore, we propose to study the genome-wide methylation status profile of Brazilian patients with COVID-19 and verify the differentially methylated regions. Material and Methods: 30 samples of male patients with COVID-19, who did not have comorbidities and/or risk factors, and aged less than 50 years, were studied. Of these, 15 samples came from patients treated at the ward and classified as having mild symptoms and 15 samples from patients admitted to the Intensive Care Unit (ICU) with severe symptoms for COVID-19. The samples were submitted to the Infinium MethylationEPIC BeadChip experiment (Illumina, Inc., San Diego, CA). The data obtained were analyzed using the R programming language (Rstudio v4.0.2) following the pipeline proposed by Maksimovic et al. (2016), with modifications. Results and conclusion: With the data obtained, a differential analysis was performed (ICU x WARD). From a dissimilarity matrix, we observed a significant disparity in the methylation status of each group, generating approximately 25,000 differentially methylated regions (DMRs). In the analysis of ontological pathways, it was possible to visualize the main biological processes related to genes affected by DMRs, with inflammatory processes and defense pathways against bacterial infections being the most affected by DMRs. Our study was able to uniquely chart the global methylation status among patients with severe and mild COVID-19 which suggests that epigenetic modulations contribute to the course and progression of the disease.

Key-words: COVID; methylation; epigenectis;

ID – 172

EPIDEMIOLOGICAL PROFILE OF CYTOGENETIC ALTERATIONS OF IMPORTANCE IN MEDICAL GENETICS IN THE STATE OF RIO GRANDE DO NORTE.

Adriana Damasceno Pereira Pinto Cirne [1]; Erica Aires Gil [1]; Elias Guilherme Lino [1]; Patrícia Odila Macêdo Lopes [1]; Lidiane Soares de Medeiros [1]; Josinete Oliveira Domingos da Silva [1]

[1]. Avenida Alexandrino de Alencar, 1900, Tirol. Laboratório de Genética Humana - Centro de Reabilitação Infantil e Adulto

Abstract:

Chromosomal alterations, both numerical and structural, constitute the largest and most frequent categories of genetic diseases, and are significant and relevant causes of congenital malformations, part of reproductive failures, and mental retardation. Thus, this research aimed to survey cytogenetic analyses in 737 individuals referred to the Human Genetics Laboratory - LGH - CRI/CRA, from January 2015 to December 2021. Karyotyping was performed using GTG banding technique in cells obtained from peripheral blood lymphocyte culture. Data collection used the results of examinations and patients' anamnesis records. Findings pointed to 220 (30%) cases of altered karyotypes, 489 (66%) with normal patterns, and 28 (4%) inconclusive diagnoses due to various reasons. Down Syndrome was the most frequent numerical alteration with 120 confirmed cases and a percentage of 55%; followed by 20 cases with various inversions, accounting for 9%; trisomy 18 presented 15 (7%) cases, Turner Syndrome 11 (5%) cases, trisomy 13 had 8 (4%) cases, and the remaining cases were confirmed with various alterations. Finally, we point to the analysis pattern that, among the total cases analyzed, 29 (13%) presented as mosaics. These results are in accordance with the bibliographic data gathered, thus emphasizing the importance of cytogenetic analyses to assist mainly in the diagnoses of individuals with rare diseases, infertility, and recurrent fetal losses. Therefore, improvements in public policies in clinical and laboratory genetics are recommended to minimize problems related to genetic diseases, to plan actions, epidemiological records, and genetic counseling for patients and their families, providing better quality of life.

Key-words: *cytogenetic*; *medical genetics*; *genetic counseling*;

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ID – 145

NCRNAS-DERIVED PEPTIDES IN BREAST CANCER. EXPRESSION PATTERNS ACROSS SUBTYPES AND FUNCTIONAL INSIGHTS.

Alexandre Luiz Korte de Azevedo [1]; Daniela Fiori Gradia [1]; Jaqueline Carvalho de Oliveira [1]; Carolina Viante Rossi [1]; Fabricio Klerynton Marchini [2]; Talita Helen Bombardelli Gomig Lazarotto [1]; Michel Batista [2]; Enilze Maria de Souza Fonseca Ribeiro [1]

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Abstract:
Worldwide, cancer represents a severe health problem, threatening the life quality of millions of people and directly engendering multiple fatalities, also generating a substantial financial burden on the healthcare system. Among the cancer types, breast cancer stands out as the most frequent and lethal cancer among women. However, despite the clinical and financial relevance of cancer, the molecular mechanisms involved in the biology of tumorigenesis remain only partially understood. In recent years, the discovery that 'non-coding' RNAs (ncRNAs) could potentially codify functional small-peptides added a new layer of complexity to the cancer study. These peptides can directly influence biological processes relevant to tumorigenesis and have therapeutic potential in the context of peptide-based treatments; nevertheless, the novelty of this discovery means that the expression and function of these peptides are yet to be thoroughly validated. Here, we applied mass-spectrometry-based peptidomics to investigate the expression of ncRNAs-derived peptides in breast non-tumor and tumor samples representing the major breast cancer subtypes. The sequences of predicted ncRNA-derived peptides were obtained from the literature. The expression of its source transcripts was also investigated using the TCGA-BRCA dataset. Using online databases, such as MULocDeep, IMED, and PEPstrMOD, we advanced in understanding the peptide's functions by predicting its structure, cellular localization, and immunogenic potential. Fifty-four peptides were expressed on our samples, mainly derived from lncRNAs and circRNAs. Among them, 20 were over-expressed on tumor samples, and seven were down-expressed, with an expression pattern that varies among the breast cancer subtypes. Based on the peptide's sequence, we could predict that most peptides are exposed at the cell membrane or secreted, with some presenting immunogenic capacity. The peptide (PEP37) codified the lncRNA ENST00000581621, for example, was over-expressed in non-luminal tumor samples and potentially found on the cell's membrane, presenting an immunogenic potential linked to its AQLPHTGVFGQSFSC amino acid residue. The down-expressed peptide PEP52, codified by the lncRNA NONHSAT170898.1, was only expressed on non-tumor samples, being predicted to localize on the cytosol and to have immunogenic potential (KAEDSLLAAE residue). Altogether, these peptides' identification, expression analysis, and functional insights on breast cancer represent a step toward a better understanding of cancer complexity. These peptides present an expression pattern intrinsically associated with cancer and have traits that support a functional role in the molecular imbalance that results in tumorigenesis. In the clinical context, peptide-based vaccines and therapies have been explored as options for breast cancer patient management; thus, validating new small peptides expressed on the breast tissue originates a set of targets for future studies and applications. Nevertheless, functional validation and trials must be conducted to determine to further the exact mechanisms in which these peptides take part and their clinical relevance.

Key-words: ncRNAs; Breast cancer; peptides; subtypes; mass-spectrometry

Acknowledgement

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ID – 69

CHROMOSOMAL ANALYSIS IN ACUTE LEUKEMIA: PERSPECTIVE OF CHROMOSOMAL ABNORMALITIES AND FREQUENCY OF BLAST CELLS

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Abstract:

Acute leukemia (AL) is a heterogeneous group of neoplastic hematopoietic diseases caused by malignant transformation of hematopoietic stem cells. The leukemic blasts enter in uncontrolled proliferation and the accumulation in the bone marrow and others organs starts the symptoms of the disease. The diagnosis and classification of AL is based on the concentration of blasts (greater than or equal to 20%), the morphology, characterization of cellular markers and cytogenetic from bone marrow aspirate. The chromosomal analysis provides important prognostic information. Based on this, a retrospective study was carried out in medical records from the years 2018 to 2022 with the aim of correlating the blasts concentration with chromosomal anomalies in diagnostics ALs at Techlife's Cytogenetics Laboratory. Total of 255 ALs were diagnosed in bone marrow (n=231) and blood (n=24) samples; 154 (60%) cases had altered karyotype, 74 (29%) cases had normal karyotype and 27 (11%) had no cell culture growth and in each group the average concentration of blasts was 69%, 70% and 73%, respectively. The myeloid lineage had the higher incidence being 147 of LAs with an average of 62% of blasts. The t(15;17) was the most frequent abnormality (11%). Considering the lymphoid lineage, it was 101 ALs cases with 80% of blasts at the diagnostic sample with Philadelphia chromosome present in 5% of the cases; only 7 cases were from acute leukemia of mixed phenotype with average presence of 80% blasts. Altered karyotypes were observed in 51% of cases with simple or complex rearrangements in 21% and 30% of the cases, respectively, and hyperdiploid and hypodiploid karyotypes in 21% and 12% of the cases, respectively with average of 67% of blasts. Satisfactory cell growth was hit in 90% of the diagnosed LAs with 69% blasts in average in the cultured samples. From the data obtained in cases of cell culture failure it was observed that the blastic cellularity is similar to cases with normal and altered karyotype results. One of the factors identified as responsible is the difference between the microenvironment in vivo and in vitro, which can cause interruption of the flow of the cell cycle. These cells may exit the G1 phase and enter a resting state called G0, so cells do no undergo mitosis. The observation of blast cellularity in the sample provides greater success in cell culture, as the excess of blasts increases intercellular communication and interferes with important factors within the culture medium causing saturation and cell apoptosis with failure in the capture of potential malignant clones. With this data we observed the same average blasts concentration in abnormal and normal karyotype however we confirm the importance of karyotype analysis for accurate diagnosis the ALs since we observed abnormal karyotype in 60% of samples.

Key-words: Acute leukemia; blasts cells; karyotype;

ID – 70

RELATIONSHIP BETWEEN MTHFR C677T AND A1298C POLYMORPHISMS AND OBESITY IN BRAZILIAN POPULATION.

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Abstract:

The *MTHFR* gene plays a critical role in the human body's metabolism, specifically in the process of converting homocysteine into methionine. The *MTHFR* gene has two common variations, known as polymorphisms, which are the C677T and A1298C mutations. The C677T mutation results in a change in the enzyme's shape, which can decrease the enzyme's activity, leading to increased levels of homocysteine in the blood. Elevated homocysteine levels have been associated with an increased risk of cardiovascular disease, stroke, and other health conditions. The A1298C mutation is less common than the C677T mutation and has a milder effect on MTHFR enzyme activity. It has been associated with a slightly increased risk of neural tube defects and other health issues. This study aims to investigate the prevalence of C677T and A1298C mutations and its relationship with the body mass index (BMI) in Brazilian individuals. A total of 380 individuals were included in this study, 187 with normal weight (NW) and 193 with overweight/obesity (OW/OB). Genotyping for C677T and A1298C mutation was performed by next-generation sequencing panel (Illumina). From the 380 individuals analyzed, 321 presents at least one mutation of the *MTHFR* gene: with 154 patients carrying the C677T mutation, 99 carrying the A1298C mutation, and 68 carrying both. When comparing the genotyping of individuals with NW with OW/OB, we observed a significant difference with C677T mutation more prevalent in patients with normal weight (123 NW x 99 OW/OB; $p<0,05$) and A1298C mutation more prevalent in individuals with overweight/obesity (72 NW x 95 OW/OB; $p<0,05$). These results corroborate studies that found the A1298C mutation is less common than the C677T mutation and the fact that the C677T mutation is not related to obesity. Our results also suggest that the *MTHFR* A1298C mutation may be a risk factor for overweight/obesity.

Key-words: MTHFR polymorphism; obesity; next-generation sequencing;

ID – 71

A PAN-CANCER ANALYSIS OF TRANSCRIBED ULTRACONSERVED REGIONS AS PROGNOSTIC BIOMARKERS

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Abstract:
The human genome has 481 ultraconserved regions (UCRs), and its sequences are 100% identical between humans, mice, and rats, with also high conservation in other species. They are distributed in the genome in all chromosomes except for the 21 and Y. The majority of those regions are transcribed in human tissues (T-UCRs). Since its description as being a potential new class of long non-coding RNAs (lncRNAs), those regions are studied in the context of cancer and other human diseases. However, no work investigated the expression pattern and the prognostic value of T-UCRs in a pan-cancer scenario. Therefore, we aimed to explore if T-UCRs could be used as prognostic biomarkers in cancer. For this, TCGA data extracted from the TANRIC platform (data from 20 tumors) was, and survival analysis was performed on different tumor types. Our analysis verified that many UCRs are expressed in the 20 tissues analyzed in this study. Overall, we can observe minor UCR-expressing tumors such as colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), and uterine corpus endometrial carcinoma (UCEC). The other tissues had approximately 300 UCRs being expressed. In addition, our research verified that 100 and 102 T-UCRs were related to disease-specific survival (DSS) and progression-free interval (PFI), respectively. Those T-UCRs are particularly important in kidney renal clear cell carcinoma (KIRC) and low-grade gliomas (LGG) survival outcomes. In addition, we verified that uc.44, uc.48, uc.135, uc.144, uc.153, uc.217, uc.255, uc.256, uc.344, uc.357, uc.390, uc.427 and uc.436 were associated with survival of more than one tumor. Our results demonstrate the potential application of T-UCRs as predictive biomarkers and shed light on the possibility of studying the mechanisms and specific roles of the highlighted T-UCRs in cancer.
Key-words: T-UCRs; Pan-cancer; Prognostic Biomarkers; TCGA; LncRNAs

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ID – 30

REGULATION OF *CRLF2* OVEREXPRESSION IN T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Abstract:

There are many molecular alterations identified in T-cell acute lymphoblastic leukaemia (T-ALL), however none of them are currently used as criteria for risk stratification in the current protocols. The dysregulation leading activation of CRLF2, a gene located on pseudoautosomal 1 (PAR1) region in X and Y chromosomes, is associated with a poor glucocorticoid response and a worse event-free survival in T-ALL. Recently, we demonstrated that CRLF2 overexpression associates with mutations leading to the NOTCH1 intracellular protein stabilization, however the mechanisms underlying CRLF2 deregulation in T-ALL remains unknown. As a potential poor prognosis biomarker in T-ALL and also a candidate for targeted therapy, we aimed to evaluate the molecular profile of CRLF2 overexpressed patients and delineate the mechanisms leading to this gene dysregulation. We accessed omic data available online from 264 patients (Therapeutically Applicable Research to Generate Effective Treatments) and T-ALL cell lines (Cancer Cell Line Encyclopedia and Gene Expression Omnibus). Initially, we categorized the samples according to CRLF2 expression levels in CRLF2-high and low subgroups. Subsequently, we observed an enrichment of early T-cell precursor ALL (ETP-ALL) samples in the CRLF2-high subgroup (23.08% vs 4.02%, p = 7.579e-06) and these entities showed similarities in their transcriptional activation profile. Also, EZH2 mutations were associated with higher CRLF2 levels (p = 0.0170). EZH2, the catalytic subunit of Polycomb Repressive Complex 2 (PRC2), is responsible for silencing the transcription factors (TFs) involved in early stages of T-cell development and its loss is a initial event in the ETP leukemogenesis. So, we accessed RNA-seq data available in GEO obtained from the modulation of PRC2 function in T-ALL cell lines. Here, we demonstrated that CRLF2 expression in LOUCY, the ETP-ALL cell line with the highest CRLF2 transcriptional level, was completely reduced after the restoration of PRC2 function (p = 0.0095). However, in the JURKAT cell (mature T-ALL subtype), the EZH2 silencing did not upregulate CRLF2 expression. So, using ChIP-seq data, we observed active enhancer regions in LOUCY and identified 104 TFs that recognize these regions. We filtered those TFs according to the association with ETP-ALL and the CRLF2-high subgroup and selected the MEF2C gene (5q14.3) as a potential regulator of this dysregulation in T-ALL. We performed a qChIP analysis and showed MEF2C binding capacity in motifs located on CRLF2 enhancer. In the present study, we demonstrated that CRLF2 is an oncogene associated with the ETP-ALL profile and its dysregulation may be drived by PRC loss in this immature leukaemia subset. However, this mechanism alone is not capable of leading to this gene upregulation in mature T-ALL, suggesting that a TF activation, as MEF2C, or the presence of molecular alterations in CRLF2 regulatory elements may be a crucial trigger for this scenario.

Key-words: T-cell acute leukemia; CRLF2; gene expression;

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ID – 90

NEXT GENERATION CYTOGENETICS: OPTICAL GENOME MAPPING VALIDATION IN BRAZIL

André Rodrigueiro Clavisio Pereira de Oliveira [1]; Bruna Burssed [2]; Maria Isabel Melaragno [2]; Ana Cristina Victorino Krepischi [3]; Carla Rosenberg [3]; Juliana Forte Mazzeu de Araújo [4]; Gabriel Joelsons [1]

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Abstract:

Rare diseases affect 65 in every 100000 individuals and 80% of about 7000 cataloged diseases are caused by gene and structural variations (SVs). SVs are characterized by microscopical and sub microscopical inversions, insertions, deletions, duplications, and translocations, that may result in gene disruption, loss of regulatory genetic elements, and gene dosage alterations, which are important factors for the development and maintenance of the organism of individuals. These abnormalities can be visualized by classic and molecular cytogenetics, and cytogenomics analyses, like karyotype (5 to 10 Mbp), FISH, aCGH/CMA (50 kbp), and Optical Genome Mapping (OGM) (500 bp). OGM is a new methodology with an increased resolution for the detection of all sorts of SVs that can bring light to undiagnosed cases and opens a new world to the discovery of the etiology of rare diseases. The aim of this study was to compare OGM results with gold standard cytogenetics techniques (karyotype, CMA, aCGH). Peripheral blood samples were collected from 3 patients in renowned research institutes in Brazil. The results of gold standard cytogenetics methods were collected from researchers and compared to the new technology results. Ultra-high molecular weight DNA was isolated from samples using Bionano Prep SP Blood and Cell DNA isolation kit v2 (Bionano Genomics, San Diego, CA, USA), according to manufacturer's instructions. Direct label and stain was performed using Bionano Prep DLS Labeling kit (Bionano Genomics, San Diego, CA, USA) following instructions, and the labeled DNA was loaded to a Saphyr Chip (Bionano Genomics, San Diego, CA, USA) in the instrument Saphyr to collect data. De novo assembly pipeline was run in all 3 samples to assess the results in comparison to genome reference consortium GRCh37 and GRCh38, and T2T reference. Sample 1 showed a ring chromosome 3 after manual inspection of T2T de novo assembly and a copy number loss on the short arm of the same chromosome, in concordance with aCGH and karyotype. On Sample 2, karyotype revealed a t(9;20)(q22;q13.3) and 9qh+, OGM located the translocation involving both long arms of chromosomes 9 and 20, but it was not able to detect constitutive heterochromatin event, due to its repetitive sequence. Sample 3 findings of aCGH were not confirmed by OGM, since a new deletion between duplications found on 9p24.3 might have confused the algorithm, but after manual inspection, it was possible to locate two regions of duplication in 9p24.3 and a new insertion of a fragment from 9p24.2, revealing a complex rearrangement. OGM technique revealed new findings on the samples, and showed concordance with results as well as its limitations. Manual inspections are important to locate features not detected by the algorithm, which demands the expertise of the analyst. This is preliminary data of an initial study of validation, and this technology shows promising to unravel SVs and the etiology of rare diseases.

Key-words: Cytogenomics; Genome Mapping; Rare Disease; OGM;

ID – 92

OPTICAL GENOME MAPPING REVEALS NEW FINDINGS OF CHROMOSOMAL ABNORMALITIES ON HEMATOLOGICAL DISEASE

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Abstract:

Hematological diseases result from clones induced by genetic and structural variations (SVs) that can lead to cell immortalization, uncontrolled proliferation, and growth, ultimately causing illness and death. Various techniques, including cytogenetics, cytogenomics, and molecular biology, are used to identify SVs for the diagnosis, prognosis, treatment, and monitoring of hematological diseases. Classical and molecular cytogenetics are commonly used for SVs detection, but they have limitations such as low resolution (5 to 10 Mbp), low cell culture growth, limited quantity and quality of metaphases, and the need for specific probes, among others, which can compromise their performance. Cytogenomics techniques, on the other hand, can facilitate the detection of clones and SVs, thereby improving diagnosis, prognosis, and treatment options. One such cytogenomics technique is Optical Genome Mapping (OGM), which can detect DNA gains, losses, and other types of rearrangements, whether balanced or not, such as insertions, inversions, and translocations, at the molecular level. In this study, we aimed to assess and compare the results of karyotyping with OGM using a blood sample from a patient with Sezary's syndrome, a cutaneous lymphoma. DNA isolation, direct labeling, staining, and chip loading on Saphyr (Bionano Genomics, San Diego, CA, USA) were performed following the manufacturer's instructions, and the Rare Variant Pipeline was run on the data to assess all clonal SVs in the sample. Karyotyping revealed a composite clone with 43 to 45 chromosomes, with long arm deletions on chromosomes 1, 2, 4, and 13, pericentric inversion on chromosome 6, additional material in the short arm of chromosomes 8, 9, and 17, and in the long arm of chromosomes 10 and 11. Monosomy of chromosomes 9 and 10, as well as the presence of a marker and a ring marker, were also detected. OGM, on the other hand, detected chromoplexy involving chromosomes 8, 9, 10, 11, and 17, pericentric inversion of chromosome 6, a translocation between chromosomes 1 and 13, aneuploidy gain of chromosome 8, and loss of chromosomes 9 and 10, but no deletion on chromosome 2. These results suggest that OGM may be a more resolving technique, providing insights into translocations and additional material present in chromosomes that may not be well understood through karyotyping alone. Furthermore, OGM does not require cell culture and is a time-saving methodology that could potentially improve the diagnosis, prognosis, and treatment decisions for hematological diseases. Additionally, the origin of chromosome markers could be better understood through the rearrangements detected by OGM in the sample. In conclusion, OGM may offer advantages over traditional karyotyping, providing higher resolution and more comprehensive insights into SVs in hematological diseases, without the need for cell culture, and saving time in the diagnostic process. Further studies and validation of OGM are warranted to establish its clinical utility in hematological disease management.

Key-words: Genome mapping; Cytogenomics; Karyotype; Lymphoma;

ID -31

CONVENTIONAL AND MOLECULAR CYTOGENETICS REVEALED RARE CHROMOSOMAL ABNORMALITIES IN MYELODYSPLASTIC NEOPLASM: THE CHROMOSOMAL TRANSLOCATIONS

Beatriz Ferreira da Silva [1]; Gabriela Farias Lima [1]; Eliane Ferreira Rodrigues [1]; Rayssa Pereira de Sousa [1]; Luize Otero [1]; Viviane Lamim Lovatel [1]; Teresa de Souza Fernandez [1]

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Abstract:

INTRODUCTION: Cytogenetics is a critical tool in the diagnosis, classification, and risk stratification of many hematological malignancies, such as the myelodysplastic neoplasm (MDS). MDS is a hematopoietic stem cell disease characterized by bone marrow dysplasias that impairs the normal hematopoietic process which, in turn, may lead to peripheral blood cytopenias. In addition to that, all patients with this neoplasm have an intrinsic risk of leukemic transformation to acute myeloid leukemia (AML), which occurs in around 30% of the cases. While MDS is a disease commonly observed in adults, it is rarely observed in pediatric patients. Cytogenetic abnormalities can be detected in approximately 50% of MDS patients. MDS is characterized cytogenetically by a partial or total chromosomal loss (deletion and monosomy), and a gain (trisomy). The profile of these abnormalities is very homogeneous, mostly being unbalanced with a predominance of partial or total chromosomal loss such as seen in deletions and monosomies, and less frequently a gain in genetic material such as seen in trisomies. Whereas chromosomal translocations are uncommon in MDS, being more frequently observed in leukemia patients. Objective: The aim of this study was to describe the frequency of chromosomal translocations observed in adult and pediatric patients with MDS and its association with subtypes and disease progression. MATERIALS AND METHODS: Bone marrow cells of 318 adult patients and 200 pediatric patients with MDS were clinically and cytogenetically analyzed. The analyses were performed by G-banding and fluorescence in situ hybridization (FISH). The chromosome abnormalities were described according to the International System of Human Cytogenomic Nomenclature (2020). The patients were classified according to the classification proposed by WHO 2022. RESULTS: Chromosomal translocations were observed in 3.7% of adult patients (12/318) and 1.5% of pediatric patients (3/200). Among the adult patients with translocations, only 25% of them had chromosomal translocations as a single abnormality (3/12), and 25% had a translocation associated with another abnormality, such as deletion of the long arm of chromosome 5 [del(5q)] (2/3) and trisomy of 21 (+21) (1/3), the remaining patients (50%) had translocations as a part of complex karyotypes. In pediatric patients, all translocations were seen as a single abnormality. Most patients with chromosomal translocations were classified into the advanced subgroups of MDS, being 83% of adult patients and 100% of pediatric patients. Regarding the progression of the disease, 75% and 100% of adult and pediatric patients respectively progressed to AML and/or died. CONCLUSION: This study suggests that although rare, chromosomal translocations in MDS are associated with a more advanced subgroup and an adverse prognosis.

Key-words: Myelodysplastic neoplasm; Chromosomal translocations; Leukemic evolution;

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ID – 156

CHROMOSOMAL ABNORMALITIES IN ADULT PATIENTS WITH MYELODYSPLATIC NEOPLASM: CLINICAL IMPLICATIONS

Beatriz Ferreira da Silva [1]; Gabriela Farias Lima [1]; Rayssa Pereira de Sousa [1]; Eliane Ferreira Rodrigues [1]; Luize Otero [1]; Viviane Lamim Lovatel [1]; Teresa de Souza Fernandez [1]

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Abstract:

Introduction: Myelodysplastic neoplasm (MDS) is one of the most common hematologic malignancies in adults, especially in patients above 65 years old. Patients with this group of clonal bone marrow diseases often have symptoms associated with peripheral blood cytopenias such as anemia, neutropenia, and thrombocytopenia. MDS is characterized by dysplasia in the bone marrow and a clinical heterogeneity marked by a variable survival, and approximately 30% risk of evolution to AML. Several studies have demonstrated that cytogenetics plays a relevant role in the clinical management of MDS, aiding in the diagnosis, classification, and prognostic risk stratification as the Revised International Prognostic Scoring System (IPSS-R). However, the prognostic value of some alterations is still controversial, such as trisomy 8 (+8). According to the IPSS-R +8 is classified as an intermediate risk group, although some studies have shown this abnormality to be associated with progression to AML and shorter survival. Objective: The aim of this study was to analyze the frequency of chromosomal abnormalities in a cohort of adult patients with MDS and its association with MDS subgroups, and evolution to AML. Materials and methods: Bone marrow cells of 318 adult patients with MDS were clinically and cytogenetically analyzed between 2000 and 2022. The cytogenetic analyses were performed by G-banding and fluorescence *in situ* hybridization (FISH). The chromosome abnormalities were described according to the International System of Human Cytogenomic Nomenclature (2020). The patients were classified according to the classification proposed by WHO 2022. Results: Abnormal karyotypes were observed in 37.1% (118/318) of patients. The most common cytogenetic alteration was complex karyotype present in (21.1%) (25/118) of the patients, followed by deletion of the long arm of chromosome 5 [del(5q)] (16.1%) (19/118) and trisomy 8 (+8) (12.7%) (15/118). Among the patients presenting abnormal karyotypes, 42.4% were classified into advanced subgroups (42.4%). The chromosomal alterations most associated with these subgroups were complex karyotypes, trisomy 8, and alterations on chromosome 7 [-7/del(7q)]. Concerning the outcome of these patients, the most common cytogenetics alterations associated with progression to AML or death were complex karyotypes, deletion of the short arm of chromosome 17 [del(17p)], and trisomy 8. Conclusion: This study reinforces the predictive value of specific chromosomal alterations and suggests that +8 might be associated with an adverse prognosis.

Key-words: Myelodysplatic neoplasm; Leukemic evolution; Abnormal karyotypes;

ID – 97

METHYLATION ARRAYS APPLIED TO IMPROVE THE CLASSIFICATION OF CNS TUMORS

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Abstract:
The correct classification of central nervous system (CNS) tumors is a challenge due to the wide variety of entities, which makes standardization difficult and leads to misleading decisions in clinical practice. In this sense, the World Health Organization (WHO) establishes criteria for classifying CNS tumors and recommends an integrated diagnosis, using clinical, morphological and molecular aspects, including the cytogenomic methodology of the array methylation profile for accurate detection of tumor subtypes. Our aim was to improve the classification of CNS tumors using methylation matrices. For this, eight samples fixed in formalin and embedded in paraffin (FFPE) of CNS tumors from the AC Camargo Cancer Center were used. We performed the methylation matrix assay using Infinium HumanMethylation450 and Infinium MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA). The data obtained were submitted to the Molecular Neuropathology platform (available at MolecularNeuropathology.org), which uses a machine learning algorithm to classify CNS tumors based on their methylation profile and Copy Number Variation (CNV) from raw data of the array assay. Interpretation was performed as proposed by Capper et al. (2018) evaluating a combination of entities and tumor classes. The results showed 4 cases concordant with the previous analyses, one case remained without specific classification and 3 cases could be better delineated. We were able to establishing a new classification and a conclusion diagnosis for 3 samples: High Grade Astrocytoma with Piloid Characteristics; Astrocytoma, IDH-mutant, grade 4; and Pediatric High-Grade Gliomas, RTK2, sub B, respectively. Our experience in using methylation arrays for tumor classification showed effective results to improve the analysis of CNS tumor entities, helping in challenging cases and facilitating diagnosis and treatment for patients.
Key-words: Methylation; Epigenomics; Cancer; array;

ID -101

REANALYSIS OF VARIANTS OF UNCERTAIN SIGNIFICANCE: COMPARISON BETWEEN RECOMMENDATIONS OF THE AMERICAN COLLEGE OF MEDICAL GENETICS AND THE ABC SYSTEM

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Abstract:

Copy number variations (CNV) are an important source of genetic variation and can be the cause of syndromes or disorders such as Intellectual Disability (ID) and Multiple Congenital Anomalies (MCA). Despite the methodological evolution for variant detection, the classification of variants remains a challenge. Most of the variants of uncertain significance (VUS) are rare CNVs, not present in general population databases, with no causal relationship with a clinical phenotype, which cannot be classified as benign or pathogenic. The purpose of this study was to reanalyze CNVs of uncertain significance in individuals with intellectual disability (ID)/ neurodevelopmental delay (NDD) and (or) multiple congenital anomalies (MCA) by comparing two different variant classification systems: the ACMG (American College of Medical Genetics) classification recommendations for CNVs and the ABC system for variant classification. The sample consisted of 100 subjects with ID/NDD and (or) MCA with CNVs previously classified as VUS. First, the variant reanalysis was performed using the ClinGen CNV Pathogenicity Calculator tool, according to the new recommendations of the ACMG for CNV classification. After internal validation of this tool, CNVs previously classified as VUS were reanalyzed. Second, a reanalysis using the ABC system for variant classification was performed. Among 114 variants, from 100 subjects, initially classified as VUS, 8 (7%) were reclassified after applying the ACMG recommendations: one was reclassified as benign, three as likely pathogenic, and four as pathogenic. The variant explained the patient's phenotype for only one subject with a CNV reclassified as pathogenic. In the other subjects, although the CNV was reclassified, it was not considered causative. According to the ABC system, 11 CNVs were said to be "somehow pathogenic", four of which were from "D" and "C" categories (variants that could explain the phenotype) and seven were classified as "incidental findings" ("X" category, not related to the phenotype, therefore not necessarily reported), 16 classified as "variants-of-interest" ("E" category, optionally reported), nine as "clinical VUS" ("F" category) and 77 as "functional VUS" ("0" category) both recommended not to be reported. The reanalysis of CNVs VUS allowed reclassification in 7% of cases according to ACMG recommendations. However, in only one subject it allowed diagnostic conclusion. The ABC system allowed a more comprehensive analysis, acting as a complementary resource to support variant classification and report.

Key-words: Copy number variations; Variants of unknown significance; Reclassification of variants;

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ID – 157

STUDY OF THE RELATIONSHIP BETWEEN THE VARIANT DUP 4Q26 AND AUTISM SPECTRUM DISORDER

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Abstract:

Autistic Spectrum Disorder (ASD) refers to a neuronal condition, caused by a disorder in neurodevelopment. With hypersensitivity, repetitive behaviors and other symptoms, this condition has both genetic and environmental causes. The etiology is still not fully known, but it is known that there are chromosomal abnormalities (2%), monogenic syndromes (5%), microduplications and microdeletions (10%), environmental (3%), multifactorial and epigenetic (80%). In ASD there is no single autism gene, but it is difficult to predict the number of genetic regions, chromosomes or loci that contribute to the development of the disorder. In 2004, many authors studying the first genome wide screening for chromosomal regions involved in classical autism reported over 354 genetic markers for this disorder. In this context, techniques emerged for identifying these genes for the elucidation and diagnosis of ASD, among others - the Array. Revolutionary high-resolution technique capable of investigating thousands of chromosomal regions, detecting losses and add in a single exam. With this method it is possible to detect CNVs and SNPs (variations in the number of copies and polymorphism of a nucleotide), uniparental disomy and loss of heterozygosity. Objective: With this technique, the authors of this study aimed to associate a dup 4q26 variant found with ASD and analyze its classification as a variant of uncertain meaning. Method: Genetic data from CGH-ARRAY tests performed from June/2018 to December/2022 were analyzed in a large laboratory in São Paulo- Brazil. 149 patients with variants of uncertain and/or pathogenic significance were selected. Of these, 38 cases were under investigation for ASD, characterized by the diagnostic hypothesis. The authors found 49 variants. Of these, 6.12% were detected on chromosome 1, 4.08% on chromosome 2, 4.08% on chromosome 3, 14.28% on chromosome 4, 4.08% on chromosome 5, 6.12% on chromosome 7, 2.04% on chromosome 8, 6.12% on chromosome 9, 6.12% on chromosome 10, 4.08% on chromosome 11, 2.04% on chromosome 13, 4.08% on chromosome 15, 6.12% on chromosome 16, 6.12% on chromosome 18, 2.04% on chromosome 20, 12.24% on X chromosome and 10.02% on Y chromosome. Results: The study showed that chromosome 4 had the highest number of detected variants. Therefore, we carefully analyzed all cases and 2 patients, who did not have consanguinity, had the 4q26 duplication, classified as VOUS (unclear meaning). This duplication is associated with genes TRAM1L1, LINC01378, NDST3, SNHG8, SNORA24, PRSS12, CEP170P1, LOC729218, LOC101929741, METTL14, SEC24D, SYNPO2, MYOZ2, LOC101929762, USP53, C4orf3, FABP2, LINC01061, GTF2IP12, LOC645513, PDE5A, LINC01365, LOC100996694. Of these genes, only the PRSS12 gene correlated with autism. PRSS12 (Serine Protease 12) encodes a protein secreted by neuronal cells, located in the synaptic cleft and is associated with cognitive impairment. Conclusion: The two cases studied had global delay in development with a suggestion, after medical evaluation, of ASD. The findings in the genetic exam did not show other alterations that justify the cognitive delay and the hypothesis of autism only the duplication of the PRSS12 gene. Thus, it is possible that this variant is associated with the observed phenotype and that it could be studied and reclassified as a probably pathogenic variant.

Key-words: Autism Spectrum Disorder; TEA; CGH-ARRAY;

ID – 157

OPTICAL GENOME MAPPING REVEALS A COMPLEX REARRANGEMENT INVOLVING FOUR CHROMOSOMES IN A PATIENT WITH SEVERAL PHENOTYPICAL ALTERATIONS

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Abstract:
Complex chromosomal rearrangements are defined as structural genome variations that involve at least three breakpoints in one or more chromosomes and result in exchanges of chromosomal segments. These rearrangements may be *de novo* or inherited from a parent. The patients' phenotypes may vary depending on different factors, such as genomic imbalance, gene disruption, and position effect. Optical genome mapping (OGM) is a new high-resolution technique that can detect structural chromosome alterations. We present a case report of a 4-year-old female patient with neuropsychomotor developmental delay, hypotonia, and speech delay, which was studied in search for genetic alterations that could explain the phenotype. Karyotype analysis showed a possible alteration on chromosome 12, leading to the following unclear result: 46,XX,del(12q) or 46,XX,der(12). Array analysis revealed a copy number variation (CNV) in chromosome 4q. In order to investigate genetic alterations that could explain the derivative chromosome not precisely described by karyotype and chromosome array, we performed optical genome mapping. OGM revealed a truly complex rearrangement: a three-way balanced translocation between chromosomes 12, 14, and 21, with an inversion of a chromosome 12 region, besides the 2.3 Mb chromosome 4 deletion. This was the first patient to be submitted to the OGM technique performed in Brazil. The use of a high-resolution technique such as optical genome mapping allowed for the resolution of the patient's balanced complex rearrangement, which could not have been predicted and detected by the previous techniques. The complete determination of the rearrangement, which can be achieved by sequencing the breakpoints and inferring the mechanism of formation, allows for a better correlation between the genotype and the phenotype of the patient. Financial support: São Paulo Research Foundation (FAPESP), Brazil.
Key-words: Optical Genome Mapping; Complex chromosomal rearrangement; Balanced translocation;

ID – 80

GENOMIC IMBALANCES IN INDIVIDUALS WITH "BALANCED CHROMOSOMAL" REARRANGEMENTS AND ABNORMAL PHENOTYPE

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Abstract:
Individuals with apparently balanced chromosomal rearrangements and abnormal phenotypes may present submicroscopic genomic imbalances at the breakpoints. Previous studies performing chromosomal microarray analysis identified imbalances in about 30% of cases. However, most of the studies included only individuals with *de novo* rearrangements. The aims of this study were to investigate genomic imbalances in a cohort of individuals carrying balanced chromosomal rearrangements presenting with abnormal phenotype and to review the literature, comparing cases with *de novo* and inherited chromosomal rearrangements. Thirteen individuals with apparently balanced chromosomal rearrangement - seven translocations, four inversions, and two complex chromosomal rearrangements (CCRs) - and phenotypic alterations, including intellectual disability and congenital anomalies, were included in this study. In six individuals the rearrangement was *de novo*, in four individuals it was inherited and three are still under investigation. Chromosomal microarray analysis (CMA) was performed using the CytoScan 750K Array platform and the Chromosome Analysis Suite program (Affymetrix®) was used for analyses; the classification of the variants was carried out according to the recommendations of the American College of Medical Genetics (ACMG). The literature review included searching for articles in PubMed and only studies which investigated genomic imbalances, through CMA, in individuals with balanced chromosomal rearrangements and abnormal phenotypes were evaluated. Among 13 individuals in the present study, pathogenic genomic imbalances (PGI) were identified in four (4/13 - 31%): one inherited translocation with a PGI on another genomic region, one *de novo* inversion with a PGI at the rearrangement breakpoint, two CCR carrying PGI at the rearrangement breakpoints and other genomic regions, one of them *de novo* and the other one without fathers' investigation. The literature review showed genomic imbalances in 38% (107/281) of the cases, with 63 translocations, 15 inversions, two insertions, and 27 CCRs. Among *de novo* cases, 43% (99/232) presented imbalances: 43 were found at the balanced rearrangement breakpoints, 42 on other genomic regions, 14 at the breakpoints, and also on other genomic regions. Among inherited cases, genomic imbalances were found in 19% (8/42) of cases: three of which were found at the breakpoints and five on other genomic regions. Our results support that about 30-40% of apparently balanced chromosomal rearrangements with abnormal phenotypes are indeed imbalanced. Also, most of the imbalances present in inherited cases from this cohort and the literature are found in other genomic regions. This shows that PGI at the breakpoints among inherited cases are not a common cause of the phenotype in these individuals.
Key-words: Chromosomal microarray analysis (CMA); Balanced chromosomal rearrangements; Apparently balanced rearrangements;

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ID – 191

CHROMOSOMAL MICROARRAY ANALYSIS IN AUTISM SPECTRUM DISORDER: A FIVE-YEAR EXPERIENCE AT THE HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Caroline Paula Mescka [1]; Danielli Rondam da Silva [1]; Juliana Vargas Campiol [1]; Monique Banik Siqueira [1]; Maria Luiza Saraiva Pereira [1,2]; Rejane Gus [1]

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Abstract:

Autism Spectrum Disorder (ASD) is a group of early-onset neurodevelopmental disorders characterized by difficulties in social and communicative skills, repetitive and stereotyped behaviors. Although it is still not possible to define a clear etiology for this disorder, studies have shown that genetic factors can contribute significantly in cases of ASD, and in this way, genetic investigation becomes an important tool for the patient's prognosis and family genetic counseling. Thus, this study aimed to investigate Copy Number Variations (CNVs) of genomic gain and loss, using the Chromosomal Microarray Analysis (CMA). For this work, a retrospective cross-sectional study was conducted by surveying the CMA tests performed by the Cytogenetic Laboratory of the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre, between the years 2018 to 2022. All patients in which clinical information mentioned "ASD" and "autism" were included, followed or not by other clinical data. Data were evaluated by descriptive statistical analysis. It was observed that of the 394 CMAs performed during this period, 45 (11.4%) referred to ASD/autism, and from those, 29 tests (64.4%) did not show alterations and 16 (35.6%) presented CNVs with deletions and duplications. Of the CNVs observed in these analyses, 7 (43.8%) were associated with variants of uncertain significance (VUS), and 5 (31,2%) were of variable expressivity, and therefore, they could or could not be associated with the clinical condition of patients. Finally, 4 cases were observed with pathogenic variants associated with ASD (25%), and from these, 3 patients had microdeletions at the short arm of chromosome 16, located at 16p11.2, confirming the diagnosis of chromosome 16p11.2 deletion syndrome (OMIM # 611913). One patient showed a duplication in RAI1 gene (Retinoic Acid-Induced Gene 1, OMIM * 607642), on the short arm of chromosome 17, associated with Potocki-Lupski syndrome (OMIM # 610883). Both the American College of Medical Genetics (ACMG) and the Canadian College of Medical Genetics (CCMG) suggest that analyzes for the evaluation of CNVs, such as the CMA, should be used as the first choice test in the evaluation of individuals with ASD. In our study, we verified that more than a third of patients with ASD-related clinical features had CNVs with pathogenic deletions and duplications, probably pathogenic or VUS. Moreover, 25% of these cases had pathogenic changes associated to ASD, highlighting the involvement of CNVs linked to the chromosome 16p11.2 deletion syndrome. This disease is clinically characterized by susceptibility to neuro psychomotor developmental delay, nonspecific dysmorphisms and behavioral disorders, notably autism spectrum disorder. Although the cost and technology used in CMA are still a challenge for its implementation in our reality, this study corroborates the literature that demonstrates the diagnostic benefit for patients with ASD.

Key-words: Autism Spectrum Disorder; Chromosomal Microarray Analysis; Copy Number Variations;

ID 186

ANALYSIS OF CHROMOSOMAL STABILITY OF MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN UMBILICAL CORD AFTER CELL CULTURE

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Abstract:

Mesenchymal stromal cells (MSCs) have been used for therapeutic purposes as an Advanced Therapy Medicinal Product (ATMP). For its clinical application in Brazil, it is necessary to meet the quality control criteria established by the *Brazilian National Health Surveillance Agency* (ANVISA), which has minimum requirements on the processing of cells and ATMPs aiming at safety and quality for therapy and clinical research, such as the evaluation of genomic integrity by chromosomal analysis and genotoxicity testing by the micronucleus technique, supporting the therapeutic use of these cells in humans. Therefore, the present study aimed to evaluate the maintenance of the genomic integrity of MSCs through conventional cytogenetic tests (GTG banding) and micronucleus tests after cell culture. The project was approved by the Research Ethics Committee of the Pontifícia Universidade Católica do Paraná (PUCPR) (n° 4.043.535) and by the National Commission of Ethics in Research (n° 4.094.640). Five samples of MSCs were isolated from umbilical cord (UC) stored in the Biobank from the Core for Cell Technology of PUCPR (CTC-PUCPR). The cryopreserved samples were thawed and expanded in vitro between passages four and five until the necessary number of cells to perform the genomic integrity tests was obtained. For chromosomal analysis by GTG banding, MSCs were plated at $0.5x10^6$ and $1x10^6$ concentrations, and the mitotic inhibitor Colcemid Solution (10 µg/mL) was added for four hours to obtain metaphases. Approximately 20 metaphases from all samples were analyzed, and the results were described according to the International System for Human Cytogenomic Nomenclature (ISCN - 2016). For the study using the micronucleus test, the samples were analyzed at a concentration of $0.12x10^6$. The actin filament inhibitor cytochalasin B was used for approximately 24 hours to induce nuclear division and subsequent analysis of binucleated cells. In GTG banding, metaphases were obtained in all five samples, three of which were male (46,XY) and two female (46,XX). All karyotypes were described as normal diploid, demonstrating the absence of clonal chromosomal alterations. In the micronucleus test, the Nuclear Division Index (NDI) for two samples of the negative control was 1.23 and 1.33, and for one sample of the positive control, it was 1.5, in which some micronuclei, nucleoplasmic bridges and nuclear buds were observed. For the other five samples studied, the NDI was 1.3, 1.17, 1.23, 1.29 and 1.37, and no micronuclei were observed. The presence of two nucleoplasmic bridges in 1,000 cells was identified in only one sample. Despite this, the results suggest that the culture and cryopreservation conditions were not genotoxic, as there was no significant difference from the negative controls. Therefore, the analysis of chromosomal stability demonstrated that the UCs from MSCs maintained their genomic integrity, even after cell culture, attesting to their quality for possible therapeutic use.

Key-words: Mesenchymal stromal cells; GTG banding; Micronucleus;

Acknowledgement

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ID - 176

CHROMOSOMAL ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA DETECTED BY CONVENTIONAL CYTOGENETIC

Cesar Augusto Becher dos Santos [1]; Mateus Vinicius Oliveira Pereira [2]; Julia Tomaz [1]; Clayton Voidelo Machado [1]; Gabriela Sayuri Sato de Oliveira [1]; Mariana Satiko Pastore Saito [1]; Fernanda Christina Mikulski dos Santos [1]; Milena Beatriz Martins Grochewski [1]; Pedro Natale Cavezzale Dias [2]; Alexandre Henrique de Carvalho Pedroso [2]; Maria Beatriz Rodrigues Minucci [3]; Suellen Borges Goss [3]; Jaqueline Carvalho de Oliveira [4]; Carolina Mathias [4]; Daniel Pacheco Bruschi[4]

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Abstract:

Multiple myeloma is a hematologic malignancy of monoclonal plasma cells that accumulate in the bone marrow producing monoclonal immunoglobulin (M protein) Patient's median age of diagnosis is about 65 years MM showed aberrant expansion of terminally differentiated monoclonal plasma cells resulting in symptoms described by the acronym "CRAB": Hypercalcemia, Renal failure, Anemia, and Bone lesions. In Brazil, the epidemiological data are entirely underestimated and epidemiological data incidences are obtained indirectly, resulting in a sub-estimative of cases. The genetic event driving malignant development is either (i) the acquisition of hyperdiploidy, the majority involving odd-numbered chromosomes, or (ii) a translocation involving the immunoglobulin heavy chain gene locus in chromosome 14 [t(4;14), t(6;14), t(11;14), t(14;16), t(14;20)]. Here, we investigate the karyotype of the bone marrow cell from 25 patients clinically diagnosed with MM from May to November 2022. The cytogenetic analyses were performed in bone marrow cells by G-banding, and chromosomal abnormalities were described according to the International System for Human Cytogenomic Nomenclature (2020). Our cohort was composed of 12 males (48%) and 13 females (52%). The median age was 65 (± 46-92) years. The literature reports a median age of about 65 when patients first visit their doctor with symptoms and interestingly, we observed that 48% (7 males/ 5 females) of our sample was diagnosed before 65 (64 - 46). Abnormal karyotypes were recovered in 60% (15/25) of cases. The most common cytogenetic alteration observed involved chromosome 14 (33%), in which 60% was reciprocal translocations [t(4;14); t(8;14) and t(11;14)] and 40% was terminal deletions of the long arm of this chromosome. The most common cytogenetic alteration was deletions (27%), followed by trisomies (20%), reciprocal translocations (20%), composite karyotype in two cases (8%), and chromosomal gain (6%) of cases. We highlight that among patients presenting abnormal karyotypes. The reciprocal translocation t(4;14) (p16;q32) and gain in 1q [gain(1q21)] are considered high-risk in MM risk-stratification criteria, and their detection is essential to patients management and reinforces the importance of cytogenetical analysis in MM patients.

Key-words: Multiple Myeloma; G-banding; Karyotype;

Acknowledgement

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ID 164
EARLY DIAGNOSIS OF KLINEFELTER SYNDROME: THE IMPORTANCE OF GENETIC COUNSELING

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Abstract:
Introduction Klinefelter syndrome is associated with a numerical abnormality involving the sex chromosomes, and has an estimated prevalence of 1:660 in live male births. We present here a child with karyotype 48,XXXY/49,XXXXY diagnosed early with genetic investigation started at 2 months of age. Case description: Brazilian male patient, 2-month-old child of a non-consanguineous couple, uneventful pregnancy and no history of genetic disease. He was brought in for genetic consultation due to delayed psychomotor development and congenital heart disease, had low birth weight, from the beginning he presented hypotonia, dolichocephaly, ear with low implantation, cryptorchidism, congenital heart disease and camptodactyly. The result of the GTG-banding karyotype examination revealed the mos 48,XXXY[20]/49,XXXXY[70] karyotype, which is compatible with the diagnosis of mosaic Klinefelter syndrome. The early identification of these patients has great relevance for their appropriate treatment and care, especially for minimizing the risks of infertility, feminization and the biopsychosocial implications of Klinefelter syndrome.
Key-words: G-banding; Mosaic; Sex chromosome;

Acknowledgement

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ID – 145

EVALUATION OF THE NORAD/PUMILIO/RALGAPB REGULATORY AXIS IN THE GENOMIC INSTABILITY OF BREAST CARCINOMA

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Abstract:

Genomic instability is a phenomenon that refers to changes in a cell's genetic material that can lead to a variety of DNA changes, including point mutations, deletions, amplifications, and chromosomal rearrangements. It is an essential factor in cancer development, as changes in DNA can affect genes that regulate cell growth, programmed cell death, and DNA repair. In this context, the sequence-specific RNA-binding proteins, PUMILIO (PUM1 and PUM2), control various cellular functions, including genomic integrity. A lncRNA, named NORAD (Non-Coding RNA Activated By DNA Damage), interacts with PUMILIO proteins, preventing their repressor activity on target RNAs. This interaction might have implications for cancer development. Through binding to PUMILIO, NORAD modulates the mRNA levels of their targets, which are enriched for genes involved in chromosome segregation during cell division in osteosarcoma (U2OS) and cervical carcinoma cells (HeLa). Here we used RNA-seq data from The Cancer Genome Atlas (TCGA) database of breast cancer's subtypes (BRCA), Lum A (LA) (n=560), Lum B (LB) (n=207), HER2-enriched (HER2) (n =82) and Basal-like (BL) (n=190). We compare the expression levels of RNAs that present binding sites for PUMILIO using the GDCRNATools and Limma packages. We selected PUMILIO target genes positively co-expressed with lncRNA NORAD (above the median - log2FC Cutoff). Among the genes with significant expression correlated to NORAD in the four BRCA subtypes, *RALGAPB* (ENSG00000170471) stands out ($p<0.00001$). *RALGAPB* shows a dramatic cell redistribution during mitosis, shifting from the nucleus and Golgi complex during the prophase to the mitotic spindle and intercellular bridge at cytokinesis. Knowing that one of the functions of NORAD is controlling PUMILIO availability, and since the *RALGAPB* is a target of PUMILIO, increased or decreased expression of NORAD may lead to a higher or lower expression of *RALGAPB* in BRCA. RALGAPB and NORAD were independently related to chromosomal instability, but the guiding principle of this axis remains unclear. Considering the heterogeneity between BRCA subtypes, since LA has a better prognosis, characterized by low-grade tumors, and BL has a worse prognosis and greater invasive potential, we focus on these two subtypes to evaluate the relationship between these two molecules. We induced NORAD overexpression in LA and BL cell lines (MCF-7 and MDA-MB-231, respectively) by transfecting a vector with the full sequence of NORAD or a vector with a mutated version of NORAD without the PUMILIO binding region. As a control, we used the empty plasmid. After 48 hours of transfection, we checked the expression of a set of genes regulated by PUMILIO, including *RALGAPB*. Cell division abnormalities of transfected cells are being investigated. A better understanding of the regulatory role of NORAD in genomic instability is relevant in helping the search for new therapies, targets, and prevention strategies for cancer treatment.

Key-words: NORAD; PUMILIO; RALGAPB; BREAST CANCER; GENOMIC INSTABILITY

Acknowledgement

CAPES, Fundação Araucária, CNPq

ID 167
FISH PANEL TO DETECT HIDDEN HYPERDIPLOIDY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Danielle Ribeiro Lucon [1]; Maria Eugenia Bosso [1]
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Abstract:
Hyperdiploid (>46 chromosomes) acute lymphoblastic leukemia (ALL) is most common in children and is important prognostic implications. The hyperdiploidy can be detected by conventional G-banding karyotype and application of a panel of FISH (fluorescence in situ hybridization) probes. Hidden hyperdiploid cases are characterized when the karyotype is normal and incomplete and hyperdiploidy can be detected in interphase cells by FISH panel. Conventional G-banding karyotype and FISH panel for chromosome rearrangement and numerical aberrations were performed according to standard methods using locus-specific and centromere probes (Cytocell): t(9;22) BCR/ABL1; t(12;21) ETV6/RUNX1; t(4;11) AFF1/KMT2A; t(1;19) PBX1/TCF3; t(17;19) HLF/TCF3; CRLF2 and P2RY8 (Xp22.33; Yp11) in the bone marrow and peripheral blood (with > 50% blasts) samples of the 97 patients diagnosed with ALL-B in the period from 03/2021 to 02/2023. Hyperdiploidy was detected in 49 (50,5%) of the 97 cases: 37 (75,5%) cases were found by karyotype and FISH; 9 (18,4%) cases were with normal karyotype and hyperdiploid FISH; and 3 (6,1%) cases with unsuccessful cytogenetics and hyperdiploid FISH. In all cases with normal karyotype and hyperdiploid FISH, less than 20 metaphases were analyzed. Chromosome abnormalities may be undetected using conventional karyotype because 25% of cases yield inadequate or insufficient metaphases (<20 metaphases). Hyperdiploidy can be detected by the application of a FISH panel which will detect the characteristic pattern of gain; this technique can be applied to interphase cells but the translocations such as t(9;22), t(12;21), t(4;11), t(1;19) and t(17;19) should be excluded by application of appropriate probes. Furthermore, the laboratory can decrease the rate of absence of metaphases and release the report with the result of the FISH panel for ALL-B.
Key-words: Hyperdiploidy; FISH; Karyotype; ALL;

ID 49

CHARACTERIZATION OF A RARE PARACENTRIC 5P INVERSION IN A PATIENT WITH TYPICAL CAT-LIKE CRY

Emanuely Maria Santana [1]; Andresa Hiromi Sakai [1,2]; Isabella Cristina Oliveira Carvalho [2]; Maria Eliane Longhi Barroso [1]; Tatiana Mozer Joaquim [1]

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Abstract:

Cri du chat syndrome (CDCS) is a genetic disorder caused by a deletion of the short arm of chromosome 5 (5p-). The clinical features, severity, and progression of the disease vary depending on the region of the chromosome that is deleted and whether it is terminal or interstitial. Mosaicism, inversions, and ring chromosomes are less common reported mechanisms to this syndrome phenotype. The main clinical findings are global development delay, intellectual disability, speech delay, a high-pitched monochromatic cry, microcephaly, hypertelorism, broad nasal bridge and micrognathia. Cytogenetic studies have helped identify two cryptic regions for the patient's phenotype: 5p15.3, which is responsible for the characteristic cry, and 5p15.2, which is responsible for some of other significant clinical findings. Here we report an atypical CDCS case diagnosed by an inversion of the short arm of chromosome 5. The proband, a 3 months-old boy, was referred to the Genetic Counseling Service of the State University of Londrina (SAG-UEL) for cytogenetic investigation due to cat-like cry, developmental delay and other dysmorphic features as hypertelorism, cleft lip and cleft palate. The metaphase analysis performed after temporary culture of peripheral blood lymphocytes, followed by the G-banding technique revealed karyotype 46,XY,inv(5)(p13p15.1)dn. Cases of CDCS caused by inversions are rarely described in the literature, to the best of our knowledge, at present, only 5 inversion cases, with no other rearrangements, are reported. Several genes in 5p region, such as *SEMA5A*, *CTNND2* and *hTERT* are of particular interest in CDCS´s phenotype by the roles they play during embryonic and neuronal development. Among them, the telomerase reverse transcriptase gene (*hTERT)* located at 5p 15.33 cytoband has been considered to be critical for the most common phenotypes found in CDCS. An extensive genotype-phenotype correlation study involving CDC patients and the *hTERT* gene action, showed that this gene, may be the rate-limiting component for telomerase activity, making its expression required in various human cells. Since *hTERT* expression is critical to cellular immortalization, it is speculated that *hTERT* is strictly required for normal cell growth and development in humans. Hence, some phenotypes observed in CDC patients as growth delay and intellectual disability could be related to the accelerated telomere shortening during early development. In accordance to these findings, and based on the gene content of our patient breakpoints, we believe that the disruption caused by the inversion could be contributing with the loss of function of important CDCS genes. Moreover, other molecular mechanisms such as microdeletions at the breakpoints or even the position effect may also be related to the clinical features observed in our patient. In consequence, the situation reported here, might be considered a step forward to better support the CDCS critical region on 5p. Since other chromosomal rearrangements, even those that appear to be balanced, may be related with abnormal phenotypes, this work also shows the importance of the G-banding analysis to diagnose rare chromosomal syndromes, resulting not only, in better clinical management for the patients, but also improving genetic counseling.

Key-words: Genotype-phenotype correlation; Cri-du-Chat Syndrome; Balanced chromosomal rearrangement; 5p inversion; G-band

ID 87

FLOW CYTOMETRY TECHNIQUE IN DETECTING GENETIC MATERIAL CHANGES CAUSED BY CHEMICAL SUBSTANCES

Enzo Zini Moreira Silva [1]; Karin Braun Padro [2]; Karin Braun Padro [2]; Danielle Palma de Oliveira [3,4]; Cynthia Bomfim Pestana [1]; Daniela Morais Leme [1,4]

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Abstract:

The combination of flow cytometry and fluorescence techniques opens new perspectives to study the effects of chemical substances on DNA as well as to elucidate their mechanisms of action in causing transient and permanent changes in the genetic material. In order to demonstrate the applicability of these two techniques together, this study used the flame retardant (FR) Aluminum Diethylphosphinate (ALPI) as a case study. The potential of ALPI to alter the DNA of human keratinocytes (HaCaT cell line) was investigated by different flow cytometry-based assays, as follows: H_2DCFDA assay to evaluate the generation of intracellular reactive oxygen species (ROS); FlowCellect™ Histone H2A.X Phosphorylation Assay Kit (Merck Millipore) to evaluate the histone variant γ-H2AX (a DNA damage biomarker); Immunostaining with anti-5-methylcytosine (clone 33D3, Cat. # MABE146, Sigma-Aldrich), anti-5-hydroxymethylcytosine (clone RM236, Cat. # MA5-24695, Thermo Fisher Scientific) and Propidium Iodide for the cell cycle to evaluated changes in global DNA methylation pattern. The protocols adopted in this study were first validated with negative and positive controls, demonstrating their capacity to generate qualified data to determine changes in DNA caused by chemical substances. Thus, the study of ALPI demonstrated that this FR could increase ROS levels concentration dependent. No DNA damage was observed in HaCaT cells exposed to ALPI, and no changes in global DNA methylation were observed compared to the negative control. The increase in intracellular ROS can be related to several biological pathways related to chemical toxicity, not all necessarily addressed in this study, such as inflammatory response and induction of specific types of DNA damage. Although DNA damage was not verified, γ-H2AX is a biomarker for double-strand breaks especially, and a chemical substance can damage DNA by other genotoxic mechanisms of action not covered by the γ-H2AX quantification. For 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), although no changes in global DNA methylation pattern were shown, the occurrence of changes in specific gene regions can not be excluded and still need to be elucidated. In summary, our results showed that ALPI did not cause changes in the genetic material of human keratinocytes; however, a broader investigation considering different mechanisms of action still needs to be performed to ensure their safety. Also, this study demonstrated the applicability of flow cytometry-based assays in evaluating chemical toxicity by a multi-biomarker test strategy.

Key-words: Chemical toxicity; Flow cytometry-based assays; DNA damage; DNA methylation;

Acknowledgement

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ID – 172

THE IMPORTANCE OF KARYOTYPING IN THE DIAGNOSIS OF 47,XXY SYNDROME: A CASE REPORT.

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Abstract:

With the advancement of technology, genetic testing has become a reality for all. The improvement of laboratories and the growth of studies have made it possible to identify genetic alterations in genes, chromosomes, or proteins of the human body. The results have the potential to confirm, rule out, or raise suspicions about a genetic condition. However, the importance of initially simpler and low-cost tests should not be overlooked. L.P.N., male, 15 years old (05/29/2007), from Serrinha dos Pintos/RN, was referred from a university service with a suggestion of genetic panel or exome due to suspicion of Marfan syndrome or Marfanoid features. He presented with increased height, elongated face, arachnodactyly, positive wrist and thumb signs, and a heart condition that was only observed after eleven years (suggesting risk of early worsening of the patient's overall condition). At the first consultation, he had an echocardiogram (12/20/2016), describing mitral valve prolapse, enlargement of the right chambers, pulmonary and aortic insufficiency, and dilation of the aortic ring; spine X-ray (04/03/2017) and ophthalmological evaluation (04/05/2017), both normal, and complete FBN1 gene sequencing by Alvaro Laboratory, which did not identify any mutation. The genetic panel for Marfan syndrome and thoracic aortic aneurysm (22 genes) performed at Fleury Laboratory resulted without pathogenic variants or VUS. The medical geneticist decided to restart the investigation with the karyotype, which confirmed Jacobs syndrome (47,XYY). This simple case report illustrates the importance of both performing good semiology, starting with simpler tests, and karyotype testing, regardless of the initial hypothesis for the patient's diagnosis, as the overlap of signs and symptoms between environmental, chromosomal, and genetic etiology is not uncommon.

Key-words: cytogenetics; medical genetics; semiology;

Acknowledgement

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ID - 138
RING CHROMOSOME 18: CASE REPORT

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Abstract:
Ring chromosomes are rare chromosomal disorders that often seem to occur de novo. A ring chromosome forms when, due to deletion, both ends of the chromosome fuse. Depending on the amount of chromosomal deletion, clinical manifestations can be different. Thus, 18 ring syndrome is characterized by severe mental growth retardation, as well as microcephaly, brain and eye malformations, hypotonia, and other skeletal abnormalities. In this study, we report a 12-year-old patient with clinical indication of retardation in neuropsychomotor development to be clarified. Chromosomal analysis was performed using the G-banding technique and was performed after the patient was referred to the Human Genetics Laboratory (CRI). The chromosome investigation came up as 46, XY, r(18) (p11.32 q21.32). According to the clinical characteristics of these patients, the chromosomal investigation was extremely important for the diagnosis.
Key-words: ring chromosome 18; karyotyping; retardation neuropsychomotor; G banding technique;

Acknowledgement

Centro de Reabilitação infantil - CRI SESAP - secretária de saúde pública do Rio Grande do Norte

ID 29
HYPERDIPLOID KARYOTYPE IN PEDIATRIC PATIENTS WITH MYELODYSPLASTIC NEOPLASM

Gabriela Farias Lima [1]; Beatriz Ferreira da Silva [1]; Isabelle Corrêa Gonçalves [1]; Eliane Ferreira Rodrigues [1]; Viviane Lamim Lovatel [1]; Teresa de Souza Fernandez [1]

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Abstract:
Introduction: Myelodysplastic neoplasm (MDS) is recognized as a heterogeneous group of clonal diseases of hematopoietic stem cells. MDS is characterized by bone marrow dysplasias, affecting one or more lineages, peripheral blood cytopenias and an increased risk of evolution to acute myeloid leukemia (AML). The incidence of MDS is mostly in elderly patients over 65 years old. MDS is a rare disease in pediatric patients. Cytogenetic analysis is an important laboratory tool for diagnosis, prognosis and clinical decision-making for MDS patients. Clonal cytogenetic alterations are found in 30-50% of MDS. The most frequent chromosomal abnormalities in pediatric MDS are monosomy 7 and deletion 7q. The karyotype is classified in five prognostic risk groups according to the Revised International Prognostic Scoring System (IPSS-R): very good, good, intermediate, poor and very poor. Rare cytogenetic abnormalities usually are considered as an intermediate group by the IPSS-R. A review of the literature demonstrates that there are only two reports focusing in hyperdiploid karyotype (HK) in pediatric MDS. So, the prognostic value of HK has yet to be fully determined in pediatric MDS. Objective: The aim of this study was to report rare cases of HK in pediatric MDS and their clinical implications. Material and Methods: This study included five pediatric patients with MDS from a cohort of 200 pediatric patients with MDS. Chromosomal and clinical studies were carried out between 2000-2022. Cytogenetic analyses were performed in bone marrow cells by G-banding and fluorescence in situ hybridization (FISH) according to the International System for Human Cytogenomic Nomenclature (2020). Results: The HK represented 2.5% of all cases analyzed cytogenetically (5/200). The HK was divided into two groups: patients with only chromosome gains (3/5) and patients with structural alteration (2/5). The structural alterations associated with HK were: dup(1q) (one patient), der(6)del(6)(q21) and der(12)del(12)(p11) (one patient). Most of these patients had advanced subtypes with severe pancytopenia and hypocellular BM. All these patients were indicated for allogeneic hematopoietic stem cell transplantation (aHSCT), but four had evolution to AML and three died before the HSCT. Only two patients underwent HSCT, the patient at initial MDS responded well to HSCT and is still alive. The patient with hyperdiploidy and structural alteration presented post-HSCT cytogenetic and clinical relapse and died. Conclusion: HK is an uncommon cytogenetic finding in pediatric MDS. The HK was associated mainly with advanced subgroups of MDS and evolution to AML. Our results suggest that the HK in pediatric MDS is a predictor marker of leukemic evolution and may be used to indicate these patients to aHSCT, the only treatment with the potential of cure for MDS patients.
Supported by: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ (E-26/201.2018/2022).
Key-words: hyperdiploid karyotype; myelodysplastic neoplasm; leukemic evolution;

ID - 173

CHROMOSOMAL MICROARRAY ANALYSES OF 446 BRAZILIAN INDIVIDUALS WITH NEURODEVELOPMENTAL DISORDERS AND/OR CONGENITAL ANOMALIES

Gabriela Roldão Correia-costa [1]; Samira Spineli Silva [1]; Beatriz Schincariol Manhe [1]; Andrea Trevas Maciel-guerra [1]; Gil Guerra-junior [2]; Vera Lúcia Gil-da-silva-lopes [1]; Társis Paiva Vieira [1]

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Abstract:

Since 2010, Chromosomal Microarray Analysis (CMA) has become the gold standard method to detect copy number variations (CNVs) in the clinical setting, and the first-tier genetic test for patients with neurodevelopmental disorders (NDD) and (or) multiple congenital anomalies (MCA). However, mainly due to high costs, there are few studies that performed CMA in Brazilian cohorts presenting with NDD and (or) MCA. We report the results of CMA carried out between 2010 and 2022 in the Laboratory of Human Cytogenetics and Cytogenomics from the School of Medical Sciences of the UNICAMP. The cohort includes 446 Brazilian individuals presenting with NDD and (or) congenital anomalies. Among them, 216 were performed using the CytoScan™ HD chip, 162 using the CytoScan™ 750K chip, and 46 using the Genome-Wide Human SNP Array 6.0™ chip, all from Affymetrix® - Thermo Fisher Scientific Inc. (LifeTechnologies, Carlsbad, CA, USA). The remaining 22 CMA were performed with the Agilent SurePrint G3 Human CGH Microarray 8x60K, from Agilent Technologies (Santa Clara, CA, USA). All microarrays were performed following the manufacturer's instructions and the results were analyzed using the Affymetrix Chromosome Analysis Suite (ChAS) - version 4.0 (Affymetrix Inc., Santa Clara, CA, USA) or the Agilent CytoGenomics Software - version 3.0.1.1 (Agilent Technologies, Santa Clara, CA, USA). The interpretation and reporting of detected CNVs were performed using an in-house pipeline following recommendations of the American College of Medical Genetics and Genomics (ACMG) and of a European research group. CMA revealed pathogenic CNVs in 17% (76/446) of the individuals and probably pathogenic CNVs in 1% (5/446), allowing diagnostic conclusion for 18% of the individuals. CNVs of unknown clinical significance were found in 22.6% (101/446) of the individuals, which is in accordance to previous studies that performed CMA for Brazilian individuals, and higher than the percentage of VUS in individuals from United States and Europe. This higher proportion of VUS in Brazilian cohorts is probably due to an underrepresentation of our population in the Database of Genomic Variants (DGV). Two pathogenic or probably pathogenic CNVs were found in 22 individuals, 20 of them due to unbalanced chromosomal rearrangements. Two or more CNVs classified as VUS were found in 15 individuals. Recurrent CNVs (in more than three unrelated individuals) were found in the 4p16.3, 15q11.2 and 22q11.2 regions. This study revealed a diagnostic yield of 18% for CMA and a higher rate of CNVs VUS in Brazilian individuals with NDD and (or) congenital anomalies. The inclusion of CNVs data from the general Brazilian population in international databases, such as DGV, would improve CNVs classification.

Key-words: chromosomal microarray analysis; diagnostic yield; variants of unknown significance; neurodevelopmental disorders; congenital anomalies

Acknowledgement

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Processos #2018/08890-9); Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Processos # 460422/2014-6, #309782/2020-1) e Fundo de Apoio ao Ensino, à Pesquisa e à Extensão (FAEPEX - Unicamp).

ID – 194

COMPLEX KARYOTYPE IN MULTIPLE MYELOMA: A RETROSPECTIVE ANALYSIS BASED IN A CHROMOSOME DATABASE

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Abstract:

Multiple Myeloma (MM) is a malignant transformation of immunoglobulin-producing plasma cells. MM accounts for around 1% of all cancers and approximately 10% of all hematologic malignancies and still is considered as a rare disease. The median age of patients at the time of diagnosis is about 65 years, it is slightly more common in men than in women and is twice as common in African-Americans compared with Caucasians. Determining of cytogenetic abnormalities in MM has received more importance over recent years for patient's risk stratification. The oncogenic pathway can be classified as hyperdiploid with multiple trisomies of odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, 21) or due to translocations involving the immunoglobulin (Ig) alleles, involving the chromosome 14 [t(4;14), t(6;14), t(11;14), t(14;16), t(14;20)]. Conventional cytogenetic studies in MM often identified karyotype altered (about 30%-50% of MM cases), more often in an advanced stage or a more proliferative form of disease. The complex karyotype (CK) presence has not yet been included in prognostic stratification risks of MM patients. Here we conducted a retrospective study in *Mitelman Database Chromosome Aberrations and Gene Fusions in Cancer* to evaluate the occurrence of CK in MM without change in diploid number (2n=46) with or without translocations involving chromosome 14. The aim was to review cases of patients diagnosed with MM with CK to insight about the future incorporation of CK as criteria in risk-stratification. From that, 110 cases were evaluated, representing 30% of the MM cases with karyotype 2n=46 and 5.3% of the total number of MM cases reported in the *Mitelman* database, demonstrating the relevant occurrence of CK cases. The mean age at diagnosis was 61 years and there were only eight cases of early MM, diagnosed before the age of 50. Our data retrieved a higher prevalence of MM reports in men (61%) than in women (39%), with a sex ratio of 1.55. 37.5% of the cases of MM with CK did not show classical karyotypic alterations of the disease (without involving chromosome 14 or traditional trisomies), both at diagnosis or in disease monitoring. These results highlight that the significance of CK to development disease have been neglected or poorly evaluated in MM studies. The CK are being gradually incorporated into classification and risk-stratification criteria for onco-hematological diseases. Particularly in MM, the absence of one classification based on genetic subtypes is an issue that fills the gap in need to better understand the high heterogeneities of the clinical outcome. Although MM is still considered a single disease with the extensive cytogenetic heterogeneity requires a better categorization. Currently, CK is not included in the prognostic stratification-risk of MM patients, despite that CK often represents poor prognosis outcomes in others hematological neoplasm. We are alert to the importance of better understanding the role of CK in MM and for report to medical staff, and improve the knowledge about the significance of these cytogenetic alterations in the clinical practices.

Key-words: Multiple Myeloma; Complex Karyotype; Risk-stratification;

Acknowledgement

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ID 71

THE POWER OF WHOLE-EXOME SEQUENCING IN IDENTIFYING GENETIC CAUSES OF NEONATAL PNEUMONIA: A CASE REPORT

Gil Monteiro Novo-filho [1]; Geovana de Souza [1]; Ana Carolina da Silva Santos [1]; Naila Cristina Soler Camargo [1]; Maria Beatriz Silva Oliveira [1]; Julia Cataldo Lima [1]; Marco Antônio Zonta [1]; Evelin Aline Zanardo [1]

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Abstract:

Neonatal pneumonia was the fifth leading cause of death in 2015, with a mortality rate estimated at 1.134 per 1,000 live births. Although neonates are more prone to infections due to their immature immune system, genetic predisposition has been acknowledged as an important cause of neonatal pneumonia. There are several genetic causes for neonatal pneumonia, highlighting pathogenic variants in genes related to immunodeficiencies. We report a case study of a four-month-old boy who presented with neonatal pneumonia and underwent tracheostomy in the treatment of the disease. The boy also had mild hypertonia at birth. Oral mucosa samples were collected from the patient and both parents, and DNA was extracted from the samples. Whole-exome sequencing (WES) was performed, and the resulting data was analyzed using bioinformatics tools. Exome analysis identified a novel variant, c.62-2A>G, in the *TNFRSF13B* gene. This variant, that presents an allele frequency of 0.000007 in the gnomAD database (1/140290), affects the splicing acceptor site in the first intron of the gene, which typically leads to a loss of protein function. Pathogenic variants in the *TNFRSF13B* gene are associated with common variable immunodeficiency 2, with both dominant and recessive autosomal inheritance and reduced penetrance, which leads to deficient antibody production and hypogammaglobulinemia, consequently causing bronchitis and recurrent bacterial infections such as pneumonia, sinusitis, enteritis and otitis media. The identification of this pathogenic variant in the *TNFRSF13B* gene facilitate early treatment and enables the administration of therapeutic strategies to mitigate the occurrence of new infections and for patients. Our findings suggest that WES is a powerful tool for identifying genetic causes of neonatal pneumonia.

Key-words: whole-exome sequencing; pathogenic variant; neonatal pneumonia;

ID – 101

EPIGENOMICS ASSOCIATION ANALYSIS IN PATIENTS WITH INTELLECTUAL DISABILITY AND VUS RESULTS

Gleyson Francisco da Silva Carvalho [1]; Beatriz Martins Wolff [1]; Lucas Liro Vieira [1]; Amanda da Silva Januário [1]; Leslie Domenici Kulikowski [1]

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Abstract:

Copy number variations (CNVs) are DNA fragments deleted or duplicated in relation to a reference genome. Some genome variants, classified as variants of uncertain significance (VUS), do not present a safe relationship with clinical phenotypes, which makes the diagnostic conclusion difficult. Hypermethylation of gene promoter regions often leads to transcriptional silencing, in addition to DNA methylation changes in gene and/or intergenic regions can play a critical role in genomic regulation and stability. Epigenomic investigation offers a more comprehensive understanding of modulation in gene expression associated with genomic imbalance in different disease and in different genomic variations. In order to determine the impact of methylation status, associated with genomic imbalances, in clinical phenotypes, our study evaluated 10 DNA samples from patients with intellectual disability and dysmorphic features with CNVs classified as VUS without a definitive clinical diagnosis. Thus, we performed genotyping Infinium CytoSNP 850k (Illumina) assay and Infinium MethylationEPIC (Illumina) assay to investigate CNVs and methylation status. The bioinformatics analysis of raw data (normalization, quality control, DMP/DMR investigation and ontological analisys) were performed following a pipeline in R language. We were able to identify significant differences when comparisons of methylation status were performed individually. The ontological analyzes of methylation results suggest a phenotypic impact consistent with the clinical presentation of the patients, indicating a possible association between the phenotype and the DNA methylation status. Genomic methylation, associated with genomic structural imbalances, can play a critical role in clinical features suggesting important genomic marker that can lead a more accurate diagnosis and possible treatment options in near future.

Key-words: VUS; Methylation; Epigenomics;

ID 119-
CLINICAL VERSUS MOLECULAR APPROACHES IN FRAGILE X SYNDROME

Gleyson Francisco da Silva Carvalho [1]; Beatriz Martins Wolff [1]; Lucas Liro Vieira [1]; Amanda Januário da Silva [1]; Vanessa Tavares Almeida [1]; Yanca Gasparini [1]; Alexandre Torchio Dias [1,2,3,4]; Leslie Domenici Kulikowski [1]

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Abstract:
Patients with Fragile X syndrome (FXS) presents neuropsychomotor developmental delay (NPMD), autism spectrum disorders (ASD), behavioral changes and learning difficulties, in addition to characteristic dimorphisms. Considered the second cause of intellectual disability (ID) in the general population and the most prevalent in men. Its molecular etiology is characterized by an increased number of CGG triplet repeats (>200 repeats) in *FMR1* gene promoter region. Molecular diagnosis can be performed using commercial kits that allow the unequivocal identification of patients and carriers. In this study, we evaluated 467 patients with intellectual disability associated with NPMD and/or other clinical characteristics of FXS, without age and gender restrictions, with a clinical hypothesis of FXS, using the Amplidex kit ® (Asuragen, Texas, USA). The whole cohort analysis revealed that 11 patients, all of them male, presented results compatible with the syndrome (>200 CGG repeats). We also identified two cases of premutation (55 - 200 repetitions) and six cases of intermediate mutation (45 - 54 repetitions). It was also possible to identify cases of heterozygosity in two women with normal and intermediate mutation alleles, five women with normal and premutated alleles, and three women with normal and full mutation alleles. One male patient, possibly due to mosaicism, presented a result compatible with three alleles, being, respectively, normal, premutated and full mutation alleles. The remaining 437 cases did not show alterations in the identified alleles. Based on the high prevalence described for FXS, it is expected that a large percentage of mutations in promoter region of *FMR1* gene would be identified in a clinical series composed of patients with ID associated with NPMD in addition to the clinical hypothesis for FXS. Thus, our study revealed a prevalence of 3.2% of complete mutations, a result lower than expected and found in the literature for this type of cohort. The early molecular approach can help the distinctive diagnosis in patients with ID, NPMD and/or syndromic autism, as well as allow parents to make informed decisions about family planning.
Key-words: Fragile X Syndrome; Cytogenomics; FMR1 gene;

ID – 115

CO-OCCURRENCE OF GENOMIC UNBALANCE DUE TO APPARENTLY BALANCED TRANSLOCATION T(12;22) AND 13Q DELETION IDENTIFIED BY CGH-MICROARRAY.

Ingrid Bendas Feres Lima [1]; Carlos Roberto da Fonseca [1,2]; Anna Luiza Vaz Serrão [1,2]; Lucia de Fátima Marques de Moraes [2]; Juan Clinton Llerena Júnior [3]; Elenice Ferreira Bastos [1]

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Abstract:

Most cases of balanced translocations are clinically normal, however, about 7% of them are associated with phenotypic alterations that include congenital malformations and/or intellectual disability. Some of the causes of abnormal phenotype in these cases include the occurrence of micro-aberrations at breakpoints, gene position effects, and more rarely, the presence of additional alterations involving other regions of the genome, with the translocation being truly balanced. This report aims to demonstrate the cytogenomic characterization and genotype-phenotype correlation of a patient referred due to multiple malformations, whose analysis demonstrated a rare event of genomic unbalance in the translocation breakpoints added to a loss in another unrelated chromosome. A female patient with young, non-consanguineous parents was referred for newborn genetic follow-up because of multiple malformations: hypotonic, syndromic facies, microcephaly, low implanted ears, ocular hypertelorism, agenesis of the corpus callosum, and severe ventriculomegaly. The techniques GTG-banding were performemed using the trypsin protocol and CGH-microarray using the CytoScan® 750k Array (Affymetrix®) platform according to standard protocol. The final nomenclature was described according to ISCN, 2020. A translocation was identified between the short arm of chromosome 12 and the centromeric region of chromosome 22, resulting in a pseudo-monosomy of chromosome 22 and loss only of the nucleolar organizer region [45, XX, -22, t(12;22)(p13;q11)]. Microarray analysis detected two microdeletions, both identified as pathogenic. One located on the long arm of chromosome 13 of about 15,884 kbp arr [hg19] 13q32.2q34 (99,224,026-115,107,733) x1 unrelated to the translocation and another corresponding to a segment of 3.278 kbp arr [hg19] 22q11.1q11.21(17,034,539-20,312,661) x1 of the long arm of chromosome 22. The analysis by multiple techniques defined the result as: [45, XX, -22, del(13)(q32.3-q34), t(12;22)(p13;q11). arr[hg19]13q32.2q34(99,224,026-15,107,733) x1, 22q11.1q11.21 (17,034,539-20,312,661) x1]. The deleted region of chromosome 13 encompasses about 47 genes, various of which are associated with pathologies. Monosomy 13q region is already described in the literature as a syndrome and characterized by several clinical conditions compatible with our patient: syndromic facies, low implanted ears, microcephaly and congenital heart defect. The deletion found in the q11.1q11.21 (17,034,539-20,312,661) region of chromosome 22 as a consequence of the translocation also encompasses a large genomic segment from q11.21 to the telomeric region of 22p (q11.21> pter). A number of genes already related to different pathologies are located in this region, among them the DiGeorge syndrome region, compatible with a clinical condition of orbital hypertelorism. Our case, unlike most of the additional findings, where the translocation is truly balanced, exemplifies a rare case in which two independent meiotic events occurred, leading to the loss of important genes in different regions of the genome, thus determining a very complex clinical condition.

Key-words: Chromosomal microarray analysis (CMA); phenotypic mapping; apparently balanced translocation; chromosomal microaberrations;

ID – 50

A COMPOUND HETEROZYGOUS MUTATION IN *TRAPPC9* GENE CAUSING NON-SYNDROMIC INTELLECTUAL DISABILITY IN A BOY FROM CENTRAL BRAZIL

Irene Plaza Pinto [1]; Ana Júlia Cunha Leite [1]; Nico Leijsten [3]; Martina H. A. Ruiterkamp-versteeg [3]; Rolph Pfundt [3]; Nicole de Leeuw [3]; Aparecido Divino da Cruz [1,2]; Lysa Bernardes Minasi [1]

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Abstract:

Intellectual disability is a complex and heterogeneous neurodevelopmental disorder with multifactorial clinical features and is genetically heterogeneous. The trafficking protein particle complex subunit 9 gene (*TRAPPC9*) plays an important role in the neuronal NF-kB signaling pathways. It is one of the numerous genes related to the non-syndromic form of intellectual disability. Different mutations in the *TRAPPC9* gene have been involved with autosomal-recessive intellectual disability. Next-generation sequencing technologies have led to the identification of several ID-associated genes, emphasizing the considerable genetic heterogeneity of ID. Herein, we reported a 23 years-old male with a compound heterozygous pathogenic variant in the *TRAPPC19* gene who was referred to genetic testing due to a previous clinical diagnosis of non-syndromic intellectual disability. He was born to healthy and non-consanguineous parents at 40 weeks gestation with 2.750g measuring and 45cm in length. Physical examination revealed microcephaly, facial dysmorphia, abnormal hands, eyes, and head movements, and seizure. He has difficulty walking and walks on tiptoe. He has a moderate intellectual disability and does not carry out his activities alone. The karyotyping at >550-band revealed a male karyotype (46,XY). GeneChip® CytoScanHD™ array did not reveal pathogenic copy number variants nor long contiguous stretches of homozygosity. A target gene panel analysis using exome sequencing identified the c.3573+1G>A variant inherited from the father that alters the predicted donor splice site, and the c.2565del variant inherited from the mother that is a nonsense substitution that leads to an interruption of the open reading frame by a premature stop codon. Both mutations cause a loss of function of the *TRAPPC9* gene. Whole exome sequencing followed by target gene panel analysis was an efficient approach to identify pathogenic variants in our patient with a non-syndromic intellectual disability. The combination of these two *TRAPPC9* mutations is responsible for the clinical features of our patient. Finally, it is recommended to do genetic counseling to help the family understand the familial implications of genetic contributions to disease and the chance of disease recurrence.

Key-words: WES; *TRAPPC9*; neurodevelopmental disorder;

ID – 18

INCIDENCE OF CHROMOSOMAL POLYMORPHISMS DETECTED BY CLASSICAL CYTOGENETIC APPROACHES IN ABNORMAL PHENOTYPE CARRIERS ASSISTED BY THE GENETIC COUNSELING SERVICE OF THE STATE UNIVERSITY OF LONDRINA

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Abstract:

Chromosomal polymorphisms are quantitative or positional alterations in constitutive DNA heterochromatin, occurring in the centromeric region of chromosomes 1, 9, 16 and Y. It can also be recurrently found in the short arms of acrocentric chromosomes including satellites and stalks regions. These variants are considered to be normal due to their frequency in the general population after karyotyping staining techniques. However, at present, the relationship between chromosome polymorphisms and clinical findings is still controversial. Several studies have reported a higher incidence of these variants in couples, affecting both male and females suffering from infertility, recurrent pregnancy loss, growth delay or even congenital anomalies. In order to add more evidence about the influence of chromosomal polymorphisms under the clinical changes, we collected the clinical data and the karyotype results from the patients assisted by the Genetic Counseling Service linked to the State University of Londrina between the years 2018 and 2022. The variants were diagnosed by classical cytogenetic techniques as G, C and AgNOR chromosomal bands and then classified as increase or decrease in constitutive heterocromatin (qh+/qh-); polymorphic inversions, increase of the centromeric region (cenh+); double satellites (pss) and increase in lengths of the stalks segments of acrocentric chromosomes (pstk+). Cases were primarily referred to the cytogenetic investigation due to infertility, recurrent miscarriages, developmental delay and congenital malformations. The presence of chromosomal polymorphisms was detected in 17 of the 295 studied patients (5.76%). Five of them were diagnosed with inv(9)(p12q13), 4 with 9qh+ and the remaining 8 cases were diagnosed with 13cenh+, Yqh+, 15pstk+, 21pstk+, 15ps+, 13pss, 15pss and 22pss. According to literature, the pericentric inversion of chromosome 9 is the most frequent chromosomal heteromorphism detected in humans due to its structural organization, making it more prone to breakage. As heterochromatin plays an essential role in meiosis, the presence of heterochromatic variants has been theorized to impair the formation of functional gametes. Consequently, patients who are chromosomal polymorphisms carriers might theoretically be more susceptible to experiencing an increased incidence of embryonic aneuploidy and impaired reproductive outcome. Regarding to satellite regions and stalk segments, it speculates that the increase in their size can predispose translocations or even lead to defect in centromere function. Moreover, the effect of chromosomal variants may act upon or block the binding of certain transcription factors or alter the regulation of genome-wide chromatin making it prone to the occurrence of other clinical findings. While hard to establish a correlation between the chromosomal variants and abnormal phenotypes, the mechanisms of clinical features that are associated with the polymorphisms still need to be better clarified. Further analysis with high-resolution genome karyomapping and haplotyping technology may unveil potential relationships between parental polymorphisms and embryological chromosome segregation errors giving us further insight on their interaction with embryonic development.

Key-words: Human chromosome variants; heteromorphisms; G-banding; C-banding; AgNOR banding

ID 174

GENETICAL AND CLINICAL CORRELATION IN THE CHROMOSOME 9P DELETION SYNDROME

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Abstract:

The 9p Deletion Syndrome (9PDS) is a neurodevelopmental condition in which delays in psychomotor, social interaction and cognitive development are observed, in addition to dysmorphic craniofacial features and congenital malformations. The diagnosis is confirmed by cytogenetic testing to detect deletion of the short arm of chromosome 9 (9p). Although there are studies involving families with this syndrome, there is a lack of data relating its symptomatology with the observed cytogenetic alterations. In this context, a Systematic Literature Review (SLR) protocol was applied in this study to collect genetic, cytogenetic, epidemiological, and clinical data from reported cases of 9PDS. The data was analyzed with bioinformatics, cellular and molecular biology tools for critical interpretation and correlation between the genetic and clinical findings of 9PDS. The application of the SLR protocol identified 133 articles related to 9PDS, including 115 case reports and 18 cohort studies. After data extraction, we obtained information from 146 patients with mutations in the 9p region, with the 9p22 deletion being the most frequent (17.1%), followed by the 9p24 region deletion (5.5%). In the 9p22 region, the genes that are partially or completely deleted were mapped. One of the deleted genes is *BNC2* (located in the 9p22.2-p22.3 region), which is involved in the development of the tongue and palate, which may be directly related to a high palate, a common symptom in the syndrome. The *ZDHHC21* gene (9p22.3), also correlated with the deletion, is involved in hair follicle development. In 9PDS there are reports of patients with thin, sparse, and brittle hair, which may be related to the lack of this gene. There is also the *FOCAD* gene (9p21.3), which is important in the development of male external genitalia. In 9PDS there are several reports indicating genital problems, including the XY sex reversal phenotype. Despite the cytogenetic and phenotypic variability observed in individuals with 9PDS, there are still insufficient data on the association between phenotypes and genotypes of the syndrome. The present research demonstrates that the SLR data corroborate the areas of activity of the genes involved in the target regions. Therefore, the continuity of this research has the potential to identify new molecules and functional information related to the phenotypes observed in 9PDS, including those involved with changes in neurodevelopment and brain neuroregulation.

Key-words: 9p Deletion Syndrome; Neurodevelopment; Epidemiology;

ID – 99

ASSOCIATION OF THE RS3781907 GENETIC POLYMORPHISM OF THE UCP3 GENE AND CHILDHOOD OBESITY

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Abstract:

Obesity is considered a worldwide public health problem, due to the increasing number of individuals diagnosed with the disease. This disease is responsible for changes in body metabolism that will lead to excess body fat, influencing the individual's weight. When diagnosed in childhood, individual follow-up can be developed in order to reduce the impacts of the disease in adult life. Obesity is considered a complex multifactorial disease and complex disease with multivariate manifestations. The genetic influence of the disease has been investigated in order to assist in individual monitoring and improve the patient's prognosis. The UCP3 gene is located on chromosome 11 and its expression is observed in skeletal muscle, related to energy expenditure and body fat deposition. The aim of this study was to evaluate the relationship between the rs3781907 genetic polymorphism of the UCP3 gene and childhood obesity. The work consists of a case-control study, with 225 children with an average age of 9 years attended at the Children's Hospital, residents of Goiás-Brazil. The children were examined by a child endocrinologist, where clinical data and peripheral blood samples were collected. The collected biological material was forwarded to the Replicon Research Center at PUC Goiás for transmission of genomic DNA and Real Time Polymerase Chain Reaction (q-PCR) for genotyping of the rs3781907 polymorphism. After the analyses, it was possible to observe that individuals with the homozygous mutant allele (GG) had higher mean Z-BMI values, showing a statistically significant difference in the genotype distribution in the obese group compared to eutrophic group. It was identified that there was a significant difference between the proportions of individuals who had the risk allele and were eutrophic in relation to obese individuals who had the wild-type allele. The rs3781907 genetic polymorphism was not significantly associated with obesity causality, but it was possible to observe that the presence of the G allele was associated with a higher Z-BMI value in the analyzed population, due to the multifactorial nature of the disease.

Key-words: SNP; uncoupling proteins; pediatric obesity;

ID – 59

FREQUENCY OF CHROMOSOMAL ABNORMALITIES OBSERVED IN A CYTOGENETICS LABORATORY IN THE NORTHERN REGION OF RIO GRANDE DO SUL (RS)

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Abstract:

Chromosome disorders are alterations resulting from the change in the number or structure of chromosomes during the cell cycle, which are responsible for most gestational losses, infertility, congenital malformations, deficit, and mental retardation. Cytogenetic analysis is an essential methodology for the detection of several chromosomal disorders that provides the diagnosis of abnormal phenotypes. Thus, this study aimed to analyze the frequency with which chromosomal alterations were found in individuals treated at a cytogenetics laboratory in Passo Fundo, Rio Grande do Sul state. We carried out a retrospective analysis of the karyotype results of 1060 individuals with suspected chromosomal disorders, from January 2015 to December 2022, through the analysis of patients' records treated at the Cytogenetics Laboratory of Hospital São Vicente de Paulo in Passo Fundo. Cytogenetic analysis was performed by the culture of peripheral blood lymphocytes with subsequent G-banding. From the 1060 cases studied, 872 did not present alterations (82%) and 188 presented chromosomal anomalies, 151 numerical (14%), and 37 structural (3%). The predominance of numerical alterations to structural ones is already well-known in the literature. Among the autosomal aneuploidies, the most frequent was Down syndrome (69%). Free trisomy 21 occurred in 101 cases and Robertsonian translocation in three. Edwards Syndrome occurred in 26 cases (17%) and Patau Syndrome in six cases (4%). In 1% of cases, there was monosomy of chromosome 22 and another one with Robertsonian translocation between chromosomes 13 and 14. Regarding the sex chromosome anomalies, Turner Syndrome was the most frequent, in four cases (3%), Klinefelter Syndrome in three cases (2%), and Triple X Syndrome in two cases (1%). In two cases, trisomy 18 and XXY (1%) were found in the same karyotype. Among structural chromosomal anomalies, the most common were deletions and inversions. The chromosomes with the most changes were chromosomes 9 (18 cases) and X (nine cases). In 5 cases (14%) an inversion of chromosome 9 was observed, such alteration is considered a variant of normality. As for the X chromosome, in four cases (11%) the deletion was on the long arm, and in three cases the deletion was on the short arm (8%). In general, patients with balanced structural changes have a normal phenotype, while those with unbalanced changes develop phenotypic anomalies. The diagnosis of structural alterations has fundamental importance to patients, since they can result in infertility or malformed fetuses. This study was able to identify a high frequency of chromosomal anomalies, emphasizing the importance of incorporating the cytogenetic test in the routine investigation of patients for subsequent referral to genetic counseling. On the other hand, it is important to point out that, even with a normal karyotype result, there is the possibility of not seeing small alterations due to the method limitations, requiring more specific and/or complementary exams for a correct diagnosis.

Key-words: Chromosomopathies; Passo Fundo; cytogenetics; chromosomal aberrations; diagnosis

ID 46
FISH RESULTS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Abstract:
Introduction: Monoclonal gammopathy of undetermined significance (MGUS) is a condition characterized by the presence of monoclonal serum proteins, related to the proliferation of clonal plasmocytes. The diagnosis of GMSI is performed through quantification of the serum monoclonal component and bone marrow analysis. Fluorescence in situ hybridization (FISH) testing performed on bone marrow provides prognostic information. Objective: to investigate data on cytogenetic alterations by means of FISH in cases with a diagnostic hypothesis of MGUS. Methodology: A survey of FISH data performed in bone marrow for plasma cell neoplasms was carried out from January/2020 to January/2023, whose clinical suspicion was GMSI. Results: 56 cases were obtained, of which 27 showed cytogenetic abnormalities, 26 were normal, and 3 did not have plasma cells for analysis. In total, 73 cytogenetic alterations were found, and the percentage was calculated separately for each alteration, since within the same case the alterations can be concomitant. Thus, 33.3% had the RB1 gene deletion and the IGH-CCND1 rearrangement, and 29.6% had an additional copy of the CKS1B gene and trisomy 17. The less frequent abnormalities were: additional copies of the MAF, MAFB and CDKN2C (3.7%); ATM, CDKN2C, MAF, IGH and FGFR3 gene deletions (3.7%). The IGH-FGFR3 rearrangement was the least frequent (3.7%). Conclusion: The changes found in cases of GMSI were diverse, from those with standard risk prognosis such as IGH-CCND1, to the deletion of the RB1 gene, which when associated with other abnormalities, results in an unfavorable prognosis. Finally, trisomy 17 was detected, which may be associated with other trisomies, configuring odd-numbered chromosome hyperdiploidy, which cannot be detected in the FISH test because the panel of probes performed did not evaluate all the odd-numbered chromosomes. Because it is a gene analysis, we cannot state that there is hyperdiploidy, so it is necessary to make an association with the medullary karyotype.
Key-words: MGUS; Cytogenetics; FISH;

ID – 192

HDAC6 INHIBITION DECREASES CHEMORESISTANCE, MIGRATION AND INVASION OF MELANOMA CELLS IN 2D AND 3D CULTURE MODELS ALTERING B-CATENIN AND E-CADHERIN EXPRESSION.

João Marcos Oliveira da Silva [1]; Leilane Sales Oliveira [1]; Carolina Berraut Chiminazo [1]; Rafael Fonseca Miranda [1]; Carlos Vinicius Expedito de Souza [1]; Alexandre Ferro Aissa [1]; Graziela Domingues de Almeida Lima [1]; Marisa Ionta [1]; Angel Mauricio Castro-gamero [1]

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Abstract:

Histone deacetylase 6 (HDAC6) plays a critical role in tumorigenesis and tumor progression, including proliferation, chemoresistance, migration, and cell motility. This activity is mainly related to its ability to deacetylate histones and non-histones and its regulatory role in microtubule architecture and stability. Therefore, we investigated the effects of HDAC6 inhibition on melanoma, the most malignant form of skin cancer, using CHL-1, WM1366, and SK-MEL-147 cell lines. After treatment with WT161, a highly selective HDAC6 inhibitor, we identified reduced cell survival in cells cultured in two (2D) and three dimensions (3D). In addition, we examined the effects of WT161 combined with temozolomide (TMZ) or dacarbazine (DTIC), which are commonly used in melanoma chemotherapy. Using Chou and Talalay's median effect method, we obtained a combination index for the simultaneous and sequential treatment regimens. In all cell lines, the combination of WT161 with a chemotherapeutic agent produced a synergistic effect, mainly in simultaneous treatments. These results were corroborated in 3D culture models of CHL-1 and WM1366 cells treated with the WT161+TMZ combination, accompanied by increased chemoinduced apoptosis, as demonstrated in WM1366 cells. HDAC6 inhibition also decreased migration and invasion, and increased cell adhesion, suggesting a detrimental effect on melanoma cell motility. These results were accompanied by alterations in the levels of β-catenin and E-cadherin depending on the evaluated cell type. In conclusion, we demonstrated for the first time that combination therapy with WT161 is effective in overcoming chemoresistance of melanoma cells cultured in 2D and 3D. In addition, the combined treatment reduced the migratory and invasive properties of melanoma cells, associated with changes in the expression of important players in the Wnt pathway. It is expected that continued progress in this area, including chromosomal instability associated to dynamics and control of microtubules, will lead to improved anti-melanoma treatments and the development of new and more effective preclinical analogs.

Key-words: Melanoma; HDAC6; WT161; tumor progression;

Acknowledgement

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ID – 207

HIGH RESOLUTION MELTING AS STRATEGY TO SCREENING PATIENTS DIAGNOSE WITH ACUTE MYELOID LEUKEMIA (AML) AND *FLT3* MUTATION

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Abstract:

Acute myeloid leukemia (AML) is a malignant transformation of immature hematopoietic cells in marrow bone. Clinical recommendations for Acute Myeloid Leukemia (AML) classification and risk-stratification remain heavily reliant on cytogenetic findings at diagnosis, which are present in around <50% of patients. The chromosomal translocations, as t(1;19), t(15;17), t(12;21), t(8;21), t(6;9) are examples of cytogenetic findings used to classify AML genetic subtypes. Gene mutations are being gradually incorporated into classification and risk-stratification criteria for AML patient; however, in Brazil the access to genetic reports is very limited due high cost. The Fms-like tyrosine kinase-3 (*FLT3*) is a class III receptor tyrosine kinase located on chromosome 13q12 have been included in World Heath Organization (WHO) as an important biomarkers to prognostic stratification-risk in AML. Several evidence of the significant increase of risk hazard in patients with this mutation. Here, we test the use of High-Resolution Melting (HRM) as strategy to screening patients with AML with *FLT3* mutation due to the methodological ease in addition to ensuring faster and more efficient responses to patients. In this context, we evaluated patients (n=17) diagnosed with AML and karyotype shown t(15;17) (n=6) and without this rearrangement (n=11). We confirm by PCR assays that patients without t(15;17) also didn't shown any of chromosomal aberrations t(1;19), t(15;17), t(8;21), t(12;21), t(6;9). Within the group of patients without cytogenetic alterations (n=11), three potential patients with the *FLT3* mutation were found by the HRM technique. These samples will be analyzed by the Sanger sequencing. By confirming the efficiency of the technique to discriminate patients with the mutation in this gene, the objective is to expand the analyzed sample in order to verify parameters of specificity and sensitivity. These findings may significantly contribute to greater molecular tracking of patients, especially in the context of the Brazilian unified health system.

Key-words: Acute Myeloid Leukemia; FLT3 mutation; High-Resolution Melting; Biomarker; molecular tracking of patients

ID – 3

CHROMOSOMAL MICROARRAY ANALYSIS OF GERMLINE MUTATIONS IN THE OFFSPRING OF A POPULATION OCCUPATIONALLY EXPOSED TO CESIUM-137 RADIATION

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Abstract:
The radiological accident in Goiânia in 1987 resulted in a serious human, animal, plant, and environmental contamination episode. They were exposed to cesium-137 chloride (137CsCl), which caused accidental and occupational exposure to ionizing radiation. Ionizing radiation (IR) is one of the environmental components that most cause cellular stress in complex organisms. Cellular exposure to ionizing radiation induces double-strand breaks, single-strand breaks, damage to bases and cross-links in nucleic acids, mainly DNA. Furthermore, the mutagenic effects of ionizing radiation are worrying, as it can lead to the accumulation of mutations in germ cells, which cause an increase in hereditary disorders. Chromosomal microarray analysis (CMA) is a cytogenomic approach to detecting losses and gains in a wide spectrum of genome regions. In the present study, we propose to analyze the effect of IR exposure on the formation of CNVs in the offspring of a population occupationally exposed to cesium-137 ionizing radiation during the accident in Goiânia. The exposed group consisted of 07 families, in which at least one of the parents was occupationally exposed to cesium-137, including 25 individuals. A group of 11 families was used as a control, including 33 individuals with no history of exposure to IR. Microarray genotyping was conducted using the GeneChip® CytoScanHD™ (ThermoFisher, USA), followed by analysis using the ChAS® software. The statistical tests were done using the Shapiro-Wilk, Mann-Whitney U, Spearman correlation, discriminant function analysis, binomial test, and χ^2 test. All analyzes were performed using the SPSS® 21.0 statistical package, with a significance level of 5% ($p<0.05$). CNV frequencies were estimated by loss/generation, gain/generation, and burden/generation, representing $3.9x10^{-5}$, $6.8x10^{-6}$, and $4.6x10^{-5}$, respectively, for the exposed group. The control group's frequencies were $2.1x10^{-5}$, $5.9x10^{-6}$, and $3.1x10^{-5}$, respectively. Thus, the frequencies of CNVs showed statistically significant differences between the exposed and control groups by the Mann-Whitney U test. Therefore, our data showed that CNVs are induced by IR exposure in a human population, while losses were more frequent than the gains within the exposed group. Additionally, progeny from an occupationally exposed IR population showed ~1.15x more de novo CNVs than controls. Therefore, with the present study, it was possible to validate the use of a high-resolution methodology to describe a mutagenic exposure signature by IR, thus legitimizing the use of CNVs as a useful biomarker to assess germline mutation of occupationally exposed to IR population. Also, to validating the use of this marker, the study was also a pioneer in the investigation of germline mutation in humans occupationally exposed to IR.
Key-words: CNVs; occupational exposure; microarrays; 137CsCl;

ID – 27

CONVENTIONAL KARYOTYPING IS A HELPFUL INSTRUMENT FOR DIAGNOSIS AND MONITORING OF FAMILIES WITH DOWN SINDROME: CASE REPORT

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Abstract:

Introduction: Robertsonian translocations (RT) occur between acrocentric chromosomes, corresponding to the fusion of centromeres with loss of chromosomal satellites. Down syndrome (DS) is a chromosomal aneuploidy defined by the trisomy of chromosome 21. Trisomy can manifest itself in three different ways: (I) trisomy free of chromosome 21, constitutive, characterized by the presence of an extra chromosome 21 in all the cells; (II) mosaicism, when aneuploidy carrier has two lineages of cells, one of then contains an extra chromosome 21; and (III) translocations, being more common the RT, which can occur between chromosomes 13, 14, 15, 22 and 21. RT involving chromosome 21 corresponds to 2 to 4% of diagnosed cases of DS. This report deals with a family with parents phenotypically normal and a 6-month-old daughter with the clinical phenotype of DS, including epicanthus, hypotonia, ADNPM (Neuropsychomotor Developmental Delay). Objective: Establish the karyotype of the proband and their parents. Materials and methods: Cytogenetic analysis of the patient and family was performed on metaphase chromosomes obtained from cultured T lymphocytes from peripheral blood samples sent by the doctor to the human cytogenetics service of BioVida laboratory. Chromosomes were stained by GTG banding and sorted according to the International System of Cytogenetic Nomenclature (ISCN, 2020) and 20 metaphase cells from each individual were analyzed. Result: The proband exhibited an extra chromosome 21 due to a RT involving two chromosomes 21, confirming the clinical suspicion of DS (46,XX,+21,rob(21;21)(p12;p12)). The mother also presented the same RT (45,XX,rob(21;21)(p12;p12)), but balanced. The father did not present karyotypic abnormality (46,XY). Conclusion: The centric fusion between maternal chromosomes 21 poses additional risks for future conceptions. Since it compromises the eggs produced by this woman. Then, 50% of the eggs will be nullisomic for chromosome 21, an incompatible situation with the viability of zygotes, and the other half of the eggs will be disomic for chromosome 21, that, after being fertilized, will constitute a karyotype with trisomy 21. Therefore, all viable ferns of the couple will have DS. The present case reinforces and illustrates the importance of cytogenetic analysis of the parents of people with DS and how this information is fundamental for the genetic counseling and for the couple's reproductive decisions.Genetic counseling provides the necessary conditions for making informed decisions by the family.

Key-words: Robertsonian translocation; Genetic counseling; Transgeneration;

ID – 145
IDENTIFICATION OF 15Q11.2 LOSS CNV IN TWO PATIENTS WITH AUTISM SPECTRUM DISORDER DETECTED BY CHROMOSOMAL MICROARRAY ANALYSIS

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Abstract:
Prader-Willi syndrome (PWS; OMIM#176270) is a neurodevelopmental genomic imprinting disorder caused by lack of genes expression inherited from the region of chromosome 15q11-q13, usually by paternal 15q11-q13 deletions or by maternal uniparental disomy 15. PWS affects one in 15,000 to 20,000 live births, with more than 400,000 individuals worldwide. Classic clinical manifestations of PWS are characterized by severe hypotonia, hypogonadism, cryptorchidism, hyperphagia, obesity, typical facial dysmorphism, short stature, global developmental delay, intellectual disability, and behavioral disorders that can co-occur with autism spectrum disorder (ASD). Herein, we reported two cases of two boys, referred to the Núcleo de Pesquisas Replicon of the Pontifícia Universidade Católica de Goiás, with syndromic aspects and clinical indication of ASD, who presented genomic losses in 15q11.2 detected by Chromosomal Microarray Analysis (CMA). The GTG banding karyotype showed a male karyotype 46,XY, without visible numerical and structural alterations for both boys. CMA was performed using the GeneChip® CytoScanHD™, and we identified in both boys a likely pathogenic loss CNV spanning approximately 150 kb in the 15q11.2 region, encompassing the *PWRN2* gene (OMIM*611217). Genomic and epigenetic alterations that cause PWS to lead to a loss of expression of paternally expressed genes present in the 15q11.2q13 region. The lack of these genes or the failure to express them causes an absence of expression in the affected individual. The *PWRN2* gene is located close to the central region of PWS, and the exact function of this gene in determining the syndrome still needs to be elucidated. The CMA technique efficiently identified loss CNVs on chromosome 15q11.2 involving the *PWRN2* gene in two patients with ASD and syndromic features. However, specific methylation tests are required to confirm the diagnosis of PWS. In addition, genetic counseling is recommended to help the families to understand the syndrome, the family implications of the genetic contribution, and the chance of recurrence.
Key-words: autism spectrum disorder; Prader-Willi syndrome; chromosomal microarray analysis;

ID - 45

PRESENCE OF A RARE COMPLEX REARRANGEMENT, INVDUPDEL(8P), IN A PATIENT WITH MODERATE INTELLECTUAL DISABILITY: CASE REPORT.

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Abstract:

INTRODUCTION: Microdeletion and microduplication syndromes are characterized by the presence of submicroscopic alterations in the genome, usually not visible at karyotyping because they involve very small segments. They are found in patients with different clinical manifestations, such as global developmental delay, epilepsy, schizophrenia and intellectual disability, thus showing extensive phenotypic heterogeneity. OBJECTIVES: To report a novel case of a patient with intellectual disability, cardiac and skeletal problems, hypotonia and facial dysmorphisms presenting a complex chromosomal rearrangement, invdupdel(8p), associated with two pathogenic variants. METHODOLOGY: Retrospective analysis of the medical record of an individual with a molecular diagnosis of two associated pathogenic variants, not yet described in the literature, confirmed by microarray CGH. RESULTS: The patient is a 25-year-old male, with no family history of genetic disorders, whose examination (a-CGH) identified 2 pathogenic copy number variations (CNVs), a deletion of approximately 7.5 Mb involving 67 genes in 8p23.3p23.1 and a duplication with size of approximately 19.9 Mb involving 166 genes in 8p23.1p12de. The patient has moderate intellectual disability, with difficulty identifying and naming colors and numbers, and has not developed writing skills, in addition to attention deficit. A delay in motor development was also identified, with balance problems persisting into adolescence, in addition to skeletal alterations and hypotonia. He also presents speech difficulties and sialorrhea and does not require continuous medication. CONCLUSION: Alterations of this nature (deletion followed by duplication) in the 8p23 region may suggest the presence of a complex rearrangement, known as invdupdel(8p). Alterations with the presence of deletions of the telomeric region of chromosome 8 (8p23-pter) and an inverted sequence duplication in the 8p11.2-p22 region are considered rare complex rearrangements, with an estimated incidence of 1 per 10,000-30,000 live births.

Key-words: Intellectual disability; Submicroscopic alterations; a-CGH;

ID – 134

DIFFERENT BALANCED TRANSLOCATIONS IN INDIVIDUALS WITH INFERTILITY IN A SUPPORT LABORATORY.

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[1]. Rua Engenheiro Cândido Gomide, 65 - Jardim Guanabara - Campinas - SP - CEP: 13073-200. Sollutio - Soluções Diagnósticas Integradas

Abstract:
Introduction: Infertility is defined as the inability to conceive after one year of regular unprotected sexual intercourse. The genetic causes are varied and include chromosomal alterations that can be elucidated through the karyotype. Objectives: Search for chromosomal alterations that justify infertility. Methodology: Whole blood samples were received from 3 individuals, 2 females and 1 male aged 35, 37 and 34 years old, respectively, all presenting a diagnostic hypothesis of infertility. Whole blood lymphocytes were stimulated with mitogen for 72 hours in culture. Cell division was stopped in the metaphase, followed by the hypotony and fixation steps. After preparing the slide, the G-banding technique was used to analyze the chromosome under a microscope with subsequent karyotyping using an imaging system. Results: Different balanced translocations were found in each individual, involving the long arms of chromosomes 12 and 13 in the q13.1 and q34 regions, respectively; involving the long arms of chromosomes 8 and 10 in the q24 region in both; and involving the short arm of chromosome 7 and the long arm of chromosome 11 in regions p22 and q23, respectively. Conclusions: Karyotyping is an important diagnostic tool for infertility. Most balanced translocations are unique and typically benign, but their presence can increase the risk of subfertility, infertility, and miscarriages due to the possibility of incorrect segregation during meiosis.
Key-words: Infertility; Karyotype; Translocation;

ID – 102

SELECTION OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA-ASSOCIATED SNPS FROM PUBLIC GWAS DATABASES REVEAL POTENTIAL LNCRNA REGIONS FOR BRAZILIAN POPULATION ASSOCIATION STUDIES

Marco Antonio Campanário Sampaio [1]; Ana Laura Bernhard Beal [1]; Wesley Rodrigues Zabot [1]; Carlos Gabriel Alves de Lima [1]; Letícia Pontamianos [1]; Mara Albonei Dudeque Pianovski [2]; Carolina Mathias [1]; Jaqueline Carvalho de Oliveira [1]

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Abstract:

Among childhood cancer, acute lymphoblastic leukemia (ALL) is the most common. ALL is a heterogeneous disease comprised of multiple subtypes with distinct somatic genetic alterations, with different outcomes for the patients. Additionally, the genetic basis of ALL susceptibility has been supported by its association with certain genetical disorders and, more recently, by several genome-wide association studies (GWAS). Even in GWAS, most studies focused on coding portion of human genome, being the analysis of 98% of the genome (which does not code proteins) under-explored, including the analysis regions that transcribe long non-coding RNAs (lncRNAs). LncRNAs are RNA transcripts longer than 200 nucleotides with important regulatory role in several signaling pathways, including cancer processes. These molecules are closely related to genomic instability, which is characterized as one of the central hallmarks of tumor development. Besides that, these molecules have been received great attention and represent novel candidates with diagnostic, classification, prognosis, and treatment response potential markers. Genetic polymorphism in lncRNA regions have been also associated to susceptibility and protection in cancer types, but studies focused in SNP-lncRNAs in ALL are not available. Furthermore, the impact of genomic variations on these molecules in the context of genomic instability is still little explored. Therefore, in order to deeper investigate lncRNAs-SNPs associated with pediatric ALL risk, we conducted a GWAS data mining analysis, to select some potential SNP candidates, that will be evaluated the in a Brazilian ALL cohort. For this, we selected single nucleotide polymorphisms (SNPs) associated with ALL based on public genome-wide association studies (GWAS) data. To reach this goal, all variants associated with the phenotype were extracted from GWAS Catalog database (https://www.ebi.ac.uk/gwas/). SNPs associated with secondary phenotypes other than ALL were excluded from the analysis, also variants found in cohorts older than 19 years and with odds ratio less than 1.2. The *in silico* analysis found 12 potential ALL-associated SNPs in children (rs7156960, rs630662, rs10018622, rs11978267, rs11155133, rs7738636, rs75777619, rs28665337, rs4617118, rs2069426, rs9290663, rs10170236) in 10 different lncRNA regions (AC016526.2, AC025508.1, AC079921.1, AC124014.1, AL035446.1, AL355612.1, CCDC26, CDKN2B-AS1, KCNMB2-AS1, MMADHC-DT). Some of them are poor-studied regions, while other are characterized by tumor suppressor and cell cycle regulation activity. The data curated in this study should contribute to association studies and functional analysis in cancer biology, specifically in childhood leukemia biology of the Brazilian population.

Key-words: lncRNA; SNP; GWAS; ALL; polymorphism

ID - 35

THE IMPORTANCE OF THE PUBLIC HEALTH SYSTEM IN THE CHROMOSOMAL ABERRATION DIAGNOSIS - THE CONTRIBUTION OF A PARANÁ GENETIC SERVICE

Maria Clara Chita Raposo [1]; Andresa Hiromi Sakai [1,2]; Isabella Cristina Oliveira Carvalho [2]; Maria Laura Marques dos Santos [1]; Maria Eliane Longhi Barroso [1]; Tatiana Mozer Joaquim [1]

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Abstract:

Constitutional chromosomal abnormalities arise during gametogenesis or early embryogenesis and may affect all or a major portion of an organism's cells. Their estimated incidence is around 20% to 50% of all human conceptions. The major clinical consequences of the chromosome and its changes may include congenital malformations, intellectual disability, infertility and recurrent pregnancy loss. By these reason, the study of the chromosomes has become an indispensable tool for the diagnosis of many genetic disorders. Even today, when high-resolution genetic analysis can be conducted easily, the discovery of a patient whose disorder is caused by a gross chromosomal abnormality is heralded as a valuable resource for locating the disease gene. The aim of this study was to trace a casuistic profile of the 92 patients assisted by the Genetic Counseling Service of the State University of Londrina (SAG-UEL) throughout the year 2022. The main referral reasons include the diagnosis of known chromosomal syndromes, dysmorphic features with global development delay, intellectual disability, short stature, sexual differentiation disturbs besides multiple congenital malformations, recurrent miscarriages, idiopathic infertility and screening of families with a known member carrying an abnormal karyotype. Metaphase chromosomes were obtained from peripheral blood lymphocyte culture. The G-banding analysis were performed using standard protocols. At least 20 cells were routinely analyzed. In cases of mosaicism or suspected mosaicism this number was increased to 50 or more. Chromosomal abnormalities were found in 14 (15.2%) out of the 92 cases reported. Numeric disorders were found in 9 (64.2%). Among them, the trisomy of chromosome 21 (Down syndrome) was the most frequent described anomaly (7). Structural chromosome changes were observed in 4 (28%) affected patients, including translocation, inversion, deletion and one case with additional material on the long arm of the chromosome X (add(Xq13)). Mosaicism was reported for both numerical and structural rearrangements, with higher incidence in Turner syndrome cases including one karyotype described as: 45,X[23]/46,X,del(X)(q25)[72]/47,X,del(X)(q25),+mar[5]. These results are all in accordance with the prevalence data showed in other Brazilian studies. While G-banding was able to resolve the cause of more than 15% of the investigated patients, it is necessary to recognize that we are probably missing chromosomal anomalies that are below of the G-banding resolution, such as microdeletion, microduplication, lower level of mosaicisms, gene mutations or even other small chromosomal rearrangements. In these cases, the evaluation by molecular techniques is required. Regarding the scope, in this period, SAG-UEL managed to spread out its services for more than 17 cities in the Paraná state, showing its relevance to the population in the interior region of the state. Facing these data it is, therefore, important to emphasize the need of incorporation of higher resolution methods into the public system, in order to increase the rate of diagnostic success in patients with clinical suspicion of cytogenetic aberrations. Moreover, this work reinforces the need of assistential support to the patients and their families as well, thus the implementation of new genetic services, turning it accessible to all the Paraná state community should also be considered.

Key-words: Human Cytogenetic; Chromosomal Abnormalities; Genetic Counseling; Karyotype; G-banding

ID – 95

CHROMOSOMAL ABERRATIONS IN NEWBORN AT THE MATERNITY SCHOOL UFRJ - RIO DE JANEIRO

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Abstract:

Clinical genetics has been gaining increasing importance in society and public health systems due to the advanced scientific and technological development scans in recent years. Cytogenetic study is one of the most traditional methodologies in human genetics, used to detect numerical and structural chromosomal alterations. In this work, we present the results of the cytogenetic analysis performed in blood of newborns from ME - UFRJ, during the period from 2007 to 2022 studied in the cytogenetics laboratory of ME. This evaluation contributes to the clinical diagnosis and clinical guidance to family members, demonstrating the importance of cytogenetic study, providing a differentiated care service in a teaching hospital, because in addition to care it enables the professional training of participating students. Peripheral blood was used in 340 NB samples; cord blood in 54 fetuses; intracardiac puncture blood in 2 Stillborn. The samples were sent to the Laboratory of Cytogenetics of the Maternity School, coming from the Uti-neonatology, obstetric center (stillborn sand fetuses), blood samples were collected by venous puncture. In stillborns and dead fetuses, blood was collected from the umbilical cord and/or intracardiac puncture was performed. The study was conducted from cultures of peripheral blood lymphocytes, we used the nutrient Linfogrowplus (CYTOGEM). After 1 hour of colchicin (16ug/ml) (CULTILAB); 30 minutes of hypotonization with KCL (0.0075M), we performed chromosomal analysis with GTG bandeamento and the karyotype was determined by analysis under optical microscope coupled to the LUCIA Imaging System (CYTOGEM). After completion of the cytogenetic study, the results were delivered to patients and/or family members. From a total of 545 samples (3.3%) (18 samples) of the cultures did not grow. It was possible to obtain cytogenetic results in 527 samples, and in 340 cases (64.5%) the karyotype was normal and altered in 131 cases (24%). Of these 131 cases, we obtained 79 cases of free trisomy 21 (60.3%); 5 cases of trisomy 21 by translocation (3.8%); 21 cases of trisomy 18 (16%), 12 cases of trisomy 13 (9.2%); 1 case of trisomy by translocation (0.8%), 3 cases of deletion (2.3%); 3 cases of translocation (2.3%); 3 cases of r-ring (2.3%); 2 cases of Klinefelter Syndrome (1.5%); 2 cases of Triploidy (1.5%). We can conclude through the cytogenetic study in NB and Stillbirth rare cases such as Triploidies; partial trisomy; ring chromosomes, are present in the population. Thus, the cytogenetic study becomes essential for the etiological diagnosis, helping in the appropriate genetic counseling for both the patient and being able to guide the risk of family recurrence.

Key-words: CITOGENÉTICA; DIAGNÓSTICO; ASSISTÊNCIA;

ID - 222
CHROMOSOMAL STABILITY OF URINE-DERIVED STEM CELLS

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Abstract:
The therapeutic potential of urine-derived stem cells (USCs) has been increasingly understood, having similar mechanisms to mesenchymal stem cells (MSCs), with a high potential for differentiation and immunomodulation. The clinical use of this cell type is still low. However, the use of USCs in research has been increasingly frequent. Thus, the characterization of USCs with both markers of this cell type and MSCs and evaluating the genetic stability is necessary. The *in vitro* culture of stem cells represents an increased risk of genetic instability compromising the gene expression and the possibility of cancer-related abnormalities. The assessment of the genetic stability of these cells is essential to show the reliability of the results in research and minimize the risk of tumorigenesis in clinical applications. This study aimed to demonstrate the genetic stability of urinary stem cells. Cells were obtained through the urine of healthy subjects with their consent. For USCs isolation, the samples were washed with PBS and antibiotics and centrifuged. The cells were cultivated in a 6-well plate precoated with 0,1% gelatin in a specific culture medium (DMEM/F-12 + REBM) supplemented with REGM and FGF2. Subsequently, the cells were characterized by flow cytometry using antibodies to MSCs (CD14, CD19, CD29, CD45, CD73, CD105, and HLA-DR); renal tissue and USCs markers (CD13, CD324, Vimentin, Fibronectin, and β-catenin). For the assessment of genetic stability, the classical cytogenetics technique was applied (G-banding). After immunophenotypic characterization, the USCs showed a positive expression for CD13, CD29, CD73, CD105, CD324, Vimentin, Fibronectin, and β-catenin and a reduced expression for CD14, CD19, CD45, and HLA-DR. Furthermore, the cytogenetics analysis USCs showed normal karyotypes with no clonal chromosomal abnormalities. The cells showed MSCs and USCs markers and normal karyotypes. Through this study, it was possible to observe the similarity of USC with mesenchymal stem cells, which could be an alternative source for cell therapy. These cells are easy to obtain; the collection is minimally invasive without risk to the donor and showed genetic stability during cultivation. The genetic stability of these cells provides more controlled/precise experiments in basic research, providing homogeneous gene expression and a biosafety aspect in clinical application.
Key-words: mesenchymal stem cells; genetic stability; G-banding;

Acknowledgement

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ID – 216
INTERSTITIAL 13Q DELETION ASSOCIATED WITH BILATERAL CLUBFOOT

Marta Marques de Carvalho Lopes1,2 [1,2]; Paula Belline3 [3]; Nelson Gaburo Junior3 [3]; Robenilson Almeida Souza3 [3]; Rejane Alves de Carvalho Monteiro1 [1]; Adriano de Paula Sabino2 [2]; Lorena Cristina Lima da Silva1 [1]; Leniza Pola Silva1 [1]; Patrícia Caroline Angelo1 [1]; Mariana Guimarães1 [1]; Fernanda Louise Freitas Oliveira1 [1]; Keila Rivelly Pinheiro Dias1 [1]
[1]. RUA VIOLETA 988 ESPLANADA BH. INFOCITO CONSULTORIA E TREINAMENTO EM CITOGENÉTICA LTDA; [2] UFMG; [3]. R. Engenheiro Cândido Gomide, 65 - Jardim Guanabara - Campinas - SP. SOLLUTIO DIAGNÓSTICO

Abstract:
Marta Marques de Carvalho Lopes[1,2]; Paula Belline[3], Nelson Gaburo Junior[3], Robenilson Almeida Souza[3], Rejane Alves de Carvalho Monteiro[1]; Adriano de Paula Sabino[2]; Lorena Cristina Lima da Silva[1]; Leniza Pola Silva[1]; Patrícia Caroline Angelo[1]; Mariana Guimarães[1]; Fernanda Louise Freitas Oliveira[1]; Keila Rivelly Pinheiro Dias[1] [1] Infocito Consulting and Training [2]Postgraduate Program in Clinical and Toxicological Analysis, Department of Clinical and Toxicology Analysis, College of Pharmacy, Federal University of Minas Gerais, Belo Horizonte [3] Sollutio Diagnoses Structural rearrangements involving chromosome 13, especially deletions, are already well established. The correlation between the phenotype and the lost gene segment is a challenge, as affected individuals have a wide spectrum of congenital anomalies, being difficult to correlate the genotype-phenotype, especially due to the variable sizes of the missing segments and the mapping of the breakpoints. Patients with partial deletions on the long arm of chromosome 13, both interstitial and terminal, may have moderate to severe intellectual disability, facial dysmorphisms, growth retardation, severe defects in the renal, cerebral, genital, gastrointestinal and cardiac systems, ocular anomalies, abnormalities in the limbs, neoplasms, among others. Thus, the discussion of cases of individuals with such alteration is essential, as it contributes to a more refined elucidation of the genetic condition of each affected person, as well as associating it with the phenotype presented. Within this context, we present the case of a newborn with an interstitial 13q deletion, presenting with a congenital malformation of the lower limbs. The genetic alteration was detected by carrying out the constitutional karyotype exam, using the G-banding technique. This is a male neonate, who at birth had bilateral clubfoot, with a 46XY,del(13)(q14q22) karyotype. According to the DECIPHER database, around 282 genes are mapped in the region comprising 13q14q22, corresponding to a size of approximately 26.60 Mb. Determining whether the size of the lost segment was exactly 26.60 Mb by itself is not possible using only conventional techniques. Musculoskeletal abnormalities, such as bilateral clubfoot, are already well established in 13q- syndrome, however, there is still no specific gene described located in 13q14q22 that establishes such a phenotype by itself. In the 13q14 region, the *RB1* gene is present, which is a tumor suppressor gene. Changes in the pattern of expression of this gene predispose to an increased risk of developing neoplasms, with retinoblastoma being the most frequent in children. The investigation of chromosomal alterations in carriers of congenital anomalies is essential in the search for a diagnostic answer and in genetic counseling. In the G-band karyotype test, it is possible to visualize the entire chromosome set of the individual, and nationwide it is the most accessible genetic test for the population, being the starting point for the diagnosis. The result found in our service, through the performance of the constitutional karyotype exam using the G-banding technique, corroborates the data described in the literature, allowing to correlate the phenotype of the newborn with its genetic condition. Although the clinical information provided was limited, it was possible to associate the interstitial 13q deletion with the congenital clubfoot phenotype, thus elucidating the cause of its congenital malformation. Thus, the understanding of the interaction and gene contribution and the clinical phenotype in patients with rare syndromes, enables a better therapeutic and prognostic approach, in order to provide a better quality of life for them. Keywords: Interstitial 13q deletion; congenital bilateral clubfoot; conventional cytogenetics
Key-words: Interstitial 13q deletion; congenital bilateral clubfoot; conventional cytogenetics;

Acknowledgement

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ID - 67

CHROMOSOMAL STABILITY OF STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Mateus de Oliveira Lisboa [1]; Gabriel Silva Morais [1]; Valderez Ravaglio Jamur [1]; Ana Helena Selenko [1]; Tatiane Mie Arakaki Moraes da Silva [1]; Isabel Carvalho Oliveira [1]; Giovanna de Araujo Ricardo Rossano [1]; Paulo Roberto Slud Brofman [1]; Letícia Fracaro [1]

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Abstract:

Stem cells from human exfoliated deciduous teeth (SHED) comprise a valuable source of stem cells that have gained significant attention in regenerative medicine. SHED are relatively easy to obtain since deciduous teeth are odontological waste, eliminating invasive procedures for obtaining mesenchymal stem cells (MSCs), such as bone marrow aspiration. Like other types of MSCs, SHED have immunomodulatory properties and the ability to differentiate in several tissue types. However, because of its ectodermal origin, SHED seems more likely to differentiate into neuronal-like cells when compared to other sources. These neuronal differentiation capabilities make SHED a promising candidate for cell-based therapies for neurological disorders like Parkinson's disease and spinal cord injury. However, in vitro expansion is necessary to obtain enough cells, which can increase the risk of genetic instability. Genetic instability can have significant implications for the therapeutic potential of stem cells. Mutations and chromosomal abnormalities can alter the properties of stem cells, such as their differentiation capacity and immunogenicity. Additionally, these genetic alterations can increase the risk of tumorigenesis, a concern for stem cell-based therapies. Although the genetic stability of bone marrow-derived MSCs are extensively described, little is known about the genetic stability of SHED. Therefore, more research is needed to verify the genetic stability of these cells. This study aimed to verify the chromosomal stability of SHED. Healthy teeth from five donors (8.8 ±1.8 years) were collected, and SHED were isolated and expanded in vitro. Following the International Society for Cell and Gene Therapy (ISCT) guidelines, the cells were characterized by immunophenotyping and differentiation in two lineages (adipogenic and osteogenic). Conventional cytogenetics analyses were performed in passage four using G-banded karyotyping. The metaphases were analyzed and interpreted according to the International System for Human Cytogenomic Nomenclature (ISCN 2016). SHED showed characteristics of MSC according to the ISCT criteria. The five samples analyzed were devoid of clonal chromosomal abnormalities. However, tetraploid cells were found in all the cases. On the one hand, the absence of clonal abnormalities suggests a possible safety in using these cells. On the other hand, the presence of polyploidy in the form of tetraploidy, a feature known to increase the risk of genetic instability, suggests the need to routinely verify the genetic stability of these cells both in basic and clinical research. The verification of the genetic stability of SHED may guarantee that these cells will perform their expected function and diminish the risk of adverse outcomes in patients. In conclusion, SHED are a valuable source of stem cells that seems genetically stable, as shown by Giemsa banding, making them an attractive candidate for regenerative medicine applications.

Key-words: Dental pulp; mesenchymal stem cells; g-banding;

Acknowledgement

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ID – 182

KARYOTYPE ANALYSIS OF ADVANCED THERAPY SPECIMENS IN A ROUTINE CYTOGENETIC LABORATORY

Mauren Fernanda Moller dos Santos [1]; Renata Kiyomi Kishimoto [1]; Roberta Maria da Silva Oliveira Safranauskas [1]; Amanda Aparecida Cardoso Coimbra [1]; Gilmara de Sousa e Silva [1]; Jason de Lima Silva [1]; Raquel de Melo Alves Paiva [1]; Micheli Severo Sielski [1]; Elvira Deolinda Rodrigues Pereira Velloso [1,2]

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Abstract:
Cytogenetic study is a prerequisite for the therapeutic use of cellular/advanced therapy products required by regulatory agencies (ANVISA). This aims to guarantee the safety and quality of the cells for clinical use, preventing the use of extensive cell manipulations with chromosomal alterations. In this study we evaluated the frequency of cytogenetic abnormalities in samples from advanced therapy services received at the laboratory between March/2015 and January/2023, also analyzing the cellular origin of the samples and the purpose of the test (treatment, research). Samples of cells to be used as an advanced therapy product were evaluated by conventional karyotyping, performed according to the standard technique for cultures of adhered and suspension cells, GTW banding and reported according to ISCN standards. 167 samples were evaluated, some of them coming from the same individual or cell lineage due to the use of cells in different culture passages. 101 (60.5%) samples were received from our own institution and 66 (39.5%) samples were sent by external services. The evaluated cell type [and tissue origin] were: 95 (56.9%) mesenchymal [30 bone marrow (18.0%), 17 muscle (10.2%), 14 palate periosteum (8.4%), 14 adipocytes (8.4%), 13 umbilical cord (7.8%), 7 tooth pulp (4.2%)], 17 (10.2%) NK cells [umbilical cord blood], 12 (7.2%) chondrocytes [cartilage], 11 (6.6%) cell lineage [7 HEK293-3F6 (4.2%), 3 K562 (1.8%) and 1 OCI-AML2 (0.6%)], 10 (6.0%) iPSC [urine epithelial cells], 8 (4.8%) lymphocytes [peripheral blood], 7 (4.2%) renal cells [kidney], 6 (3.6%) CAR-T [peripheral blood] and 1 (0.6%) fibroblast. The application of cells was primarily designed for treatment [120 (71.9%)]: 39 (23.4%) chondral lesions and osteoarthritis, 37 (22.2%) urinary incontinence, 21 (12.6%) neoplasms hematological, 14 (8.4%) COVID lung injury and 9 (5.4%) cytomegalovirus - post HSCT; and for research [47 (28.1%)]: 19 (11.4%) culture validation and cell expansion, 11 (6.6%) cell authenticity, 10 (6.0%) neurological processes associated with Down syndrome and 7 (4.2%) chronic kidney disease. Among the samples designated for treatment, 87 (72.5%) samples presented a normal karyotype result, 5 (4.2%) normal karyotype with non-clonal alterations, 9 (7.5%) absence of metaphases and 19 (15.8%) karyotype with clonal alterations. Among the clonal alterations detected, 10 (52.6%) cases had trisomies, 7 (36.8%) structural alterations, 1 (5.3%) tetraploidy and 1 (5.3%) loss of the Y chromosome. Among the samples designated for research, all samples that showed complex karyotype [10 (66.7%)] were cells originating from a cell lineage for the purpose of evaluating cell authenticity; the others had trisomies [3 (20.0%)] and structural alterations [2 (13.3%)]. Extensive manipulation of cells and advanced therapy products can contribute to instability, which may increase the risk of cytogenetic alterations and tumorigenic potential, suggesting that products with clonal cytogenetic alterations should be discarded.
Key-words: advanced therapy; cell therapy; karyotype;

ID – 174

CYTOGENOMIC CHARACTERIZATION OF Y-CHROMOSOME IN PATIENTS WITH MOSAIC TURNER SYNDROME

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Abstract:

Turner syndrome (TS) is a common human aneuploidy, cytogenetically resulting from complete or partial absence of the second sex chromosome in ~50% of cases. The other cases present mosaicism, involving a normal or abnormal sex-chromosomes. We performed G-banding, fluorescence in situ hybridization and cytogenomic Y regions investigation (*SRY* at Yp11.31, *ZFY* at Yp11.31 and , *UTY* at Yq11.221) by MLPA - P095 Aneuploidy kit - in 8 TS patients. A total of 4/8 (50%) of TS patients showed Y-chromosome regions: one case with one copy of SRY_1, SRY_2, ZFY and UTY probes; one case with one copy of SRY_1 and SRY_2 probes; one case with one copy of SRY_1, SRY_2 and UTY probes and one case with duplication (two copies) of all probes (SRY_1, SRY_2, ZFY and UTY). The presence of Y-chromosome regions in TS patients can contribute to phenotype variability at TS. Patients with *SRY* and other genomic determinant sex sequences are more likely to progress to gonadal lesions. In addition, the *ZFY* gene is expressed in testis and in other tissues, such as esophagus, urinary bladder and bone marrow. The *UTY* gene acts directly with cell cycle control genes, histone modulation and apoptosis. Thus, the unambiguous characterization of the Y chromosome regions present in mosaic TS patients may contribute to understanding the genome architecture, to the refinement of the genotype-phenotype relationship and to the appropriate therapeutic approach for these patients.

Key-words: Turner Syndrome; Y-chromosome; Cytogenomic technique; Karyotype; MLPA

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ID – 188

PARTIAL TRISOMY SYNDROME OF LONG ARM OF THE CHROMOSOME 4: A CASE REPORT

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Abstract:

A partial trisomy 4q is a rare syndrome of chromosomal anomaly, which results from the duplication of the long arm of chromosome 4. Individuals with this anomalous condition have variable phenotypic characteristics, such as psychomotor delay, intellectual disability, craniofacial dimorphism (microcephaly, low implantation, prominent ears, etc.). In addition, there are also reports of malformation in external organs such as: heart muscle, kidneys, cryptorchidism, hypotonia and hearing impairment. This case report describes a female patient, seven years and two months old, weighing 50.7063 pounds and measuring 3.64173 feet. The patient has low ears, low implantation, bulging forehead, ocular hypertelorism, shortened FPs, low nasal band, palate with complete cleft, cleft lip on the left, extensive heterochromia on the right thigh, dorsolumbar scoliosis on the left, brachydactyly with hypoplasia in the 3rd metacarpal and absence of phalanges on the right hand; Duplicate rays showing two phalanges on supernumerary finger in left hand; The skull has a microcephalic configuration and a retrocerebral arachnoid cyst on the left; Patent ductus arteriosus, without hemodynamic repercussions. The patient has already undergone plastic surgery to correct ectrodactyly and in the ear to reduce episodes of recurrent otitis. Objective: The goal of this work was to investigate possible numerical and structural genomic variations using the karyotyping method and CGH Array to correlate chromosomal rearrangements with the observed phenotype. Materials and methods: A peripheral blood sample was collected with heparinized syringes to perform a T-lymphocyte culture, followed by a karyotype test with G banding and treated with trypsin, followed by a CGH SNP-Array test. Results: After the G-band karyotype exam showed 46.XX, which is expected for females, the SNP-Array test was performed, which detected a chromosomal alteration classified as pathogenic, compatible with the duplication of the interstitial segment of 3.4 Mb of the long arm of chromosome 4. According to the DECIPHER database, the duplicated segments are mapped to 32 genes, 15 of which code for proteins, five of which are already associated with diseases. Conclusion: The size and gene content of the 4q31.21q31.23 duplication explain the presence of the clinical picture.

Key-words: trisomy; SNP-Array; Karyotype;

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ID 17-
DETECTION OF CLINICALLY RELEVANT STRUCTURAL VARIANTS IN AUTISM SPECTRUM DISORDER BY OPTICAL GENOME MAPPING - A CASE REPORT

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Abstract:
Autism Spectrum Disorder (ASD) is the term for a group of pervasive neurodevelopmental disorders characterized by impaired social and communication skills, along with repetitive and restrictive behavior. The genetic complexity of ASD reflects its phenotypic complexity. It is known that patients with ASD often go through a "diagnostic odyssey" when trying to find a genetic explanation for their conditions. This study aimed to elucidate these questions by combining genetic techniques, sophisticated bioinformatics analysis, and phenotypic data. We report a male patient, 11 years old, followed in the genetics sector of the *Hospital do Servidor Público Estadual "Francisco Morato de Oliveira"*. The patient was born at term at 37 weeks, by cesarean delivery, weighing 3,945g, 49 cm, with no complications during pregnancy, childbirth, or the neonatal period. There is a report of a significant delay in neuropsychomotor development, he had independent gait at three years of age and sphincter control also at three years of age, within the expected range for his age. Language development evolved with delay, first words at four years old, and short sentences at five years old. Currently, the patient has difficulties in understanding expressive verbal and non-verbal communication. The patient does not present any genetic alteration previously detected by the Guthrie Test, Karyotype, Molecular Study of Fragile X Syndrome, Multiplex Ligation-dependent Probe Amplification, and Exoma. Due to the lack of conclusive results detected by the previous methodologies, we focused on the detection of de novo protein truncation and missense variants in genes involved in ASD (high impact de novo variants) detected by the new methodology called Optical Genome Mapping (OGM). Our goal was to determine the potential contribution of all forms of structural variation in genomic regions to ASD. We performed a genome-wide assessment for structural genetic variants via OGM. This technique detects structural variations such as: Heterozygous Insertions/Deletions greater than 500pb (99% sensitivity). Balanced or unbalanced translocations, greater than 50kb (95% sensitivity). Inversions greater than 30kb (99% sensitivity). Duplications greater than 30kb (97% sensitivity). From the results found, genomic analysis tools were used to account for the phenotypic effect of these alterations on ASD. Descriptive analyzes were performed in the R programming language. The OGM was able to detect 67 structural variants (SVs), categorized into: Deletions (55%), Insertions (34%), Inversions (6%), and Duplications (5%). The genes contained in these structural variants are related in different degrees to ASD, according to the literature, corroborating the patient's clinical picture. Dissecting the cytogenomic architecture of the results obtained with the OGM, we found structural variants that occur in gene regions related to neurodevelopment, in addition, some of the detected genes were haplo-deficient. These preliminary results highlight biological insights, particularly related to neurological function, and establish that the OGM can be very productive in the short term to detect cytogenomic alterations in ASD. In conclusion, our

preliminary findings reinforce the important implications of using the OGM methodology to understand the genetic underpinnings of ASD compared to other methodologies.
Key-words: Autism Spectrum Disorder; Structural variation; Optical Genome Mapping;

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ID - 118
CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF RING CHROMOSOMES: PRELIMINARY RESULTS

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Abstract:
Ring chromosomes are rare rearrangements, formed by the breakage of terminal regions of both arms of the chromosome followed by the fusion of the proximal regions, generating a circular structure and causing losses at terminal regions. This configuration results in chromosomal instability at mitosis leading to secondary changes. The phenotype of carriers of ring chromosomes varies according to the extent of deletions and the chromosome involved. This study aimed to investigate the instability of ring chromosomes and characterize their breakpoints. To date, five individuals were investigated, one ring chromosome 6, one 14, two 22 and one X. G-banded karyotyping, with a count of 200 metaphases, was performed for all cases. Chromosomal microarray analysis (CMA), using the CytoScan™ 750K and HD chips from Affymetrix® - Thermo Fisher Scientific Inc. (LifeTechnologies, Carlsbad, CA, USA), was performed for the individual with ring 6 and two individuals with ring 22. Secondary cytogenetics changes were found in all cases, with the loss of the ring being the most common. The secondary changes were found for the ring chromosomes 6 (1.5%), 14 (9.5%), 22 (4.5%), 22 (4%) and X (95%) and the percentages of loss in each case were 0.5%, 8.5%, 4%, 2.5% and 94% on ring chromosomes 6, 14, 22, 22 and X, respectively. Other secondary changes found were: two ring chromosomes (rings 6, 14 and 22), dicentric rings (rings 6, 14, 22 and X) and open rings (ring 22). The CMA analysis on chromosome 6 and both 22 showed different deletion sizes and breakpoints. Ring chromosome 6 showed deletions at p25.3-p25.2 region (GRCh37 chr6:156,974-3,184,710), of 3 Mb, and q26-q27 region (GRCh37 chr6:163,233,738-170,919,482), of 7.6 Mb. In one of the cases of ring chromosome 22, there was a deletion at region q13.31-q13.33 (GRCh37 chr22:48,309,424-51,197,766) of 2.9 Mb. In the other, there was a deletion at q13.31-q13.33 region (GRCh37 chr22:43,906,771-51,197,838) of 7.3 Mb. In conclusion, the secondary changes found in different percentages showed that the chromosomal instability varies from case to case, even when the involved chromosome is the same. CMA allowed the precise identification of deleted regions, which also showed heterogeneity of breakpoints, improving the genotype-phenotype correlation. The investigation of ring instability in cells from buccal smear will be performed by fluorescent in situ hybridization and the breakpoints will be further refined by low-coverage genomic sequencing.
Key-words: Ring chromosomes; chromosomal instability; chromosomal microarray analysis; ;

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ID – 153

CYTOGENETICS AND CYTOGENOMICS REVEALED HYPERDIPLOIDY ASSOCIATED WITH *IKZF1*, *CDKN2A/B* AND *ETV6* DELETIONS AND *BCR-ABL* AND *ETV6-RUNX1* TRANSLOCATIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Rayane de Souza Torres [1,2]; Valeska Vieira [2]; Viviane Lamim Lovatel [1]; Maria Socorro Pombo de Oliveira [2]; Teresa de Souza Fernandez [1]

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Abstract:

Childhood Acute Lymphoblastic Leukemia (ALL) is the most common malignancy accounting for 25% of all pediatric cancer. The cytogenetic pattern is crucial to determine prognostic and therapeutic decisions. The most common clonal chromosome aberrations in ALL is numerical (about 30%) and divided into low or near-hyperdiploidy (47-49 chromosomes) and high hyperdiploidy (HeH, more than 50 chromosomes). Also, it is related to a favorable prognosis. However, associations of hyperdiploidy with other alterations such as deletions and/or translocations may change prognosis. The most frequent deletions involved are *IKZF1* gene (coding for the IKAROS transcription factor), *CDKN2A/B* gene (coding for tumor suppressor proteins) and *ETV6* gene (Transcription factor involved in B-cell development). The most frequent translocations involved are *BCR-ABL1*, *TCF3-PBX1* and *ETV6-RUNX1*. Besides, the literature review showed a few studies focused on hyperdiploidy associated with structural chromosomal alterations. Therefore, the aim of this study was to analyze the frequency of hyperdiploidy associated with structural chromosomal alterations (deletions and translocations) in a cohort of BCP-ALL. Karyotyping by G-banding was performed in 50 cases diagnosed with BCP-ALL. MLPA SALSA Probemix P335 was used to detect deletions and RT-PCR were performed to identify fusion genes associated with recurrent chromosomal translocations. Conventional karyotyping enabled the identification of hyperdiploidy in 12/50 cases. Ten cases were HeH and 2 cases were Low Hyperdiploidy. Using complementary methodologies (MLPA and RT-PCR) it was possible to detect hyperdiploidy associated with other alterations in 6 cases: HeH and *ETV6* deletion (1 case), HeH and *CDKN2A/B* deletion (2 cases), HeH and *IKZF1* deletion (1 case), HeH and *BCR-ABL1* (1 case) and HeH and *ETV6-RUNX1* (1 case). The most frequent trisomies observed were +4, +6, +8, +10, +14, +21 and +22. In this study, we showed the importance of combination of cytogenetics, cytogenomics and molecular methods to characterize the chromosomal pattern in pediatric patients with ALL to aid in prognosis and in treatment choice, as in the case of HeH and *BCR-ABL* the use of tyrosine kinase inhibitor.

Key-words: Hyperdiploidy; BCP-ALL; Cytogenetics; Cytogenomics;

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ID – 33

FREQUENCY OF ADDITIONAL CHROMOSOMAL ALTERATIONS IN CHRONIC MYELOID LEUKEMIA: ANALYSIS BY CONVENTIONAL CYTOGENETICS AND FISH

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Abstract:

OBJECTIVE: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm of clonal origin in hematopoietic stem cells, hallmarked by the specific cytogenetic alteration t(9;22)(q34;q11), the Philadelphia chromosome (Ph). The Ph carries the oncoprotein BCR-ABL1 fusion gene, which causes cell proliferation, inhibits cell differentiation, and cell death. CML can classify into three phases: an indolent, early phase known as the chronic phase (CP) and a more advanced aggressive phase, consisting of an accelerated phase (AP) and a fatal blast crisis phase (BC). Currently, the gold standard treatment of CML is with tyrosine kinase inhibitors (TKIs). Despite the effectiveness of TKIs, a minority of patients develop resistance to TKIs and may progress to a CML-AP through BCR-ABL-dependent or independent mechanisms. Additional cytogenetic alterations (ACAs) can be detected before the clinical evolution of CML. ACAs are considered a reflection of genetic instability and are more frequent in CML-BP. Furthermore, patients with ACAs are associated with a worse therapeutic response. In this sense, this study aimed to report the frequency of ACAs in CML. MATERIALS AND METHODS: We cytogenetically analyzed 201 patients from *Instituto Nacional do Câncer* diagnosed with CML over 8 years by G-banding and fluorescence *in situ* hybridization (FISH) (Vysis LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe Kit). Chromosome abnormalities were described according to the International System of Human Cytogenomic Nomenclature (ISCN, 2020). RESULTS: Adults patients represented 94% (189/202), and the mean age was 48 years, 69 females and 120 males. Most patients were classified as CML-PC (88%), while CML-AP and CML-BP represented 5% and 7% of cases respectively The Ph was presented as an isolated alteration in 90% (170/189), while ACAs were observed in 10% (19/189). The ACAs observed in this study were: 3 (15%) cases with double Ph, 6 (30%) with complex karyotypes, 5 (25%) trisomy 8, 1 (10%) t(9;10;22), 1 (10%) t(1;9), 1 (10%) with a gain of chromosome Y, 1 with deletion 5p (10%), 1 (10%) t(9;12;22). ACAs observed in 90% of patients with CML-CP and 10% in CML-AP. In pediatric patients, the mean age was 11 years, with 5 females and 7 males. All pediatric CML were classified as CML-PC and had Ph without ACAs. CONCLUSION: The early detection of ACAs helped to identify patients who had the progression of the disease, showing the importance of conventional and molecular cytogenetics in the follow-up and choice of treatment for these patients. This study was supported by Ministério da Saúde.

Key-words: Chronic myeloid leukemia; Philadelphia chromosome; Additional cytogenetic alterations;

ID – 214

CLINICAL FINDINGS AND CYTOGENETIC OF PATIENTS WITH DELETION 9P SYNDROME (SYNDROME DI ALFI)

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Abstract:

Deletion 9p syndrome or di Alfi syndrome is a rare chromosomal disorder and presents wide spectrum of clinical features. This syndrome was reported for the first time in 1973 by Alfi and results in partial monosomy of the short arm of chromosome 9. The major clinical features are psychomotor delay, craniofacial dysmorphism (trigonocephaly, palpebral fissures, low-set ears), cardiac defects, abnormal genitalia and hypotonia. Most patients have either pure terminal deletions and *de novo* (sporadic), with the breakpoints usually occurring between bands p22 to 24. The phenotype of each patient depends on the size, location and which genes are involved, although deletions only in 9p24 are sufficient to characterize the syndrome. There are few reports of patients who present syndrome, and most cases are identified with ages and dysmorphic signs varying, which makes it difficult to delineate a standard phenotype, especially when dealing with low prevalence alterations. In this study were included a total of 7 patients, 4 females and 3 males detected by conventional cytogenetic analysis. The ages ranged from 5 days to 19 years. Three patients had developmental delay and learning disability. The trigonocephaly was observed in three other patients, one of which also included low set ears, in addition to these another patient exhibited hypotonia. The results of the chromosomal analysis of these patients revealed a karyotype with terminal deletion of the short arm of chromosome 9 varying between points p21-p24. In this segment, more than 220 genes are mapped comprising a region of approximately 20Mb, according to the DECIPHER database. Some genes found in this region play important roles, such as *FOXD4, KANK1, DOCK8* and their deletion causes behavioral abnormalities, intellectual disability and delayed speech. We postulate that the haploinsufficiency of these genes would be involved with the alterations observed in three of our patients. The *CER1* and *FREM1* genes are being suggested in several studies as being responsible for trigonocephaly, although many patients with this phenotype do not have these genes deleted. In this way, we can infer that they are not the only genes responsible for this characteristic. Currently, a critical region for this phenotype is suggested to be within the 9p23 segment. The three patients presented here who manifested this clinical condition have this region deleted, suggesting that this segment may indeed contain an essential regulatory sequence for the clinical condition described here. The only patient who had global hypotonia was a boy with a distal deletion at 9p24. A specific gene that corroborates the observed phenotype has not yet been described, however, several patients with the syndrome present this clinical condition, including those described by Alfi, and this correlation is already well established. Considered a rare syndrome, the publication of new cases helps in the knowledge of the clinical characteristics, helping in the early diagnosis. Thus, well-described clinical signs and better definition of breakpoints have been advocated by several authors, in order to relate which gene would be modulating each phenotype. Accurate diagnosis is essential to exclude other causes and provide adequate genetic counseling.

Key-words: Deletion 9p syndrome; trigonocephaly; rare diseases;

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ID – 42

CYTOGENOMIC CHARACTERIZATION OF THE HUMAN COLORECTAL CARCINOMA CELL LINE HCT-8

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Abstract:

Colorectal carcinoma (CRC) is the third most common malignancy in the world. Numerous genomic changes associated with the onset and progression of this type of cancer have been identified. Part of these results have been obtained from studies using cell lines, which are excellent models for *in vitro* studies. In this sense, the cytogenomic characterization of cancer cell lines is an important step in order to better understand and interpret results concerning altered cell pathways, mutations in critical genes for cancer, and anti-cancer drug tests, among others. The human cell line HCT-8 has been extensively used in studies to evaluate different aspects of CRC, including genetic predisposition and antitumor drug development. However, no genomic characterization has been performed so far. Thus, in order to confirm the occurrence of genomic changes in HCT-8 that validate it as a model for studies related to CRC, we performed the cytogenomic characterization of this cell lines through cytogenetic analysis and aCGH. For this purpose, metaphase chromosomes and genomic DNA were obtained from HCT-8 cells, and used for conventional staining and aCGH experiments. The results revealed a modal diploid number 2n= 46. However, despite of maintaining the normal human diploid number, a high frequency of copy number alterations (CNAs) was observed, distributed in almost all chromosomes (except pairs 21 and 22). The main gains were found in pairs 14, 18, and X, and losses mainly in pairs 3, 7, and 18. A series of altered genes previously described in tumor samples and directly associated with colorectal carcinoma carcinogenesis (*TP53, KRAS, DCC, SMAD4, PTEN*) were also affected by CNAs. In conclusion, the cytogenomic characterization of the HCT-8 cell line confirmed that it is a potential model for *in vitro* studies for understanding the carcinogenesis of CRC, as well as for understanding the therapeutic response, and testing compounds with anticancer potential.

Key-words: aCGH; Cancer Cell Line; CNAs; Colorectal Carcinoma; Cytogenomics

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Instituto Evandro Chagas

ID – 146

DETECTION AND EVALUATION OF COPY NUMBER VARIATION OF UNCERTAIN CLINICAL SIGNIFICANCE: COMPARISON BETWEEN DATA FROM CHROMOSOMAL MICROARRAY ANALYSIS AND WHOLE EXOME SEQUENCING

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Abstract:

The limitations for detecting copy number variations (CNV) in data from Whole Exome Sequencing (WES) are well known. However, this approach is routinely used since WES is recommended as the first-tier diagnostic test for individuals with neurodevelopmental disorders and several diseases such as hearing and vision impairment, among others. The analysis of CNV in WES data, combined with sequence variant analysis, increases the diagnosis rate in these cases. Therefore, studies about the accuracy of CNVs detection on WES data are still important. The aim of this study was to compare the CNVs detected by chromosomal microarray analysis (CMA) and WES data with focus on those classified as variants of unknown significance (VUS). Data from 35 individuals with neurodevelopmental delay/intellectual disability and/or multiple congenital anomalies, with CNVs classified as VUS previously detected by CMA, were included. CMA was performed using the CytoScan™ 750K chips or HD array (ThermoFisher®) and data was analysed with the Chromosome Analysis Suite software (ThermoFisher®) (hg19). We considered a minimal number of 25 probes for losses detection and 50 for gains detection, according to the manufacturer's instructions. WES was performed using the Agilent SureSelect Target Enrichment V5 (Agilent®) capture kit (hg19), followed by sequencing on the Illumina HiSeq platform (Illumina®). CNV calls on WES data were obtained by the CoNIFER (Copy Number Inference From Exome Reads) algorithm that considered a minimum of three consecutive exons to the calls. The comparison between CNV calls by both methods, independent of class, was performed with data from 20 individuals. With CMA (n=20) 453 CNVs were called, 112 gains (Sizes: Max= 3.9 Mb; M_{in}= 8 kb; M_e= 411 kb) and 341 losses (Sizes: M_{ax}= 3.3 Mb; M_{in}= 1 kb; M_e = 74 kb). Using CoNIFER, 321 CNVs were called in WES data, 209 gains (Sizes: M_{ax}= 51 Mb; Min= 0.6 kb; Me= 125 kb) and 112 losses (Sizes: M_{ax}: 2,1 Mb; Min: 1.5 kb; Me: 148 kb). Only 48 CNVs were called by both methods, 24 gains (M_{ax}=3,5 Mb M_{in}=20 kb M_e= 586 kb) and 24 losses (M_{ax}=960 kb, M_{in}=15 kb, M_e= 151 kb) all of them encompassing protein coding genes. Only one intragenic loss identified in WES data and not detected by CMA, encompassing the *PCLO* gene (6/25 exons), was considered potentially relevant. Regarding CNVs VUS, from data of 35 individuals, we identified 42 CNVs by CMA, 30 (71.4%) of which were also identified by WES. Among 12 CNV-VUS not identified, some harboured small genes with few exons, large intronic regions with few exons, small parts of genes, or large intergenic regions compared to the gene portion. Furthermore, three CNVs that encompass the *SHOX* gene region (pseudoautosomal region) were not detected by WES, because the pseudoautosomal region is not included in the analysis. There was a high discrepancy of CNV calls by the two methods, which can be expected due to differences of genomic coverage between the microarrays and the capture kit. Another reason is the high rate of false calls by algorithms used for CNV calls on NGS data, already described in the literature. However, the majority of CNVs classified as VUS were also detected by CNV analyses from WES data using CoNIFER. In addition, an intragenic loss was detected in WES data, but not by CMA.

Key-words: copy number variation; variants of unknown significance; whole exome sequencing;

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ID – 16

XP22.33-22.13 DUPLICATION IN A PATIENT CARRYING A RECOMBINANT CHROMOSOME X DERIVED FROM AN INHERITED INTRACHROMOSOMAL INSERTION

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Abstract:

Insertions are structural chromosomal rearrangements that occur when a single chromosome breaks in two places and a piece of the chromosome is reinserted into another non-homologous chromosome (interchromosomal insertion) or a different location of the same chromosome (intrachromosomal insertion). Since these rearrangements require at least three break events they are classified as complex chromosomal rearrangements (CCR). The phenotypic consequences of insertions may vary by several different ways, such as increased gene expression due to the duplicated segment, loss or gain of function by disruption, cryptic chromosome imbalances at the breakpoints or even abnormal gene expression due to position effect. Here we describe the genotype and the phenotypic effects of a patient carrying a recombinant chromosome X that arose from a maternal balanced intrachromosomal insertion. The imbalance resulted from a meiotic crossing-over between the displaced Xp chromosomal segment and its normally located homologous region. The proband, a 13 years-old boy, was referred for clinical genetic evaluation due to developmental delay, intellectual disability, behavioral disorders and mild dysmorphic facial features. The karyotype of the proband was initially described as 46,XY,inv(9)(p12q13). His mother, with normal phenotype, was also evaluated with karyotype: 46,XX,inv(X)(p22.1),inv(9)(p12q13). The paternal karyotype was normal. Array-CGH analysis showed that the proband was a carrier of a 13.05 Mb duplication located on chromosome X (Xp22.33Xp22.12) including 99 genes. FISH studies, using BAC customized probes, performed in the patient and his mother revealed that the patient´s duplication was located at the terminal portion of the long arm of chromosome X (Xq). Its analysis suggested that the Xp duplication was the consequence of a balanced insertion inherited from his mother, rather than an inversion as initially described. The occurrence of the intrachromosomal pericentric insertion for both the proband and his mother (balanced insertion carrier) was confirmed by M-Band analysis. The G-banded karyotype of the proband was thus redefined to be 46,XY,rec(X)dup(Xp)ins(X)(q26.1p22.33p22.13)mat whereas the maternal karyotype was reported as 46,XX,ins(X)(q26.1p22.33p22.13). While hard to establish a correlation between the duplication of single genes and our patient's phenotype, there are several reports in the literature associating the duplication of Xp22.33Xp22.12 region with X-linked intellectual disability, neurodevelopmental disorders, cardiovascular problems and mild dysmorphic facial features. We suggest that the synergistic action of the 99 genes presented in this duplication may be collectively contributing to the clinical findings. We also speculate that position effects leading to facultative inactivation of genes adjacent to the duplicated Xp region or even other genetic mechanisms such as the X-inactivation may be a consideration to the observed phenotype. Furthermore, this study has provided the importance of the combination of molecular cytogenetics and cytogenomic approaches to better characterize complex chromosome rearrangements. The association of FISH, M-Band and aCGH has provided not only a precise definition of the breakpoints at X chromosome, as well as the parental origin of the rearrangement, resulting in better clinical management for the family and improving genetic counseling.

Key-words: Complex chromosomal rearrangement; Xp duplication; G-band; M-band; FISH

ID – 201

THE INVOLVEMENT OF CHROMOSOME 11 IN THREE CASES OF COMPLEX KARYOTYPE OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

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Abstract:

Complex karyotype (CK) is defined by the presence of three or more cytogenetic numerical and/or structural aberrations in the same clone, detected by chromosomal G-banding analysis. In chronic lymphocytic leukemia (CLL), CK cases with three or four aberrations (low-CK and intermediate-CK, respectively) followed aggressive disease courses only in the presence of deletion of chromosome 17p and/or *TP53* mutations. High-CK is defined when five or more abnormalities are observed. Patients with high-CK exhibit unfavorable clinical outcomes, independently of the clinical stage in CLL, *TP53* aberrations, and the expression of somatically hypermutated (M-CLL) or unmutated immunoglobulin heavy variable genes. Patients with CK and trisomies of chromosomes 12 and 19 show an indolent course. In our cytogenetic study with 49 patients with CLL, we observed three cases with CK, all with involvement of chromosome 11. Two of them exhibited involvement of the long arm of chromosome 11, the first one with a deletion at 11q and the second with a derivative chromosome with additional material at 11q. The karyotype of these patients were: 45~48,XY,del(11)(q2?3)[4],+12[3],+mar(?19)[3][cp10]/46,XY[12] and 44~46,XY,X,der(11)t(?;11),del(13)(q?21),+2mar,inc[cp28]/47,XY,+12[4]/46,XY[7], respectively. The trisomy of chromosome 12 was present in both cases. In both cases, the FISH test showed the deletion of *the ATM* gene, located at 11q22. These two patients were diagnosed with anemia, thrombocytopenia, and leukocytosis and required treatment. The third patient exhibited involvement of the short arm of chromosome 11, and the FISH test showed normal results for *the ATM* gene. The karyotype was 45~46,XY,del(1)(q32), add(11)(p10),12[2]./41~44,XY,del(6)(q?24)[2]/35~46,XY[24]. This patient was diagnosed with leukocytosis but without anemia and thrombocytopenia. One year after the diagnosis, the patient developed spinocellular carcinoma. The cytogenetics tests were done after the treatment for CLL and carcinoma. The additional material attached to band 11p10 already was reported in two cases of this type of carcinoma. Our results highlight the significance of combining G-banding and FISH assays to better understand the CK em CLL and reinforce the importance of incorporating CK information into the classification and risk-stratification criteria of patients with this hematological neoplasm.

Key-words: complex karyotype; CLL; chromosome 11; ATM gene;

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ID – 73

DISRUPTION OF *DLGPA2* OR *DLGAP4* IN PATIENTS WITH MILD INTELLECTUAL DISABILITY

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Abstract:
Intellectual disability (ID) is a neurodevelopmental disorder, that derive from an altered function of the brain. ID is characterized by 3 features: cognitive impairment, deficit in adaptive function, and onset during the developmental period. Thus, it impacts social interactions, as well as the cognitive and practical skills of affected individuals. Causes of ID include genetic abnormalities and environmental factors. Thousands of genes encode proteins that are needed for an efficient brain function, therefore resulting in a myriad of possible genetic causes for ID. The aim of this work was to describe two unrelated families with mild ID and autism spectrum disorder with a detailed cytogenetic investigation. Both families were evaluated at the Clinical Genetics Unit of University Hospital of Brasília. Family 1 consists of monozygotic twin sisters born from consanguineous parents and Family 2 consists of two siblings and a similarly affected mother. Cytogenetic investigation included Karyotype, Chromosomal Microarray Analysis using CytoScanTM 750k platform, Mate-Pair Sequencing and Optical Genome Mapping. In family 1 karyotype analysis revealed a balanced translocation between chromosomes 9 and 20 [46,XX,t(9;20)(q22;q13.3),9qh+,9qh+], with a very large heterochromatin region on both chromosomes 9. Chromosome microarray analysis did not reveal any imbalances. Mate-pair sequencing and Optical genome mapping both disclosed that the chromosome breakpoint on chromosome 20 interrupts the *DLGAP4* gene. Family 2 had a normal karyotype. Chromosome microarray revealed a 760 Kb duplication in 8p23.3 that overlaps *DLGAP2* gene, leading to its disruption [arr[GRCH]8p23.3p23.3(1,570,315-2,330,754)x3]. Also, the duplicated region encompasses four genes: *CNL8, ARHGEF10, KBTBD11*, and *MYOM2*. The duplication was identified on both siblings and their mother. Optical genome mapping confirmed a direct duplication on chromosome 8p and delimited the distal breakpoint to intron 5 of *DLGAP2*. The DLGAP family is composed of four homologous genes (DLGAP1-4) that encodes proteins that play an important role in postsynaptic density (PSD), a complex structure composed of thousands of densely packed proteins. Alterations in these proteins have been associated with neurodevelopmental diseases, such as autism spectrum disorder, schizophrenia, and attention deficit hyperactivity disorder. Therefore, disruption of *DLGAP2* and *DLGAP4* adds more evidence to a possible role of these genes in the etiology of neurodevelopmental disorder.
Key-words: Intellectual disability; Chromosomal rearrangements; DLGAP2; DLGAP4;

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ID – 14
INFANTILE NEUROAXONAL DISTROPHY: A CASE REPORT

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Abstract:
Infantile neuroaxonal dystrophy (INAD) is a rare autosomal recessive disease (RARD), with almost 150 cases reported worldwide, integrating a complex group of neurodegenerative disorders associated with phospholipase A2. The illness is characterized by a progressive neurodegeneration, which in general, is related to a bioaccumulation of iron in the brain tissue. Deposit of iron in basal ganglia, nigra substance and vermis cerebellar indicate the diagnoses.The phenotypic signs of INAD include delay and regression psychomotor, neurological displays such as dysarthria, dystonia and hypotony, which may lead to tetraparesis and death. To confirm the diagnoses, there must be identified the pathogenic variations involving the *PLA2G6* gene, either in homozygosis or compound heterozygosis. The present case report brings out a child of masculine sex, 6 years old, that was attended in the department of medical genetics. The proband showed signs of speech delay, neurological regression, myoclonic epilepsy, optic nerve atrophy and axonal demyelinating polyneuropathy, which was confirmed by electroneuromyography. The child and his family were sent to the department of genetic counseling with clinical indication of not specified epilepsy to investigate. Firstly, the genetic counselor asked questions to create the familiar historic of the proband, which reveals parental consanguinity. Secondly, the patient was physically examined to obtain the clinical phenotype. According to the information collected by the doctor and the genetic counselor, it was suggested that the proband do a panel of Next Generation Sequencing of epilepsy-related genes to clarify the condition. There were identified two identical pathogenic variations of the *PLA2G6* (chr22:38.565.227-38.565.392) in homozygosis, causing the deletion of the exon 2 in both alleles. In parent's genome, the variants were in heterozygosis, confirming a case of identical by descent (IBD) alleles. The data bases of rare diseases have reported 150 cases of INAD worldwide, with clinical phenotypes and hypervariable onset. Additionally, all cases that were reported have similar phenotypes. Therefore, it was concluded that the proband is affected by the disease, being one more classical case of the INAD infirmity, a RARD by IBD. The genetic counseling was able to teach the family about mutation and its consequences, discuss the inheritance on evidence, estimate the risks of recurrence for the decedents, forward to multidisciplinary areas to ampere and discuss possible therapies that can ease the proband's symptomatology, aiming his better quality of life.
Key-words: Infantile Neuroaxonal Distrophy; Case Report; Genetic Counseling;

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ID 37

CYTOGENETIC ALTERATIONS IN PEDIATRIC MYELODYSPLASTIC NEOPLASM: AN OVERVIEW OF CYTOGENETIC RISK STRATIFICATION

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Abstract:
Pediatric myelodysplastic neoplasm (MDS) comprises less than 5% of malignant hematologic neoplasms in childhood. Pediatric MDS is a rare disease of clonal origin of hemopoietic stem cells characterized by dysplasias, inefficient hematopoiesis, and cytopenias in peripheral blood. Its clinical course is variable; however, around 10-40% of cases progress to acute myeloid leukemia (AML). The only curative therapy for these patients is the hematopoietic stem cell transplant (HSCT). Cytogenetic analysis is essential to the diagnosis, prognosis, and for clinical decision-making as the indication for HSCT. Clonal cytogenetic alterations can be detected in approximately 55% of pediatric MDS, mainly in advanced subtypes. Unlike AML, the MDS cytogenetic hallmarks are partial or total chromosomal losses (deletions or monosomies) or chromosomal gains (trisomies). The prognostic value of cytogenetics has been studied extensively in adult patients with MDS, however knowledge of cytogenetics in pediatric MDS is still limited, except for alterations involving chromosome 7. The aim of this study was to analyze the frequency of chromosomal alterations in pediatric MDS patients and their impact on the evolution from MDS to AML. We have studied 200 pediatric patients with MDS. The G-banding was performed for all patients at diagnosis from bone marrow cells in cultures for 24 hours. Fluorescence *in situ* hybridization (FISH) was performed as a complementary tool to confirm and characterize the cytogenetic alterations. In total 50.5% (101/200) patients showed cytogenetic alterations, these alterations were more frequent in patients with more advanced subtypes representing 86.36% (57/66). Among the initial subtype, the abnormal karyotype was observed in 31.34% (42/134). The most frequent alterations were -7 (22%), del(11)(q23) (10%), +8 (9%), and harbored a complex karyotype (10%). Rare chromosome alterations such as biclonal, hyperdiploid karyotype, and chromosomal translocations were present at 2%, 2.5%, and 1% respectively. The distribution of the pattern of cytogenetic alteration did not show an association with a specific subtype of pediatric MDS. Leukemia evolution was present in 29% being associated with -7, followed by +8, del(11)(q23), complex karyotypes, and rare chromosome alterations. Our study provides new information on the role of common and rare cytogenetic abnormalities in pediatric MDS with important clinical implications.
Key-words: pediatric MDS; cytogenetic alteration; leukemia evolution;

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Citogenética Vegetal

ID – 89

ORGANIZATION AND DIVERSITY OF CLUSTERED AND HOLO-DISTRIBUTED SATDNA FAMILIES IN *ELEOCHARIS* R. BR. (CYPERACEAE)

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Abstract:
Repetitive sequences may represent the main fraction of medium and large plant genomes and participate in different ways in the composition and organization of chromosomes. Satellite DNA (satDNA) families are part of this fraction, and can accumulate at different chromosome domains, mainly associated with (peri)centromeres and subtelomeres. The presence and functional roles of satDNA have been widely studied to understand centromere organization in both mono- and holocentric chromosomes. Species of *Eleocharis* (Cyperaceae) have holocentric chromosomes, and karyotypes showing high variation in chromosome number ($2n$ = 6 to 196). The distribution of kinetochore proteins along chromatids makes karyotype variations, such as chromosome fissions and fusions, more frequent in comparison to monocentrics. In this study, five *Eleocharis* species, from different phylogenetic clades, were sequenced by Illumina MiSeq at low coverage. The short reads were used to characterize the most common repetitive sequences, mainly satDNA families and transposable elements. The search for repetitive sequences was performed using the RepeatExplorer/TAREAN tools in a comparative analysis. SatDNA monomers were annotated and validated using Dotter b/l tool. Selected satellites were physically mapped by FISH and the (peri)centromeric histone variant H2AThr120ph was *in situ* localized by immunostaining. The LTR retrotransposons were the most abundant elements, mainly members of Ty1/copia and Ty3/gypsy superfamilies. However, the distribution of these superfamilies varied considerably among genomes. *Eleocharis maculosa* and *E. geniculata,* which belong to the same clade, showed the largest accumulation of satDNAs, mainly in *E. maculosa*. The EmSat14 probe was distributed along the chromatids in *E. maculosa*, *E. geniculata* and *E. filiculmis* but not uniformly, suggesting a differential accumulation within and between chromosomes. This distribution was compatible with the dispersed localization of anti-H2AThr120ph signals, which suggests a holocentric organization in the genus. The other satellite probes displayed clustered, block-like signals on a single chromosome pair at terminal or interstitial regions, such as EgSat112 in *E. geniculata,* EeSat99 in *E. elegans* and EpSat248 in *E. parodii*. Satellite probes obtained from *E. montana* exhibited a larger number of terminal and interstitial FISH signals. Generally, some satDNA families were more accumulated in genomes from species of the same clade, like EmSat14 in *E. maculosa*, *E. geniculata*, and *E. filiculmis*, and others were species-specific. When the monomers were compared with the partially sequenced genomes available in databases using the Repeat Masker tool, we observe the presence of some satellite sequences in species of other clades. However, hybridization signals were not noticed, suggesting that there was differential accumulation within clades or even species-specific.
Key-words: holokinetic chromosomes; karyotype evolution; satellite DNA.

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ID 159
GENOME SIZE IN *AVENA STRIGOSA* AND *AVENA BREVIS* GENOTYPES

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Abstract:
Among cultivated cereals, oat stands out as one of the main winter crops in southern Brazil. The diploid species *Avena strigosa* Schreb. (2n=2x=14) is widely used as an annual winter forage for grazing and soil cover. Due to its importance, genetic breeding seeks to incorporate genetic variability into programs, which includes interest in *A. brevis*, also with 2n=2x=14. However, cytogenetics studies have been restricted to some cultivated *Avena* species and the data from other genotypes may contribute to characterize the wild germplasm. The 2C DNA is defined as the nuclear DNA content of an unreplicated diploid cell (in G1 phase) and is used both to get an estimate of genome size and ploidy level. This study aimed to estimate the genome size, in order to evaluate intra- and interspecific genome size variation and provide insight into the evolutionary history of *A. strigosa* and *A. brevis*. Four samples of each species *A. brevis* (BRS Centauro) and *A. strigosa* (BRS Pampeana, IAPAR 61, PFA201702 and UPFA 21 Moreninha) were used to quantify the nuclear DNA content via flow cytometry. Young leaves (20-30 mg) from the *Avena* genotypes were macerated with the same amount of *Vicia faba* L. leaves (reference standard, 2C = 26,90 pg) on a Petri dish containing 1 mL of cold LB01 buffer to obtain a nuclear suspension. Finally, 25 µL propidium iodide was added to the filtered nuclear suspension. The analysis was performed in a cytometer BD FACSCalibur™, and at least 10,000 nuclei were quantified for each sample. Histograms were obtained using CellQuest™ software and analyzed using WinMDI™ 2.8 software. Nuclear DNA content was estimated in picograms (pg) by comparing them with the G1 peak position of the reference standard. The estimates of genome size were subjected to analysis of variance and the Tukey test ($p < 0.05$) within R software (R Core Team, 2022). The coefficient of variation obtained by flow cytometry when determining genome size was 2.08%, demonstrating experimental precision. The nuclear DNA contents of 2C nuclei was 9.17 pg for IAPAR 61, 9.21 pg for UPFA 21 Moreninha and BRS Pampeana and 9.32 pg for PFA201702 and BRS Centauro, showing no significant differences, which is very close to the published in the literature. The genome size of *A. brevis* was previously reported at 8.98 pg and that of *A. strigosa* at 9.07 pg. Other older studies reported values of 8.9 and 9.5 pg for *A. brevis* and 9.7 and 8 pg for *A. strigosa*. We found very little variation of genome size among genotypes within the *A. strigosa* species and between the species *A. strigosa* and *A. brevis*, suggesting that no important events leading to divergence within a genome type. In conclusion, the genome sizes reported here should provide consistent and reliable benchmarks to assist in further characterization of the evolutionary relationships among these important species.
Key-words: Oat; flow cytometry; C-value;

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ID – 185

REPETITIVE SEQUENCES OF FUNCTIONAL REGIONS OF THE CHROMOSOMES DE *UROCHLOA HUMIDICOLA* (RENDLE) MORRONE & ZULOAGA ACCESSIONS

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Abstract:

Most of the plant genome is composed of dispersed repetitive sequences (TEs) or in tandem (multigene and satDNA families). These sequences, especially satDNA, have been used as cytogenetic markers, as their organization in clusters favors karyotyping via FISH, revealing chromosome-specific, species-specific or genome-specific marks. Therefore, identifying and locating these sequences on chromosomes is important for cytotaxonomic and evolutionary studies and phylogenetic comparisons. *Urochloa humidicola* (Rendle) Morrone & Zuloaga is an important perennial species of forage for pastures under waterlogging conditions. However, the ancestry relationships, polyploidy origin, and genomic composition are still inconclusive. The study aimed to comparatively analyze the location of tandem repeats and satDNA sequences on the chromosomes of accessions H016 (cultivar BRS Tupi) with 2n=6x=36 (apomictic) and H031 with 2n=6x=36+1 (sexual), both used as parents in crosses for the production of hybrids. The roots were pretreated with cyclohexamide and amiprofos-methyl (APM) (2:1) for 2h, and the slides were prepared using the cell dissociation technique and air-dried. FISH was performed with probes SAT_2, 127 bp and SAT_3, 115 bp, isolated from the genome of *U. humidicola* (accession CIAT26155) and SAT_4, 361 bp, from *U. decumbens* of 'brizantha' complex, labeled via PCR. The 18S rDNA probe was used to identify the 35s rDNA regions. Accessions H031 and H016 showed four and five 18S rDNA signals positioned in the terminal regions of the chromosomes. The SAT_2 and SAT_3 sequences revealed marks exclusively in the terminal regions. SAT_2 intensely marked 10 and 18 chromosomes in accessions H031 and H016, respectively. The SAT_3 probe hybridized on 10 and 17 chromosomes from accession H031 and H016, respectively, with a pair of chromosomes with markings at both arms end in H031 and only one chromosome in H16. The SAT_4 sequence from the 'brizantha' complex marked the centromeric region on five and 16 chromosomes in H031 and H016, respectively. The FISH signals obtained with the three sequences showed size heteromorphism between the chromosomes that are related to the variable number of repeats. The results showed differences in the number of chromosomes hybridized with the satDNA sequences in the chromosomal complement of the two hexaploids. The greater number of chromosomes with SAT_4 centromeric marking demonstrates that parental H016 shares common sequences with the *'brizantha'* complex, probably belonging to one of its genomes. Specific types of satDNA, and probably also retrotransposons, may be present in the composition of centromeres of allopolyploid genotypes and, as well as sequences with terminal marks, may be helpful to investigate the dynamics and evolution of centromeres and telomeres in *Urochloa*. Polyploidy associated with post-polyploidization changes, differences in the mode of reproduction and hybrid origin of the aneuploid H031 are among the factors that may contribute to differences in the chromosome complement of the evaluated *U. humidicola* genotypes.

Key-words: Brachiaria; FISH; satDNA;

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FAPEMIG, Capes e CNPq

ID – 79

WHAT POLLEN CAN TELL US ABOUT THE POLYPLOIDY IN *HERBERTIA* (TIGRIDIEAE, IRIDACEAE)?

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Abstract:
Polyploidy is considered one of the most important evolutionary mechanisms of diversification and speciation in Angiosperms, responsible for affecting ecological, morphological, physiological and reproductive species characteristics. The genus *Herbertia* (Tigridieae, Iridaceae) comprises eight species with remarkable morphological and cytogenetic variation. Polyploid series have already been reported for two of these species: *Herbertia lahue (2n = 2x = 14, 2n = 6x = 42, 2n = 8x = 56)* and *Herbertia pulchella (2n = 2x = 14, 2n = 4x = 28, 2n = 6x = 42)*. However, so far only the tetraploid cytotype of *H. pulchella* has been found in Rio Grande do Sul (Brazil). In order to characterize aspects related to the pollen grains of *H. lahue* (*2x*, *6x* and *8x*) and *H. pulchella* (*4x*) in Rio Grande do Sul and to correlate these data with the ploidy levels found in both species, flower buds from seventeen populations were collected, fixed in ethanol:acetic acid and macerated in Alexander 2%. The resulting slides were used for three different analyzes: (1) pollen stainability, where 500 grains per individual were counted and classified into viable and nonviable grains through Alexander's method; (2) quantification using the Neubauer Chamber, to estimate the number of grains per anther and per flower and (3) pollen morphology, with the measurements of the polar axis (P) and equatorial diameter (E) determined with a Zeiss Axioplan photomicroscope. Besides that, statistical analysis was performed with the R software. Regardless of ploidy level, both species presented similar pollen stainability (Kruskall-Wallis, $p = 0,6138$) and high viability (ranging from 93,02% to 94,96%), which suggest that polyploid cytotypes are well established in natural populations. Concerning the quantification, ANOVA and Dunn's test showed statistical differences [$F(3, 58) = 15,75$; $p < 0,001$] between the amount of pollen produced by the diploid cytotype, which is much higher than the polyploids. Differences like this could be evidence of an alternative breeding system followed by polyploids, such as selfing or even clonal propagation. Previous experiments of manual pollination leading by our research group indicates that the octaploid and the hexaploid cytotypes are self-compatible with fruits formed by self fertilization. In the other hand, the diploid cytotype is self-incompatible. Considering the occurrence of mixed populations, the existence of distinct forms of reproduction may favor the coexistence of the cytotypes. The ratio between the polar axes and the equatorial diameter indicates that all ploidy levels have sub-spheroidal pollen grains. Significant differences in the pollen size dimensions between the groups were found by the Kruskal-Wallis test. Octaploid and hexaploid individuals presented the largest pollen grains, while diploids had the smallest, possibly due to the "giga" size effect in polyploids. However, these differences were not enough to determine the ploidy level of the cytotypes only with the pollen measurements. In addition, significant differences were observed between individuals of the same cytotype belonging to populations from different locations, which may indicate the presence of environmental factors acting on pollen grains.
Key-words: Polyploidy; Pollen grains; Iridaceae;

ID – 211

ENVIRONMENTAL VARIABLES MAY EXPLAIN THE VARIATION IN GENOME SIZE IN SECTION *VIPERELLA, SISYRINCHIUM* (IRIDACEAE)?

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Abstract:
The *Sisyrinchium* (Iridaceae) genus comprises species that occur across the American continent. Recent studies have indicated environmental variables, like altitude, as possible determining factors of the large morphological variation in this genus. In some situations, interaction between genetic and environmental factors, such as climatic conditions, can result in wide changes in the morphology of a given species. Some species of *Viperella* are limited to the Pampa and Chaco regions, and others extend to the Atlantic Forest or even to the north-central Andes. In *Sisyrinchium*, the *Viperella* section has the highest number of records regarding genome size (2C) and chromosome number. We observed variation in the genome; most of them have 2C values ranging from 3.54 to 5.3 pg, *S. marchioides* has 2C = 2.68 pg and three other species with larger genomes but which have polyploid (2C = 7.36 to 8.4 pg). Considering geographic distribution of *Viperella* species and their available genome size data, this study aims to investigate a possible relation between genome size variation and climatic variables. To test if genome size variation is influenced by climatic variables, we tested the correlation between genome size with climatic variables and also geographic distribution. The genome size of *S. congestum*, *S. bromelioides*, *S. coalitum* and *S. restioides* was estimated by flow cytometry following Dolezel et al (2007) method, using field collected specimens that are currently listed in the ICN herbarium. Genome size of these species were estimated and follows the pattern found in the rest of the group. We compiled genome size information from previous studies and from genome size databases. The chosen climate variables were precipitation, radiation, average, minimum and maximum temperature. All of them extracted from the BioClim platform. These variables were obtained from climatic rasters using geographical coordinates of the specimen used in flow cytometry. For investigating the existence of correlation between the occurrence in different biomes with the genome size, we created three categories in terms of distribution: 1) species that occur only in Pampa and Chaco; 2) Pampa and Chaco plus Atlantic Forest occurrences; and 3) species also present in the north-center Andes. Thus, our sampling covered 12 species. We performed Pearson's correlation tests for normally distributed data and ANOVA to test the correlation between genome size and environments. All cited analyses were performed in R v.3.2.1. Considering the dataset used, we found no association between genome size and the variables employed. These results indicate that the variation found for genome size seems to be more dependent on genomic factors, such as the presence of transposons and other repetitive sequences. Therefore, we suggest a study including a larger number of samples to test our hypothesis from a more comprehensive sample. The new genome sizes obtained, increase the dataset available for section that can be used in future works. More studies are being carried out with the objective to obtain the chromosome number for these species.
Key-words: flow cytometry; chromossome number; cytogenetic;

Acknowledgement

CNPq, Capes and Fapergs

ID – 215

IS THERE A RELATION BETWEEN CYTOGENETIC AND PHENOLOGICAL SHIFTS? WHAT DOES *CYPELLA PUSILLA* (TIGRIDIEAE: IRIDACEAE) TELL US ABOUT IT.

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Abstract:

Cypella pusilla (Iridaceae) stands out from other species of the genus due to its peculiarities in relation to phenology, reproductive biology and pollination ecology. Unlike the other species of the genus, *C. pusilla* has two flowering peaks (spring and autumn), but presents low rates of pollinator visitation and fruit production in both flowering events. In Iridaceae, there is an important diversity in relation to chromosome number, genome size and karyotypic architecture. So far, only five out of 36 *Cypella* species have known chromosome numbers and genome sizes. According to literature, polyploidization and genome size variation can affect phenological and reproductive traits. Thereby, this work aims to investigate whether populations of *C. pusilla* in distinct phenological periods present any cytogenetic variations. Three populations of *C. pusilla* were sampled in Rio Grande do Sul: São Gabriel (SG) and Caçapava do Sul (CA1 and CA2). Data collection was carried out in 2022 during the months of April (Aut) and November (Spr). The phenology of *C. pusilla* was tracked throughout in both seasons and individuals were marked to verify if the same plant blooms in both phenological events. The number of flowers were counted during the two flowering peaks. For cytogenetic data at least five individuals from each population were sampled. For slides preparation, root tips were submitted to an enzymatic pool maceration and stained with Giemsa. Genome size estimation was performed using flow cytometry following the methodology proposed by Dolezel et al. (2007). T-test and Analysis of Variance were used to analyze the difference between the means of genome size in all populations, with p-values < 0.05 being considered statistically significant. The number of flowers varied between populations and seasons: SG/Spr = 46, SG/Aut = 67, CA1/Spr = 0 and CA1/Aut > 5000 (CA2/Spr/Aut were not evaluated). Interestingly, the phenological study showed that the individuals that bloom in each flowering peak are not the same. All populations studied presented the same chromosome number 2n = 14, despite a substantial variation in genome sizes. The São Gabriel population showed a statistically significant difference between seasons: 2C = 3.43pg±0.03 (SG/Spr) and 2C = 3.59pg±0.02 (SG/Aut). The two populations of Caçapava do Sul were analyzed only in autumn and presented similar 2C values: 3.67pg±0.02 (CA1/Aut) and 3.61pg±0.07 (CA2/Aut). There were no statistically significant differences among all those populations sampled during autumn. Bearing in mind that the individuals that bloom in spring are not the same as those in autumn and that there is a significant difference in their 2C values, such variations could potentially be linked to a temporal segregation among conspecific individuals within the same population. However, it is important to keep in mind that genome size is just one of many factors that can impact phenology, and additional studies are needed to fully understand these relationships. Subsequently, a comparative analysis will be conducted between the karyotypic characteristics of the investigated populations.

Key-words: threatened species; genome size; phenology;

Acknowledgement

CAPES, CNpq, BIC-UFRGS and FAPERGS

ID – 26

POLYPLOID INDUCTION IN STYLOSANTHES GUIANENSIS (ABUL.) SW. (FABACEAE)

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Abstract:

Polyploidy induction is one important tool for plant breeding programs including fodder legumes.This process enables the development of new varieties with desirable morphological and physiological traits. The polyploidyzation process may occur naturally via non-reduction of gametes, naturally via somatic cells duplication, or can be artificially induced by doubling the number of chromosomes in somatic cells treated with mitotic blockers in tissue culture. In this experiment, a protocol for in vitro polyploid induction of Stylosanthes guianensis (Fabaceae), an important forrage legumen with 2n=2x=20 chromosomes, was studied by using two different concentrations of colchicine (0,1% and 0,05%) in three different times of exposition (6h, 12h and 24h). As explant, seeds germinated in MS medium culture enriched with the 2 concentrations of colchicine at the 3 different times were utilized (50 seeds for concentrations/time totalizing the utilization of 300 seeds). Flow cytometry analysis was used to confirm the ploidy level of seedlings, demonstrating that a total of 10 tetraploid (2n=4x=40; 2C=5,72 pg) and 40 mixoploids (with diploid and tetraploid cells) individuals were obtained, while 210 seedlings remained diploids (2n=2x=20; 2C=2,84 pg) and 40 seedlings died. All polyploids plants were obtained in medium cultured enriched with 0,05% of colchicine for 24h of treatment and the mixoploids were obtained in all treatments utilized. Additional tests using calli and organogenesis proccesses will be applied in the future to obtain the best methodology for chromosome duplication in Stylosanthes guianensis.

Key-words: chromosome; polyploidy; flow cytometry;

Acknowledgement

FAPEMIG, CNPq, CAPES, UFJF.

ID – 112
POLLEN VIABILITY IN *UROCHLOA HUMIDICOLA* ACCESSIONS BY COLORIMETRIC TESTS

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Abstract:
Pollen quality is mainly to ensure the formation of viable seeds in the genus *Urochloa*. Sexual genotypes depend on fertility for successful fertilization and endosperm fertilization. In apomictic accessions, the development of viable seeds depends on the viability of the pollen grains for fertilization of the endosperm. In this way, the development of the endosperm is compromised because of pollen infertility, the seeds do not develop and become "empty seeds". The fertility rate is related to the number of viable seeds produced and the success of crossbreeding. Pollen viability can be assessed directly by the ability of the pollen to fertilize and germinate, and indirectly by the coloring pattern in colorimetric tests. Colorimetric tests are widely used and represent a fast and effective alternative in determining genotype fertility. There is no ideal dye for the determination of pollen viability. In the genus *Urochloa*, colorimetric tests have been shown to be genotype specific. Thus, in the present study, two dyes were used in the evaluation of pollen grain fertility of nine apomictic accessions and one sexual accession of *U. humidicola*. The dyes tested were 1% propionic carmine and 2% Alexander's reagent. Pollen grains with homogeneous coloration in the protoplasm and normal shape were considered viable, and non-viable ones with weak or no coloration, abnormal shape and reduced protoplasm. The colorimetric tests using 1% propionic carmine showed a variation between 91.24% viable pollen grains in the access HumG and 97.73% in the access HumF. When the staining was done using Alexander's reagent 2% the variation was from 92.12% in access HumJ to 98.12% in access HumD. The average of the values obtained with the two dyes was similar, 95.86% for propionic carmine 1% and 95.613% for Alexander's reagent. Pollen fertility showed no statistically significant difference between the dyes ($p>0.05$). It can be concluded that for these accessions there was no genotype-specific response to the dyes used.
Key-words: 1% propionic carmine; Alexander's reagent 2%; viable seeds;

Acknowledgement

Capes, Embrapa, Unipasto, Universidade Estadual de Maringá

ID – 136

THE TROPICALIZATION OF THE MAIZE (*ZEA MAYS*) INBRED LINE B73 PROMOTES A PUTATIVE EPIGENETIC CHANGE IN SEED PHENOTYPE

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Abstract:
Maize is a worldwide cultivated crop of high economic relevance and a key model organism for genetic studies, being present in all continents with different climate conditions. This specie is constituted for many types of lines and are more diverse from each other than the human to chimp comparison. Among this line variety, the B73 inbred line has been chosen to this work, because have its genome sequenced and used as reference for genetic studies. This line was born in the temperate climate of Iowa State, United States. Due to the lack of a tropical maize inbred line as a reference, the B73 must be used. Recently, the Maize Genetics Cooperation Stock Center donated B73 seeds to be introduced to Brazil, beginning its tropicalization. Since then, two cycles of self-pollination were carried out during the summer of 2018-2019 and 2021-2022. In this work, a comparative analyze was made between the seeds of the original B73, the S1 progeny with one cycle of tropicalization and the S2 with two cycles of tropicalization. The seeds were evaluated for area, Feret diameter and mean gray value by image analysis using the ImageJ software. Additionally, the seeds weight was measured. Differences were found in all parameters analyzed. The results showed that some traits may vary along with generation, suggesting that not only the climate difference can affect the seeds, but also an epigenetic accommodation with accumulation for some parameters. Chromosome analysis shows a perfect karyotype constitution without variation through generations. All the heterochromatins appear in a perfect morphology without significative differences. No additional chromosome markers are observed. Cytosine methylation in a global analysis is apparently stable, and detailed molecular maker analysis using MSAP are in progress to map specific regions with differential methylation states. This study reinforces the important of monitoring genotypes from other climate conditions, especially the ones used as genetic model. Furthermore, these results can allow us to understand how maize was able to adapt to various environments and be successfully productive in most of them.
Key-words: maize; epigenetics; phenotype; tropicalization;

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ID – 166

DESCRIPTIVE AND COMPARATIVE ANALYSIS OF CHROMOSOME NUMBER AND GENOME SIZE OF *HYMENOPHYLLUM* SM. SUBG. *SPHAEROCIONIUM* (C. PRESL) C. CHR. (HYMENOPHYLLACEAE, POLYPODIOPSIDA).

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Abstract:

Hymenophyllaceae Mart. is a fern family of pantropical distribution with approximately 450 species. The *Hymenophyllum* Sm. genus comprises around 250 species organized within 10 subgenera. Among these, *Sphaerocionium* (C. Presl) C.Chr. displays high diversity in the neotropics. Phylogenetic analyses of Atlantic Forest species evidence the monophyletic nature of *Sphaerocionium*. However, the group has a highly variable morphology, which makes taxonomic delimitation difficult at more specific levels. Cytological data can provide additional taxonomic and phylogenetic criteria. Thus, this work aims to perform a descriptive and comparative analysis of chromosome number and genome size for genus *Hymenophyllum* with emphasis on subgenus *Sphaerocionium*. Fourteen species from six subgenera were sampled at Atlantic Forest formations in Southern Brazil. Sori fixed in ethanol:acetic acid (3:1) were subjected to meiotic analysis following the squashing method. Total DNA quantification was performed through flow cytometry according to Dolezel and collaborators (2007). Analysis of Variance was applied to genome size data, *p*-values<0,05 were considered statistically significant. The genome size (2C) was estimated for fourteen species from subgenera: *Sphaerocionium*, *H. delicatulum* Sehnem (43,74pg), *H. crispum* Kunth (41,12pg), *H. elegans* Spreng. (35,84pg), *H. fragile* (Hedw.) C.V.Morton (38,77pg), *H. vestitum* (C.Presl) Bosch (38,11pg), *H. rufum* Fée (36,80pg), *H. trichophyllum* Kunth (32,37pg) and *H. pulchellum* Schltdl. & Cham. (32,53 pg); *Myrmecostylum* (C. Presl) Ebihara & K.Iwats., *H. magellanicum* (Klotzsch) Willd. ex Kunze (36,58pg); *Mecodium* C.Presl ex Copel, *H. sturmii* Bosch (25,81pg); *Hymenophyllum*, *H. megachilum* C.Presl (30,01pg), *H. fucoides* (Sw.) Sw. (134,26pg); *Globosa* (Prantl) Ebihara & K. Iwats, *H. caudiculatum* Mart. (46,74pg), *H. asplenioides* (Sw.) Sw. (38,36pg). A statistically significant difference in genome size was observed when considering the whole *Hymenophyllum* genus and within *Sphaerocionium*. The haploid chromosome number was *n*=26 for *H. sturmii*, *n*=27 for *H. magellanicum*, *n*=13 for *H. megachilum*, *n*=36 for *H. crispum*, *H. delicatulum*, *H. pulchellum*, *H. rufum*, *H. elegans* and *H. fragile* (ongoing analysis for the remaining species). No previously reported genome sizes were found for *Sphaerocionium* species. In contrast, chromosome numbers were found for nine species, eight of which had *n*=36 and one had two values *n*=36 and *n*=72. Despite the stability in *Sphaerocionium* chromosome numbers, a significant genome size variation was unveiled, ranging from 32,76pg to 43,74pg. High genome size variation is not an exclusive characteristic of this group. In the subgenus *Hymenophyllum*, variation reaches up to 10 fold (30,28pg - 134,26pg). However, contrary to *Sphaerocionium*, the chromosome number is not fixed for the *Hymenophyllum* subgenus. Previous studies have witnessed a positive correlation between fern species diversification and genome size evolution, which might be the case for Hymenophyllaceae. Future research directions involve acquiring and investigating cytogenetic data for other Atlantic Forest *Sphaerocionium* species. This information will be integrated with the group phylogeny in order to advance the current knowledge on fern evolution and diversification.

Key-words: Filmy ferns; Cytogenetics; Atlantic Forest;

Acknowledgement

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ID – 9

THE HETEROCHROMATIN ADDITIVE EFFECTS ON MAIZE PHENOTYPE USING A TB9SB TRANSLOCATION.

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Abstract:

Flowering time is an agronomic characteristic of high interest in maize breeding programs. Recently, the presence of the knob on the short arm of chromosome 9 (K9S) was associated with shortening flowering time. To continue the investigation of the influence of the K9S on the flowering time, the B73 and B73+TB9Sb were compared. The TB9Sb is a B-A translocation containing the K9S and the genetic marker *C1*, which produce anthocyanin in the aleurone and the embryo. Two repetitions of the experiment were carried out using B73 and B73-TB9Sb, and for the late, the yellow and purple seeds were considered separately. The days for the germination and the flowering time were measured, and the inbred lines were maintained by selfing and sibling fertilization. Chromosome number counting was performed on metaphases stained by DAPI. For the first experiment, germination time was faster for B73-TB9Sb with purple seeds. In both replications, the male and female flowering times remained stable. The backcross progeny B73-TB-9Sb (purple) (female) and B73 (male) showed seeds with colorless embryo and yellow aleurone and seeds with embryo and purple aleurone in a 1:1 ratio. The presence of the K9S in the B73-TB9Sb does not express changes in flowering time. On the other hand, the presence of B73-TB9Sb accelerates germination speed. Cytogenetic analysis showed 2x=20 chromosomes for B73 and the yellow seeds of B73-TB-9Sb; and 2x=20 + 1 TB9Sb for purple seeds of the B73-TB-9Sb, demonstrating that the chromosome system was suitable for the experiment. Finally, this work is essential to better understand the functions of maize knobs heterochromatin.

Key-words: Translocation B-A; Maize; Heterochromatin; Flowering Time; Phenotype

Acknowledgement

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ID 168 –
DISCOVERY AND CHARACTERIZATION OF A NEW REPETITIVE DNA FAMILY HIDDEN IN THE K180 CLUSTER OF THE MAIZE B CHROMOSOME

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Abstract:
Satellite DNA refers to non-coding and highly repetitive *in tandem* sequences located in certain areas of the chromosomes, mainly in the centromeric and telomeric regions. In maize, we can mention the families of the centromeric region (Cent-C) and knob (K180). B chromosomes are additional supernumerary, and dispensable chromosomes added to a normal set of a given species, being smaller in size, almost entirely heterochromatic, and do not contain homologies with A chromosomes. It is assumed that B chromosomes originated through structural modifications from A chromosomes during cell divisions. This work aims to study satellite DNA diversity on maize B chromosome and to screen its entire sequencing to uncover hidden repetitive sequence motifs. For this purpose, data mining of all K180 knobs sequences of the maize B chromosome was realized, and a new satellite DNA family was described, characterized, and compared to other well-known repetitive sequences. Alignment between the sequences and the construction of dotplot comparisons with other satellite DNA families (B_specific, Cent_C, Cent4, K180_1, K180_2) were also performed. A total of 14 well-conserved motifs were found intermingled into the K180 arrays of the B chromosome at the positions 206 Kbp, 227 Kbp and 735 Kbp. All the motifs are 189 bp long. In a search for this new satellite DNA across the A chromosomes revealed a cluster of 478 motifs in the short arm of chromosome number 4, between the Cent-C and Cent-4 regions. These motifs were divided into seven groups according to their size, ranging from 55 bp to 187 bp. These sequences have only a small similarity with Cent4 and B-specific repeats and have no similarity with any other family of satellite DNA previously analyzed.
Key-words: Satellite DNA; B chromosome; B specific;

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ID – 75

CORRELATION BETWEEN THE MEIOTIC INDEX AND PURE SEED YIELD IN UROCHLOA HUMIDICOLA GENOTYPES

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Abstract:

Evaluating meiotic behavior is a fundamental stage of the Urochloa breeding process for developing new materials. Cytogenetic analyses allow us to determine compatibility and fertility levels in each genotype, resulting in successful crossbreeding. Nowadays, one of the breeding program's goals includes stabilizing seed production by selecting fertile and stable individuals. In this way, the goal of the present study was to evaluate cytogenetically ten accessions of Urochloa humidicola from Embrapa Cattle Beef Breeding Program and to determine the correlation between meiotic stability and seed yield. We analyzed ten apomictic accessions of Urochloa humidicola. The inflorescence collected in the field at Embrapa Cattle Beef were fixed in ethyl alcohol, chloroform, and propionic acid (6:3:2 v/v) for 24 hours in 70% alcohol at 4ºC until the analyses. The analyses were performed in the Plant Cytogenetics Laboratory of the State University of Maringá, Maringá, PR, Brazil. For meiotic analyses, the anthers were squashed in 1% propionic carmine. The tetrad cells were analyzed to determine the percentage of abnormalities and the Meiotic Index. Seed yield evaluations, data provided by Embrapa Cattle Beef Researchers, were used. Afterward, Pearson's correlation (r) was used to observe the association between the Meiotic Index and total seed yield, pure seed yield, and purity percentage. The presence of micronuclei was variable, considering the different microspores of the tetrad. However, all accessions showed a high incidence of micronuclei in one microspore, ranging from 42.3% in access HumJ to 76% in access HumD and HumH. The evaluation of post-meiotic products results in calculating the Meiotic Index, representing the frequency of viable products for each genotype. The highest index was observed in the access HumI (80.77%) and the lowest HumJ (25.60%). The abnormal end products of meiosis will form unviable pollen grains that cannot fertilize the endosperm of apomictic plants, resulting in "hatched" seeds. So, a correlation was investigated between the Meiotic Index and the evaluations of seed yield, total seed production (Kg/ha), pure seeds (Kg/ha), and the percentage of pure seeds produced. The correlation estimate obtained among the analyzed variables revealed significance ($p<0.05$) according to the t-test between the Meiotic Index and percentage of pure seeds ($r = 0.67$), between pure seed production (%) and percentage of pure seeds (Kg/ha) ($r = 0.65$) and between total seed production (Kg/ha) and pure seed production (Kg/ha) ($r = 0.79$). These observations indicate that, in this case, the Meiotic Index can be used to quickly interpret the future percentage of "full" seed production. Materials with high Meiotic Indices are likely to produce higher percentages of pure seeds, as there exists a positive and significant correlation between the Meiotic Index and the percentage of pure seeds.

Key-words: Meiotic behavior; Micronuclei; Abnormalities; Propionic carmine;

Acknowledgement

Capes, Embrapa, Unipasto

ID 72
CHARACTERIZATION OF DNA SATELLITES IN SPECIES OF *PIPER L.*

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Abstract:
Piper L. is one of the largest genera of Piperaceae and has great socioeconomic importance, as many species are used as spices, flavorings, medicinal and ornamental plants. Due to these gastronomic and phytotherapeutic characteristics, these *Piper* species stand out in the group of basal angiosperms. In order to understand the genome organization of these species, the objective was to identify and characterize satellite DNA sequences in the genome of ten *Piper* species. Genomic sequencing data were obtained from the ENA bank (*European Nucleotide Archive*) and pre-processed on the *RepeatExplorer* platform. Satellite DNA sequences were identified via TAREAN tool. The satellite repetition structures were self-confronted on the Dotmatcher - EMBOSS platform, forming Dotplot graphs for confirmation of tandem arrangement. The similarity and identity between these satellite sequences were obtained by multiple local alignment adopting the MAFFT algorithm in the UGENE software and used to classify the variants. The satellite DNAs were ordered by length and abundance of reads and the abundance visualized in the form of a Bubbles graph built in the R software. No satellite sequences were identified in four *Piper* species (*P. amalago*, *P. commutatum*, *P. darienense* and *P. insectifugum*), indicating a particular organization of the repetitive fraction of their genomes. For the other species (P. *borbonense*, P. *hederaceum*, *P. mestonii*, *P. ponapense*, *P. umbellatum* and *P. nigrum*), 21 satellite sequences were identified. The monomer length of the satellites ranged from 36 (P. *ponapense*) to 529 base pairs (*P. borbonense*) and heterogeneity was observed in the abundance pattern. One satellite (311bp) was the only one shared among all species, with abundance ranging from 154 to 994 reads. One satellite of P. *mestonii*, one of P. *borbonense* and six of P. *ponapense* were exclusive to the respective species, with abundance ranging from 23 to 598 reads. The remaining 12 ones were shared by two to five species, with abundances from one to 646 reads. The variability of satellites among the species indicates the existence of ancient sequences, shared by many or all species, and more recent ones, exclusive or shared by few species.
Key-words: tandem repetitions; plant genome; repetitive DNA; basal species;

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ID – 204
KARYOTYPIC VARIABILITY IN HYBRIDS OF *CYNODON* RICH (POACEAE)

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Abstract:
In the genus *Cynodon* Rich., as well as in other grasses, intra and interspecific hybridization can be widely explored in a breeding program to obtain forages with high quality, productivity, and adaptation to different edaphoclimatic conditions. Usually, the resulting hybrid genotypes are initially selected based on phenotype but need certification regarding karyotype features, such as chromosome number, ploidy level, and location of sequences of functional structures of the chromosome. This study aimed to determine the chromosome number and genome size of new genotypes (EGL16-23 and EGL17-126) obtained from inter-population crosses between half-sib families of *Cynodon sp*. The slides used for FISH were prepared by dissociation with acetic acid and air drying. In addition, FISH was performed with telomeric and 18S rDNA biotin- or digoxigenin-labeled hybridizing probes obtained from PCR reactions. Genome sizes of EGL16-23 (2C = 2.19 pg) and EGL17-126 (2C = 2.17 pg) were not significantly different according to the Tukey test ($p \leq 0.05$). The EGL16-23 genotype showed 2n=4x=36 chromosomes. FISH combining telomeric-18S rDNA probes allowed the identification of two chromosomes showing an interstitial 18S rDNA signal in a distended region. Therefore, it was possible to observe four chromosomal segments very close to each other, one ending with a telomeric signal and the other with only the 18S rDNA signal. The EGL17-126 genotype showed 2n=4x=36+2 also with two interstitial 18S rDNA FISH signals. One of the chromosomes demonstrated the 35S rDNA region distended, similar to observed in EGL16-23 chromosomes, delimited by the telomeric signals. Additionally, the telomere probe revealed two extra chromosomes since they showed telomere signals in both terminal regions. These extra chromosomes can either be a result of rearrangements that led to aneuploidy or B chromosomes, both common events in grasses. Our results demonstrate that the cytogenetic characterization of new *Cynodon* genotypes, mainly resulting from hybridization, is an important step in the screening of materials for decision-making by breeders to develop new cultivars.
Key-words: Chromosome number; rDNA sites; telomere;

Acknowledgement

Financial support: CAPES, FAPEMIG.

ID – 61

EXTINCTION OF DINOSAURS AND RISE OF POLYPLOIDS: GENOMIC CONSEQUENCES OF CRETACEOUS-PALEOGENE (K-PG) MASS EXTINCTION ON THE REPEATOMES OF THE PALEOPOLYPLOID MALVATHECA CLADE (MALVACEAE)

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Abstract:
The Cretaceous-Paleogene (K-Pg, 66 Mya) transition is characterized by a mass extinction event that resulted in a major turnover of earth's biota, including the extinction of non-avian dinosaurs. The apparent concentration of independent whole genome duplication (WGD) events around the K-Pg transition in unrelated plant families may point to a favorable scenario explaining the success of polyploidy lineages. The 65 million years old Malvatheca clade (Malvaceae) represents an interesting model to study genomic consequences of ancient WGD events (paleopolyploidy) because it comprises two lineages with distinct evolutionary trends: (i) the Bombacoideae subfamily, which retained high chromosome numbers (> 2n = 86) and (ii) the Malvoideae subfamily, which evolved lower chromosome numbers (> 2n = 14). We characterized the repeatome of 14 Bombacoideae and 11 Malvoideae species in order to comparatively investigate evolutionary trends of repetitive elements, based on protein-specific domains of LTR retrotransposons (LTR-RT) and repeats abundance and similarity. These data were interpreted on a plastome topology using Phylogenetic Comparative Methods (PCMs). In general, protein domains of different transposable elements lineages showed contrasting patterns: Ty3/Gypsy Ogre and CRM resulted in an unresolved topology and the other LTR-RTs showed a clear split between Bombacoideae and Malvoideae elements. These data suggest a heterogeneity in the evolutionary rate of LTR-RT apparently not related with paleopolyploidy. The repeat-based phylogenies (repeat abundance and similarities) were not entirely congruent with the plastome phylogeny, suggesting that repeats may lose phylogenetic signal in ancient lineages. Furthermore, branch length in the repeat abundance topology was longer in Malvoideae than in Bombacoideae, resulting in negative correlation between chromosome number vs. fluctuations in repeat abundance. Furthermore, a significant correlation between chromosome number and repeat diversity was found. We hypothesize that high repeat diversity in Bombacoideae may be a consequence of (i) a higher rate of associated crossing over (chiasmata) in karyotypes with high chromosome numbers; (ii) a slow repeatome evolution, conserving old repeat classes; (iii) rapid turnover rate of repeats in Malvatheca, leading to fixation/increase of some repeat classes and elimination of others. It is fascinating to note that the high chromosome numbers of Bombacoideae were probably generated from a Cretaceous WGD, with subsequent diploidization of the genomes without reduction in chromosome number. The conservation of this plesiomorphic features may be associated with the predominant arboreal habit. This implies in longer generation times and slower rates of repeatome evolution than in the mostly herbaceous fast evolving Malvoideae. Although genomics indicate that the Malvatheca clade experienced a single Cretaceous WGD, our results suggest that the global environmental stress that led to mass extinctions was overcome in Bombacoideae and Malvoideae through different karyotypic and genomic trends.
Key-words: Bombacoideae; Malvoideae; Paleopolyploidy; Repetitive DNA; Evolution

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ID - 104

CHROMOSOMAL ASSOCIATIONS AND MEIOTIC BEHAVIOR IN *UROCHLOA HUMIDICOLA* (RENDLE) MORRONE AND ZULOAGA

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Abstract:

Urochloa humidicola, popularly known as "Quicuio-da-amazônia", is one of the tropical forage species of the genus *Urochloa*, which were introduced in Brazil in the 1980s. The adaptation of *U. humidicola* to Brazilian climate and soil conditions, such as poorly drained areas with seasonal flooding in the Pantanal and Amazon regions, resulted in its widespread use in cattle raising activities. Despite this, the composition of pastures has few species and this weakens the fields due to monoculture. Therefore, the development of new materials, through breeding, searching for favorable characteristics is extremely necessary. The polyploidy and apomixis, present in the genus, need to be overcome so that the variability can be exploited. In this context, the breeding program conducted by Embrapa Beff Cattle has been selecting the most adapted and stable materials. Thus, the objective of this study was to evaluate the meiotic behavior of apomictic accessions of *U. humidicola*. Ten accessions belonging to the genetic improvement program of Embrapa Beff Cattle - Campo Grande/MS - were analyzed. To perform the cytogenetic analyses, the material was collected during the flowering period of the plants. The collected material was fixed in ethyl alcohol, chloroform and propionic acid for 24 hours and kept under refrigeration in 70% alcohol until the slides were prepared. The preparation of the slides was conducted in the Laboratory of Plant Cytogenetics of the State University of Maringá - Maringá/PR. The slides were prepared by squashing, using 1% propionic carmine. The stages of meiosis between diakinesis and microspore tetrad were evaluated. It was counted about 1,000 cells per genotype. The chromosome associations were analyzed in the diakinesis phase and for each genotype were evalueted about 20 cells. For the evaluation of meiotic behavior, the phases from metaphase I to microspore tetrad were analyzed. The cells that presented intact cytoplasm and regular meiotic division were considered normal and those that presented variations in meiotic behavior were considered abnormal. In microspore tetrads, micronuclei were discriminated as to the number of affected microspores. The total number of cells analyzed, the percentage of abnormalities in each phase, and the average frequency of abnormalities per genotype were determined. The accessions HumF and HumH showed uni, bi, tri, tetra and hexavalent associations. The accessions HumA and HumI, on the other hand, showed only bi and tetravalent associations. The access HumG showed bi, tetra, and hexavalent compounds. In all accessions there was a higher frequency of bivalent associations. As for meiotic behavior, the accessions HumC, HumD, HumE, HumF and HumJ showed only segregational irregularities. The accessions HumA, HumB, HumG, HumH and HumI also showed convergent spindle formation during the first meiotic division. The segregational abnormalities affected the regularity of meiosis with mainly the tetrad micronuclei being the most detrimental to the normality of the final product, wich results in pollen and seed inviability.

Key-words: micronuclei; polyploidy; convergente spindle;

ID – 183

POLISOMATY AND HETEROCHROMATIC BLOCKS DISTRIBUTION IN *MIMOSA* L. KARYOTYPES

Jarbson Henrique Oliveira Silva [1]; Márcia Vieira de Sousa [2]; Loane Costa Sampaio [2]; Mariana Gomes Adriano [2]; Paulo Sarmanho da Costa Lima [3]; Lidiane de Lima Feitoza [2]; Lívia do Vale Martins [2]

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Abstract:

Mimosa L. (Leguminosae Juss.) comprises ~550 species mainly distributed in Brazil, especially in Cerrado and Caatinga biomes. The species of this genus are considered a multipurpose plant, being widely used in traditional folk, soil enrichment and recovery of degraded areas. Despite of its importance, *Mimosa* is an under-exploited genus in a cytogenetic and phylogenetic point of view. To better understand the karyotype constitution of this genus, the present work aimed to analyze the constitutive heterochromatin (CH) patterns in three species, *Mimosa caesalpiniifolia* Benth., *M. verrucosa* Benth. and *M. tenuiflora* (Willd.) Poir., by CMA/DAPI fluorochrome banding technique. *Mimosa* analyzed species showed 2*n* = 26 small meta- and submetacentric chromosomes, with constant number of heterochromatic blocks (four positive CMA bands), being the larger CMA^{++}/$DAPI^{-}$ pair (GC-rich and AT-poor, respectively) probably equivalent to the nucleolar organizer regions (NORs). Additionally, we observed, in all three species, the presence of two moderately stained CMA^{+}/$DAPI^{0}$ bands in the pericentromeric regions of one chromosome pair. In *M. verrucosa*, these bands showed a *dot-like* pattern. Interestingly, polysomatic root-tip cells were identified in *M. caesalpiniifolia* and *M. tenuiflora*¸ with diploid and polyploid metaphases in the same analyzed accession. As expected, we observed, in the polyploid cells, four larger CMA^{++}/$DAPI^{-}$ (probably NOR-related regions) strongly stained. Polysomaty has been previously reported in *Mimosa*, such as in *M. pudica, M. bimucronata* and *M. caesalpiniifolia*, and may be related to an advantage in the first developmental stages and seeding development. Our results reported, for the first time, the heterochromatin block patterns in *M. verrucosa* and *M. tenuiflora* karyotypes, with polysomatic cells related for the later species. The analysis of CH bands distribution in *Mimosa* species is a fundamental step in cytogenetics studies of this group. Further cytomolecular techniques as FISH (Fluorescent *in situ* Hybridization) using ribosomal DNA (rDNA) and dispersed repetitive DNA probes are, therefore, needed to increasing the knowledge of the distribution of repetitive DNA fraction and karyotype constitution in *Mimosa* genus.

Key-words: constitutive heterochromatin; *Mimosa*; polyploidy;

Acknowledgement

CAPES, FAPEPI

ID – 178

TROPICAL MAIZE POLLEN GRAIN FERTILITY EVALUATED BY DIFFERENT CULTURE MEDIUMS FOR *IN VITRO* GERMINATION

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Abstract:

Fertility is a determinant of maize yield, being male fertility given by the quality and longevity of the pollen grains. Pollen grains are the result of the formation and maturation of microspores originated during the meiotic division process, and their performance may be highly affected by environmental conditions. Introducing new genetic materials to a determined place requires a series of analyses, including pollen grain germination, to certify potential fertility and capability to cross with native varieties and species. Several approaches can be used to measure pollen grain viability, such as staining with specific dyes and germination in a culture medium. Pollen grain germination is a challenging approach because there is no universal protocol for all plant species, and even among varieties, results may vary in a broad range. To understand better the behavior of different sister inbred lines of maize, we analyzed pollen germination in three different culture mediums. Two culture mediums are recommended by the China government as a reference to test maize fertility. The other culture medium is considered a reference to maize in general, described in the Maize Handbook (Freeling and Walbot, 1994). Two near-isogenic inbred lines, 133425 and 133427, were used to investigate pollen grain fertility by monitoring the male tassel cycle. Plants were growth in a greenhouse under controlled environmental conditions. Pollen grains harvest started on the first day and followed until the sixth day after the flowering, using paper bags as the routine to make maize crosses. For each fresh pollen grain sample, three slides were prepared and stained with 1% aceto-carmine for viability measurement and to be used as a reference to compare with culture medium *in vitro* germination assays. Pollen grains *in vitro* germination was carried out in three replicas using the three mediums (1xPGM, 2xPGM, and MHB) described above on Petri dishes covered with moisture paper, kept in B.O.D. for 2 hours. Pollen tube growth was evaluated by counting under a microscope. A pollen grain was considered as germinated when its tube was 2x longer than its diameter. The total amount of pollen grain produced per plant was measured by weight. The pollen grain fertility by staining was 98,95% and 98,58%, respectively, to 133427 and 133425. The 133427 had 43,29% pollen grain germination at 1xPGM and 37,17% at MHB. The 133425 had 41,29 and 41,58% pollen grain germination using 1xPGM and MHB, respectively. The 2xPGM culture medium had the lowest performance for both genotypes, with 8,18% for the 133427 and 4,75% for the 133425. In total, 133427 produced 530 mg of pollen grain while the 133425 produced 440 mg. The highest pollen grain liberation occurs on the second day. This first report shows pollen grain *in vitro* germination is controlled by a interaction between culture medium and genotype.

Key-words: pollen grain; maize; fertility; *in vitro* germination; inbred lines

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ID – 94

GENOMIC AND CHROMOSOMAL DIFFERENCES BETWEEN DIPLOID AND POLYPLOID SAMPLES OF *ADENIUM OBESUM* (APOCYNACEAE)

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Abstract:

Adenium (Apocynaceae) comprises a plant group of succulent species with sculptural stems and large variations in flower shapes and colors. *Adenium obesum* is the species with the greatest ornamental interest, and most of the world's production chain is based on hybrids. Due to the lack of knowledge about genetic variations status between cultivars and hybrids, our objective was to compare diploid and polyploid samples from the productive chain, using cytomolecular techniques. The study was based on conventional cytogenetics, chromosome banding (CMA/DAPI), and FISH with rDNA, satDNA and Gypsy/Ogre retrotransposon probes using *A. obesum* with 2*n*=22 and 2*n*=44. It also involved flow cytometry and Illumina sequencing data obtained from NCBI (ERR5033363.1.1). 5S rDNA, Line and satDNA sequences predominated in relation to Copia and Gypsy retrotransposons. Karyotypes exhibited meta- and submetacentric chromosomes, and genome size ranged from 2C=2.06 to 2C=2.91 pg, which is a difference about 67.8%. The length (μm) of the polyploid complements was 70% greater than that observed in diploids, however, this value was 25% smaller than what is expected for an autopolyploid genome. $DAPI^+$ bands predominated in the proximal chromosome regions, while CMA^+ bands and rDNA sequences were terminal. In meiosis, $DAPI^+$ regions appeared as weakly condensed chromatin at interphase, whereas CMA^+ regions consistently appeared condensed. In zygotene and pachytene, $DAPI^+$ sequences were prominent in the proximal chromosome regions, as well as Ogre retrotransposons. Despite the similarity observed in the repetitive fraction, there was a reduction in the DNA C-value in the polyploid species. These analyses allowed for deepening the knowledge about the genomes in *Adenium* and showed that there was a reduction in the size of the genome after the polyploidy process. These data can be useful to add knowledge to the productive sector since the generation of hybrids and polyploids is common in the chain production of desert roses.

Key-words: CMA/DAPI bands; chromocenters; FISH; retrotransposons; rDNA

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ID - 108

INTRASPECIFIC VARIABILITY OF *HERBERTIA QUAREIMANA* RAVENNA (IRIDACEAE): A CYTOGENETIC, MORPHOLOGICAL AND ECOLOGICAL APPROACH

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Abstract:

Herbertia quareimana Ravenna is an herbaceous species with distribution restricted to a few localities in the Pampa biome near the Brazil borders with Argentina and Uruguay. Two morphotypes of *H. quareimana* have been observed in the field: the one with the smallest morphology found in Missões region and the largest one on the western border of the State of Rio Grande do Sul. Interestingly, mixed populations containing both morphotypes have not been found so far. Although the occurrence of intraspecific polyploid series is common in Iridaceae, the literature report just tetraploid plants (*2n = 28*) for this species. The polyploid process can result in ecological and morphological differentiation, which can impact the taxonomy. Thus, the present study aims to answer the following questions: i) are there really two distinct morphotypes of *H. quareimana*? ii) is there any relationship between these morphological differences and the ploidy level? iii) what could explain the geographical separation between them? To answer these questions, we used a multidisciplinary approach, including cytogenetic, morphometric analysis and niche modeling techniques. Ploidy level was accessed by chromosome counting (Giemsa conventional staining) and flow cytometry (following Dolezel et al 2007). Morphological variation was evaluated from 22 floral and 23 vegetative characters through univariate and multivariate statistics. Species distribution modeling was conducted aiming to understand the distribution pattern of *H. quareimana* through a machine learning approach, responsible for generate distribution models for each cytotype and for the species. Cytogenetic analyzes showed the existence of a diploid cytotype (*2n* = 14) that relates to the small morphotype; on the other hand, the large morphotype is the tetraploid cytotype previously reported in the literature. The results of the morphometric analyzes were consistent with the field observations and detected two very distinct morphological groups: one with only small individuals (diploid) and the other with only large individuals (tetraploid). Additionally, we identified nine floral characters with statistical capacity to distinguish the cytotypes. Analyzing the distribution of populations of *H. quareimana*, the segregation is clear: diploid populations occur in northwestern RS (Missões) and tetraploids are found in southwestern RS (Campanha region). This segregation is validated by models showing that the cytotypes inhabit distinct niches, with areas of probability of occurrence with different coverage between ploidy levels. The analysis strategies employed in the present study were successful in confirming the suspicion that diploid and tetraploid populations of *Herbertia quareimana* are distinct in morphology and climatic niche. The results contributed to a better understanding of species boundaries in *H. quareimana* and suggest new perspectives for evolutionary hypotheses. The modeling results may still be useful as input in a future review of the conservation status of *H. quareimana*.

Key-words: polyploidy; chromosome number; Iridaceae; niche modeling; morphological variation

ID - 196

THE FIRST WHOLE GENOME SEQUENCING OF PAU-BRASIL (*PAUBRASILIA ECHINATA*) REVEALS A PLEISTOCENE BURST OF TRANSPOSABLE ELEMENTS TY3/GYPSY TEKAY EXPANDING THROUGHOUT DIFFERENT CHROMOSOME DOMAINS

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Abstract:
Paubrasilia echinata ("pau-brasil") is the most emblematic, historically explored tree of Brazil and an endangered species endemic to the Brazilian Atlantic Forest. This species belongs to the Caesalpinia Group (Caesalpinioideae, Leguminosae) which presented remarkable genome-environment correlations in previous comparative genomics studies. This species has the karyotype 2n = 24, high amount of proximal GC-rich heterochromatin, DNA content of 1C = 1,378.98 Mpb, and a repetitive genomic fraction predominantly composed by transposable element (TE) Ty3/Gypsy Tekay. Despite the high number of whole genome sequences available for cultivated Papilionoideae species (beans, soybeans, peanuts, etc.), low genomic information is available for representatives of the subfamily Caesalpinioideae. In this sense, this work aims to provide the first whole genome sequencing at chromosome level of pau-brasil using PacBio-generated long reads. We characterize the genomic organization of repetitive elements, in order to discuss their dynamics and how the repeatome may mediate the remarkable genome-environment interaction in the Caesalpinia group. By combining long reads, short reads and HiC technology, we were able to reconstruct 12 scaffold-level molecules ranging from 37.50 Mbp to 152.17 Mbp, for a total length of 1,273.15 Mbp. The assembled genome was highly repetitive (69.4% of the total genome), with a remarkable dominance of TEs from the Ty3/Gypsy Tekay lineage (51.5%). The centromere specific TE Ty3/Gypsy CRM showed ubiquitous density peaks, denoting the possible centromere position within the scaffolds. As for the density of the highly abundant Tekay, we observed a non-uniform distribution throughout the scaffolds, being more prominent at the perincetromeric/interstitial regions, while less abundant at the terminals. This pattern is strikingly correlated with the previously reported CMA bands seen in the chromosomes of *P. echinata*, providing evidence that Tekay is the main component of the heterochromatin of this species. In contrast, general Ty1/Copia TEs density showed a inverse pattern, with density peaks at the scaffold terminals. Interestingly, Tekay elements present at terminal regions were mainly truncated (lacking one or more protein domains), while full-length Tekay were more restricted to the heterochromatic regions. It has been proposed that the presence of a chromodomain (CHD) in chromovirus TEs such as Tekay could facilitated target insertion at heterochromatic regions, effectively "hiding" these elements. Thus, we propose that while Tekay thrived at heterochromatic regions, amplification to the euchromatin (and a probable close proximity with genes) could have affected gene space and consequently occasioned deleterious effects. This would lead to selection of *P. echinate* lineages that successfully repressed Tekay transposition at gene-rich fractions, explaining the presence of non-functional Tekay fragments in the terminal regions. Dating of Tekay insertions revealed that most of the observed abundance of Tekay was the result of a single amplification burst approx. 2Mya, at the early Pleistocene, an ecologically challenging period. It is possible that Tekay is especially sensitive to climatic changes, and as prominent genome component of most species of Caesalpinia, it could be one of the major culprits of the genome-environment correlations observed in the group. Thus our ongoing efforts to assemble a high-quality reference genome for the symbol plant of Brazil can improve our understanding of the impact of repetitive elements on genome and species evolution. Additionally, a sequenced genome within Caesalpinioideae is going to help elucidate genome evolution in the whole legume family.
Key-words: Pau-brasil; Whole Genome Sequencing; Transposable elements; Heterochromatin;

Acknowledgement

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ID – 147

CHARACTERIZATION OF THE REPETITIVE FRACTION IN GENOMES OF SPECIES OF THE SUBTRIBE CASSIINAE (FABACEAE)

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Abstract:

Fabaceae is considered one of the three largest families of Angiosperms, containing 765 genera and 19,500 species with a cosmopolitan distribution. Several groups of the family have had their classification changed over time, as is the case of the Cassiinae subtribe, which has three genera resulting from the division of *Cassia* lato sensu: *Cassia* stricto sensu, *Chamaecrista* Moench and *Senna* Mill. Molecular and cytogenetic studies were important for the contribution of the systematics of the subtribe, but not enough to elucidate the controversies involving the relationship between the three genera. The inclusion of other genomic information is important to contribute to a better understanding of the relationships within the group. With the advent of next generation sequencing (NGS) it was possible to carry out studies that allow the identification and characterization of the repetitive fraction in the genome of a species, proving to be an interesting tool for understanding genomic evolution. Thus, our work aimed to characterize the repetitive fraction in the genome of species representing the three genera of Cassiinae. Low coverage sequencing (about 0.4x) was performed for *Cassia fistula* L., *Chamaecrista ensiformis* Vell. and *Senna occidentalis* L. using the Illumina HiSeq™ 4000 Platform. We performed a graph-clustering analysis on the RepeatExplorer pipeline for characterization and comparison of the repetitive fraction of the three species. The sequences classified as satellites in the individual analysis were identified by the TAREAN algorithm (RE2). From the clusters characterized as satellites, a dot-plot was used to confirm whether the repetition structures are tandem arrangements. The genome of *C. fistula* (0.70 pg/1C) has 41.74% repetitive DNA, *Ch. ensiformis* (0.85 pg/1C) presents 32.86% and *S. occidentalis* (0.70 pg/1C) 40.49%. The LTR elements were the most abundant (20.37% to 31.67%) in the genome of the three species, with the *Ty3/Gypsy* superfamily being the most abundant, corresponding to 13 to 20% of the repetitive fraction. The *Ty1/Copia* superfamily was less representative and more divergent among species, with 8.47% for *C. fistula*, 2.24% for *Ch. ensiformis* and 2.61% for *S. occidentalis*. 25 possible satellites were identified, 15 belonging to *Cassia fistula*, four to *Chamaecrista ensiformis* and six to *Senna occidentalis*. Of these, six sequences were confirmed as satellite through the Dotmatcher - EMBOSS platform, three for *C. fistula*, one for *Ch. ensiformis* and two for *S. occidentalis*. These sequences were predominantly unique to the species in which they were identified, with only three being shared among species. This pattern corroborates hypotheses that Cassiinae species underwent successive processes of chromosomal rearrangements during their evolution, which may have contributed to the emergence and elimination of satellite DNA families. These repetitive sequences change rapidly during evolution, where they become excellent markers that allow tracking chromosomal rearrangements. Among the three species, *C. fistula* and *S. occidentalis* presented the closest repetitive fraction in terms of abundance and families of shared elements, indicating greater distance from *Chamaecrista*. The satellite sequences identified in each species will be mapped using fluorescent in situ hybridization - FISH.

Key-words: Cassiinae; LTR elements; Satellite DNA;

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ID – 198

ALLOPOLYPLOIDY IN *VIGNA GLABRESCENS* MARÉCHAL, MASCHERPA & STAINIER SUGGESTED BY OLIGO-FISH

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Abstract:

Vigna Savi genus (Leguminosae family) comprises ~150 species, mostly with 2*n* = 22 chromosomes. As an exception, *Vigna glabrescens* Maréchal, Mascherpa & Stainier shows 2*n* = 4*x* = 44, being recognized as a natural tetraploid in the genus. Aiming to reveal chromosomal evolutionary mechanisms involved in the origin of *V. glabrescens*, we used oligopainting of *Phaseolus vulgaris* L. chromosomes 2 and 3 (*Pv*2 and *Pv*3) and oligo-FISH barcode probes of *Vigna unguiculata* (L.) Walp. Chromosome-specific painting allowed us to identify four copies of chromosome 3, with the short arm painted with *Pv*2 probe, besides a proximal region of the long arm marked with *Pv*2 probe and the remaining arm painted with *Pv*3. Additionally, four orthologs of the chromosome 2 exhibited the long arm and almost all the short arm painted with *Pv*2 probe, and a small terminal region of the short arm with a *Pv*3 faint signal. *Phaseolus vulgaris* chromosome 2 probe hybridized to additional chromosome sites in *V. glabrescens*, suggesting that complex rearrangements may be involved in the evolution of this species. The barcode probes enabled the identification of four copies of three chromosome types, with heteromorphic signal intensity for two types. Additionally, in other three chromosome types, only two copies, instead of four, were identified, corroborating the heteromorphic pattern of *V. glabrescens*. Based on these results, we suggest an allopolyploid chromosome composition for this species. Further cytomolecular analysis are needed to understand the evolutionary history of this species.

Key-words: Allotepraploidy; barcode; chromosome heteromorphism; oligo-FISH; oligopainting

Acknowledgement

Financial Support: CAPES, CNPq, FACEPE, UFPE

ID – 15

STABLE MEIOTIC CHROMOSOME SEGREGATION IN SUGARCANE (*SACCHARUM* SPP.) AND THE EXISTENCE OF THE *ZIP4* GENE, A PROMISING CANDIDATE GENE RESPONSIBLE FOR REGULATING THE PROGRESSION OF MEIOSIS

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Abstract:

The modern cultivars of sugarcane (Saccharum spp.) are highly polyploid and accumulate aneuploidies due to their history of domestication, genetic improvement and interspecific hybrid origin involving the domesticated sweet species S. officinarum ('noble cane') and the wild S. spontaneum, both with an evolutionary history of polyploidy. The first hybrids were backcrossed with S. officinarum, and selection from progenies in subsequent generations established the genetic basis of modern cultivars. Saccharum genome complexity has inspired several molecular studies that have elucidated aspects of sugarcane genome constitution, architecture, and cytogenetics. Herein, we present a comparative analysis of the meiotic behavior of representatives of the sugarcane parental species (*S. officinarum* and *S. spontaneum*), and the commercial variety, SP80-3280. *S. officinarum*, an octoploid species, exhibited regular meiotic behavior. In contrast, *S. spontaneum* and the variety SP80-3280 exhibited several abnormalities, 51.8% and 77.1% of cells exhibited abnormalities, respectively, including lagging chromosomes from metaphase I to the end of division. We reported, for the first time, the occurrence of both peri- and paracentric inversions. We visualized an inversion loop in SP80-3280 pachytene cells containing the centromere, that is typical of a pericentric inversion. Clarifying, for a pericentric inversion to occur, two breaks arise on opposite arms of the centromere; the region between the breaks is inverted, and the ends are rejoined to the rest of the chromosome. We also visualized chromosome dicentric bridges in some SP80-3280 anaphase I and II cells. When two breaks in one chromosome arm rejoin after the excised piece has inverted, not including the centromere, this results in a paracentric inversion. Furthermore, using *in-situ* hybridization techniques (FISH and GISH), we were able to determine how pairing association occurred at diakinesis and, in particular, the chromosome composition of SP80-3280, which is as follows: 80% of *S. officinarum*, 11% of *S. spontaneum* and 9% of recombinant chromosomes. Despite its interspecific origin, bivalent pairing prevails. Our findings lend weight to the idea that stable chromosome segregation occurs in modern sugarcane varieties, suggesting that a synapse regulatory mechanism exists in *Saccharum*, in which multivalent associations are resolved into bivalents towards the end of prophase I. This mechanism has been proven to exist in wheat and *Brassica*. There are gene clusters responsible for regulating the progression of meiosis and the promising candidate gene to play this role is thought to be *ZIP4*, which acts as a scaffold protein containing tetratricopeptide repeats, facilitating the assembly of protein complexes and promoting homologous crossovers. Therefore, using the Basic Local Alignment Search Tool (BLAST), we searched for *ZIP4* sequences in the parental species and the commercial variety, SP80-3280, considering the sequence similarities of *ZIP4* found in the available genome resources of sorghum and wheat. Our sequence-data and bioinformatic studies support that *ZIP4* exists in sugarcane and their parentals, explaining that, despite the chromosomal abnormalities found, meiotic segregation is regular in sugarcane. Overall, our results have implications for sugarcane genetic mapping, genomics and molecular cytogenetics, and also for other studies on resynthesized polyploids.

Key-words: meiotic behaviour; polyploid; scaffold protein;

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ID 139
DIFFERENTIAL CHROMATIN STATUS OBSERVED FOR MAIZE K180 KNOB REPEATS

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Abstract:
Maize knobs are heterochromatic regions that exhibit notable variation in number and position among different genotypes. They are composed of two main repeats, K180 and TR-1 organized *in tandem* arrays. Based on DNA sequence analysis, our research group has proposed the division of K180 into two subfamilies, named K180_1 and K180_2. Aiming to uncover more details about the structure and organization of maize knobs, we mapped the two subfamilies to the reference genome of *Zea mays* B73v5 and compared their chromatin status. Using the UCSC Genomaize (genomaize.org) tools, the distribution data was intersected with DNA replication timing profiles and chromatin accessibility patterns for four tissues: ear shoot, endosperm, coleoptilar node, and root tip. The K180_1 repeats occurs in long blocks coinciding with the cytological knobs and dispersed in smaller clusters along the chromosomes. Meanwhile, the K180_2 subfamily is distributed in small clusters, only outside cytological knobs. The dispersed K180_1 and K180_2 sequences occur in regions with early or middle replication timings, contrasting with the late replication profile observed for the regions of cytological knobs. The chromatin accessibility analysis revealed an open chromatin pattern for K180_2 in all tissues. Contrastingly, the K180_1 subfamily seems to display a swift from closed to open chromatin in a tissue-specific manner. The root tip presented the highest levels of chromatin accessibility, while the lowest level was observed in the endosperm, followed by the coleoptilar node and ear shoot. These results indicate that knobs might be more dynamic and responsive structures instead of static heterochromatic blocks.
Key-words: Heterochromatin; 180-bp knob; Repetitive DNA;

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ID – 196

ECOLOGICALLY-SENSITIVE TRANSPOSABLE ELEMENTS TY3/GYPSY TEKAY CAN DRIVE THE GENOME-ENVIRONMENT INTERACTION IN THE CAESALPINIA GROUP (LEGUMINOSAE)?

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Abstract:

The Caesalpinia Group (Leguminosae) offers an interesting model for comparative genomics studies by presenting a marked interaction between genomic traits and ecological variables. Although macroevolutionary studies have demonstrated these correlations, it is not clear how the different repetitive elements respond to environmental changes at a fine genomic scale. Within this group, the Neotropical genus *Erythrostemon* stands out by being the most diverse (22 spp.), ecologically heterogeneous and presenting a phylogenetically-corroborated disjoint distribution in Mesoamerica and South America. Here we performed a comparative genomic analysis of *Erythrostemon* species in order to test the impact of environmental variables and phylogenetics relationships over different repeat lineages. We compared the repetitive fraction and environmental variables of 9 *Erythrostemon* species (7 Mesoamerican and 2 South American) across a complete plastome phylogeny. Individually, the repetitive elements were diverse in *Erythrostemon*, especially the transposable element (TE) Ty3/Gypsy Tekay linage which is 30-folds more abundant in the South American than in the Mesoamerican species. Interestingly, the differential Tekay abundance between *Erythrostemon* clades seems to be related to previously described heterochromatic patterns divergent between Meso- and South American clades. Thus, total repeatome composition was able to strongly differentiate these lineages. Remarkably, Tekay, Athila and SIRE TE lineages showed differentiating traits. On the other hand, satDNA repeats showed a predominantly species-specific pattern, apparently without phylogenetic correlation. TEs abundance in *Erythorstemon* showed a high correlation with phylogenetic relatedness (phylogenetic signal), suggesting that TEs evolve uniformly along with speciation in this clade. Furthermore, some specific repeat lineages showed a significant correlation with temperature variables. Tekay abundance presented a strong negative phylogenetic correlation with Mean Temperature of Wettest Quarter ($r^2 = 0.83$) and Altitude ($r^2=0.80$), while all other significantly correlated TEs showed an exact opposite pattern. Remarkably, Tekay evolutionary trends described here directly mirrors the relationship between genome size and latitude/temperature described for the whole Caesalpinia group, suggesting that this element may be primarily responsible for the previously described genome-environment correlations. Although it is unclear why Tekay exhibits this behavior, we hypothesize that the presence of chromodomains (CHD) can facilitate the targeted integration of LTR retrotransposons in the heterochromatin, allowing them to avoid negative selection arising from insertion into coding regions. In this scenario, Tekay could be "hidden" of selection effects, and while under neutral evolution, would provide the condition for heterochromatin (and genome size) expansion/retraction, followed by differential ecological selection in the Neotropical region during the last 35 Mya. Future whole-genome sequencing and assembly of Caesalpinia group species may test this theory and clarify the role of specific repetitive elements in the genome-environment interactions.

Key-words: Caesalpinia; transposable elements; ecological variables;

ID – 19

THE *ZIP4* GENE, A PUTATIVE CANDIDATE FOR CONTROLLING THE PROGRESSION OF MEIOSIS IN SUGARCANE, A MAN-MADE GENOME OF INTERSPECIFIC ORIGIN.

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Abstract:

Despite having a highly polyploid and complex genome, the wild *Saccharum spontaneum* (2n = 40-128) and the cultivated species *S. officinarum* (2n = 80; the noble cane), and the modern commercial varieties of sugarcane (2n = 100-120) exhibit a predominant bivalent pairing during meiosis. The control of meiosis progression in natural polyploids has been extensively researched, especially in wheat, in which a potential candidate, the *ZIP4* gene, acts as a scaffold protein that promotes the development of protein complexes which, in turn, favors homologous crossovers. The aim of this work is to identify the *ZIP4* gene in sugarcane cultivars and parental species, which may reveal the existence of a genetic control of meiosis progression in sugarcane. In the present study, *ZIP4* was investigated in the available genomes of both sugarcane parental species, and four and three homeologous copies were found to occur in *S. spontaneum* and *S. officinarum*, respectively. In the cultivated hybrids, SP80-3280 (2n = 112) and R570 (2n = 114), four and 11 homoeologous copies were discovered, respectively. In sugarcane, *ZIP4* has a 3254-bp genomic sequence exhibiting a conserved structure of five exons and four introns in all copies. The coding sequence is 2901-bp long with at least 99.5 % pairwise identity. According to the InterProScan computational tool, the following domains are present in the 966-aa protein: SPORULATION-SPECIFIC PROTEIN 22/ZIP4 (PTHR40375), TPR-like (SSF48452), and Meiosis protein SPO22/ZIP4 like (PF08631), which are the same domains reported in wheat. The TPRpred-based analysis of the tetratricopeptide repeats (TPRs) that compose the SPO22/ZIP4 domain revealed the occurrence of five TPRs in all *ZIP4* copies. The MARCOIL, a hidden MARkov model-based program that identifies coiled coils domains, was used; however, no coiled coils were identified. Nevertheless, the number of TPRs per gene copy in sugarcane is low comparing to the number in wheat, in which it ranges from nine to 12. Our studies give support to the existence of a genetic control of meiosis progression in sugarcane; however functional studies are necessary to confirm this hypothesis.

Key-words: Saccharum; Polyploid; Homeologous pairing;

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ID 82

CHROMOSOMAL BANDING AND ESTIMATION OF DNA CONTENT IN *ARUNDINA GRAMINIFOLIA* (ORCHIDACEAE), AN IMPORTANT ORNAMENTAL SPECIES

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Abstract:
Commonly known as bamboo orchid, *Arundina graminifolia* (D. Don) Hochr (Orchidaceae) has a great importance in the ornamental plant market, due to the beauty of its flowers and for blooming all year round. However, little is known about the cytological aspects of *A. graminifolia*. Thus, in view of the economic importance and the lack of chromosomal data for the species, the objective of this work was to characterize cytogenetically specimens of *A. graminifolia* collected at different points of Juiz de Fora, Minas Gerais. In this way, analyzes of chromosome number and distribution of heterochromatin (chromosome banding) were performed. For this, we used root tips, fixed in etahnol/acetic acid solution pre-treated with mitotic blocker 8-HQ (Hydroxyquinoline, 3mM) for 9h at room temperature digested in Cellulase (2%)/Pectinase (20%) solution for 5h at 37°C. The slides were prepared using the air-drying cell dissociation technique, aged for 3 days at room temperature and stained with 30 uL of DAPI (2 µg/mL) for one hour and 30 uL of CMA (0,5 mg/mL) for 30 minutes, after which 30 uL of glycerol/McIlvaine (1:1) were added and covered with a coverslip sealed for visualization under a fluorescence microscope. The DNA content of *A. graminifolia* was also estimated using flow cytometry. Plant tissue samples (leaves) were macerated in 500 uL of WPB buffer and stained with 20 uL of Propidium Iodide (1mg/mL). The external standard species used for the analyzes was *Glycine max* Cv. Polanka (Fabaceae). The results revealed that the species shows 2n=40 chromosomes, as reported in the literature, and approximately 7.50 picograms of DNA (pg). Furthermore, the species presented around 28 interstitial DAPI bands and 2 terminal CMA bands. Our results, in addition to confirming the chromosome number of the species, introduce for the first time the DNA content and the heterochromatin pattern of *A. graminifolia*. These data are very useful information for taxonomy of the species and for future applications in the pre-breeding/breeding programs of this important ornamental plant.
Key-words: chromosome number; chromosome banding; flow cytometry; heterochromatin; ornamental plant

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ID – 25

COMPARATIVE ANALYSIS OF RETROTRANSPOSONS AMONG TOMATO SPECIES AND THE CHARACTERIZATION OF CENTROMERIC ELEMENTS

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Abstract:

The cultivated tomato (*Solanum lycopersicum* L.) is a well-described model organism, and its wild relatives a great diversity source. Along with the cultivated, there are 12 wild and 2 related species. Several tomato genomes have been published, and long-reads brought more information about the repetitive fraction, like retrotransposons and satellite DNA. Some retrotransposons, like CRM, can accumulate at the centromeric and pericentromeric regions, sometimes accompanied by arrays of satellite DNA. Species from the *Solanum* genus seem to have the CRM lineage underrepresented, this information can raise questions about the composition of the centromeric and pericentromeric regions. Knowing the low accumulation of CRM sequences in the tomato genome, this work aimed to characterize the centromeric region of the cultivated tomato and to explore the centromeric diversity in closely related species. For this, two approaches were employed, the first was the use of bioinformatic tools to retrieve, classify and map repetitive elements in pseudochromosomes of long reads sequencing. The second was molecular cytogenetic methods for the physical localization of the sequences retrieved. A chloroplast tree with 14 tomato genomes (*S. lycopersicum* and wild relatives) was built and three major groups were observed. The first group was composed of species with red and orange fruits, along with *S. habrochaites* L. (green fruits). The second was composed of species with green fruits, and the most divergent (*S. lycopersicoides* (A. Child) Peralta) formed an isolated group. With the comparative analysis implemented on RepeatExplorer, no drastic difference was observed among all species. The lineages Tekay and Athila were the most representative, both from the Gypsy superfamily, CRM which belongs to the Chromoviruses was not observed among the major lineages. Although no drastic difference, the LINE elements stand out for the great accumulation among seven green fruit species. The centromeric repeat TGRIV was mapped and the putative centromeric regions were extracted and analyzed and the set of sequences that composes the centromere of tomato species was retrieved. Firstly, we have identified a TGRIV sequence, followed by a retrotransposon-like identified as *Jinling*, which contains satellite sequences in both LTRs and, finally, a satellite family. These sequences appear to have a differential accumulation among chromosomes from the same species and among species. FISH assays revealed either scattered or clustered signals, depending on the probe analyzed. The putative centromeric satellite exhibited strong signals. Both TGRIV and *Jinling* belong to the Tekay lineage, usually probes from this lineage show a scattered profile, but this was not the case. The *Jinling* probe exhibited some signals at the centromeric but also in proximal regions. The TGRIV probe exhibited centromeric signals, but not in all chromosomes, some chromosomes with a more intense signal. Here we show that centromeric regions of tomato species are composed by sequences from the Tekay lineage in arranges interspaced with satellite sequences. We also demonstrate that the *Jinling* retrotransposon is present in the centromeric array and not only in the pericentromeric regions as previous report stated. The diversity of centromeric sequences could be observed in all wild relatives.

Key-words: centromere; *Solanum*; transposons;

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ID – 181

COMPARATIVE ANALYSIS BETWEEN PARENTS AND HYBRID BASED ON REPETITIVE SEQUENCES AND GENOME SIZE IN *UROCHLOA HUMIDICOLA* (RENDLE) MORRONE & ZULOAGA

Rafael Penha Brito [1]; Bruna Natália Veloso dos Santos [1]; Ana Gabriela Damasceno [1]; Sanzio Carvalho de Lima Barrios [3]; Cacilda Borges do Valle [3]; Vânia Helena Techio [2]

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Abstract:

Since the 2000s, a significant sample of species, accessions, euploid and aneuploid hybrids of *Urochloa* P. Beauv. have been evaluated using cytogenetic analyses. Much of this information is applied to breeding programs, aiming to produce forage grasses with high quality and productivity. The program with *Urochloa humidicola* (Rendle) Morrone & Zuloaga is focused on developing apomictic cultivars from crossing with the only sexual accession H031 (CIAT 26146) so far identified in the germplasm. This species has a basic chromosome number of x=6 and forms a polyploid series (6x, 7x and 9x), predominantly apomictic. Molecular and cytogenetic studies suggest allopolyploidy and a combination of two or even three subgenomes, which still need to be confirmed. This study aimed to compare the genome size and the location of repetitive sequences in the chromosomes of the parental accessions (H016 and H031) and of the hybrid (Hb216) of *U. humidicola*. The roots were treated with cyclohexamide and amiprophos-methyl (2:1), and slides were prepared by cell dissociation and air drying. FISH was performed with probes for the satellite sequence (sat_1) from the genome of *U. humidicola*, and for the 18S rDNA sequence of the genome of *Oryza sativa*. The DNA content was estimated by flow cytometry and the difference between genotypes was evaluated by the Scott-Knott test. Parental accessions H016 (apomictic) and H031 (sexual) showed, respectively, 2n=4x=36 and 2n=4x=36+1 (aneuploid) chromosomes and genome size 2C= 4.40pg and 4.45pg. The hybrid Hb216 is euploid with 2n = 6x = 36 chromosomes, karyotypic formula 32m+4sm and genome size 2C = 4.26 pg, with no statistical differences towards the parents. The parent H031 and the hybrid Hb216 showed four 18S rDNA signals positioned in the terminal region of the chromosomes, while accession H016 showed five signals. The sat_1 probe predominantly showed terminal signals in the parents and the hybrid. In accessions H031 and H016, 10 and 17 chromosomes, respectively, were hybridized with this sequence on the short arm, except for one chromosome pair from H016, whose signal was on the long arm. This accession showed greater variation for the sat_1 sequence with the chromosome showing a syntenic signal (in the opposite arms) and an interstitial signal, contiguous to the 18S rDNA sequence. In Hb216, the sat_1 sequence showed 14 conspicuous signals in metaphase, coinciding with the marking in interphase nuclei. The three genotypes presented one (H016) or two chromosomes (H031/Hb216) with markings at both ends. The FISH sat_1 and 18S rDNA marks show size heteromorphism, indicating a variable number of tandem repeats between chromosomes. The karyotypic variations between the parents and the hybrid can be due to hybridization processes, polyploidization rounds and post-polyploidization changes. Given that H031 has an extra chromosome and a supposed hybrid origin, genealogies involving this parent, may contribute to amplifying the effect of the genomic combination on progenies. These results show the importance of karyotypic characterization of hybrids produced in forage breeding programs.

Key-words: *Brachiaria*; Cytometry; FISH; ;

Acknowledgement

FAPEMIG, Capes e CNPq

ID – 20

SAMPLES COLLECTED IN THE PARAÍBA DO SUL RIVER (VOLTA REDONDA-RJ) SHOWED CYTOGENOTOXICITY IN THE *ALLIUM CEPA* L MODEL.

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Abstract:

Volta Redonda-RJ (VR) is one of the most polluted cities in Brazil. This pollution is consequence of diverse industries, including one of the largest in Brazil, the CSN, which releases a black powder that spreads reaching various points of the city. Another problem lies in the existence of a "mountain" present alongside the industry, called slag (localized near to Paraíba do Sul river). Slag may contain heavy metals, the presence of which has been demonstrated in air, soil and water in VR. Both the release of this black powder into the atmosphere that pollutes the river itself near the CSN and the existence of this slag near the river impact the entire municipality. The treated water that supplies VR comes from the Paraíba do Sul River and the Sewage Treatment Plants, most of the time, cannot extract these polluting agents. The purpose of this work was to analyze the water collected in the Paraíba do Sul River. Three points were sampled. One at the entrance of the river in the city of VR (P1), one in front of the storage space of CSN slag (P2) and another at the exit of the river of the city (P3). P1 is located upstream of P2 and P3 is located downstream of P2. Samples were collected on the bank of the river with the aid of a bucket. These samples were analyzed by the *Allium cepa* L. assay. Was used a completely randomized design composed of 3 replications (3 slides per treatments). Water samples and distilled water (negative control; NC) were used in this assay. *A. cepa* bulbs were exposed to distilled water for 24 hours for rooting, then exposed to treatments for 24 hours. The slides were made by squashing method and variables related to cell cycle, chromosomal alterations and toxicity were quantified. The mitotic index (MI), which reflects the percentage of dividing cells, demonstrated a significantly different result (Tukey, $p<0.05$) for the water collected in P2 in relation to the NC (cells exposed to P2 water) showed increase in MI (Tukey, $p<0.05$), corroborated by a greater observation of metaphases in the slides (increase in metaphase index) (Tukey, $p<0.05$). This result is related to the observation of a high number of c-metaphase (Tukey, $p<0.05$), an aneugenic alteration, characteristic of the malfunction of the mitotic spindle. Several other aneugenic chromosomal alterations were observed, such as late segregation, chromosomal loss, multipolarity, evidencing that the water collected in P2 has an aneugenic effect. Clastogenic changes (related to DNA breaks) were also perceived in the analysis, but most of them had no significant effect (Tukey, $p<0.05$). It is evident the presence of cytogenotoxic pollutants in the water of the Paraíba do Sul River, especially in the vicinity of the area that maintains the steel slag generated by CSN. The aneugenic effects observed in the results mean the risk of serious occurrences of genetic damage in species that depend on river water, especially the human species.

Key-words: ecotoxicology; mutagenesis; *Allium cepa*;

Acknowledgement

Fapemig, CNPq, Capes e UFJF

ID – 88

CYTOGENOTOXIC EFFECT OF THREE PHARMACEUTICALS DETECTED IN PARAIBUNA RIVER (JUIZ DE FORA-MG): A STUDY USING *ALLIUM CEPA* L. ASSAY

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Abstract:

Pharmaceutical active compounds (PhACs) are currently considered emerging contaminants, for which regulations and a greater understanding of their effects on the environment are still required. Reports of these contaminants in aquatic environments have been consistent, and environmental researchers are increasingly interested in this subject. One of the adverse effects of the presence of drugs in the environment is the cytogenotoxic effect, which is related to disturbances in the cell cycle and DNA, as well as toxicity. One of the main models for the investigation of the cytogenotoxic action of pollutants in an ecotoxicological context is the species *Allium cepa* L. In this work, we investigated the effect of three PhACs found in the Paraibuna River in the municipality of Juiz de Fora-MG in high concentrations: metformin, valsartan and atenolol. From the concentrations found in Juiz de Fora-MG (0.004471 mg/L of metformin; 0.000110mg/L of valsartan and 0.000106mg/L of atenolol), we obtained a mixture of these drugs. This treatment was called CJF (concentrations in Juiz de Fora-MG). Two concentrations greater than CJF (100xCJF and 10xCJF) and two lower concentrations than CJF (1/10CJF and 1/100CJF) were also used in the assay. Distilled water was used in the negative control (NC). The experiment was carried out using a completely randomized design with three replicates (bulbs) per treatment. The bulbs were exposed to the treatments for root emergence for 24 hours. After exposure, the roots were fixed in ethanol:acetic acid (3:1 v/v) and stored for 24 h, subsequently hydrolyzed (HCL 5N for 10 min), and the meristematic region collected. The preparation of the slides followed the squash technique. For each replicate, 1 meristem was analyzed by microscopy, and approximately 1500 cells were counted per slide, totaling 4,500 cells. The mitotic index (MI) decreased in all treatments compared to NC, except for the treatment of 1/100CJF (on average these reductions were 34% in relation to the NC) (Tukey, $p<0.05$). An increase in the percentage of prophases was observed in the two highest concentrations investigated (100xCJF and 10xCJF), 1.64X and 1.23X respectively. (Tukey, $p<0.05$). Regarding chromosomal alterations, all variables analyzed (percentage of clastogenic, aneugenic, and toxic alterations) showed an effect in all treatments investigated, except for the treatment of 1/100CJF (Tukey, $p<0.05$). The significant increases for these variables were 1.11X; 1.32X and 1.39X, respectively (considering all treatments with significant effects). The main alterations with a significant effect were adherent chromosomes, c-metaphase, and chromosomal bridges for the treatments with significant effect. The main significant effects were observed for the 100xCJF and 10xCJF treatments, but even in the observed concentration of these drugs in the municipality of Juiz de Fora (considered a low concentration) we observed effects. The conclusion of this work is that even in the concentrations of metformin, valsartan, and atenolol drugs found in Juiz de Fora-MG, we observed a cytogenotoxic effect, and these effects remain even when we dilute these concentrations up to 10x. The risk associated with the presence of these drugs in the environment is evident.

Key-words: emerging contaminants; ecotoxicological risk; DNA damage;

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ID – 210
HOW VARIABLE CAN IT STILL BE? UNBOXING PANDORA'S BOX MAIZE GENOME

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Abstract:
Maize is a crop species, genetically variable and distributed over different climatic conditions, altitudes and latitudes. Landraces and racial complexes are representative of this wide genetic variability. Maize landraces, by definition, are a set of local varieties that share common genetic characteristics, which are adapted to environments, and associated with certain cultural and social contexts. Recent studies revisited the classification of maize landraces for the lowlands of South America and contributed with a new look at the diversity of local varieties in Brazil and Uruguay. One of the steps to identify local varieties is through cytogenetic characterization. Based on this information, it is possible to assemble karyotypes of each variety, assessing the differences between them, in order to validate the groups formed through phenotypic characterization and infer the intraspecific dynamics of the maize genome. In this context, the aim of this work was to estimate, for the first time, the genome size (GS) of different landraces belonging to Uruguay and Brazil and to analyze the intragenomic variation of heterochromatic knobs within the landrace called Cateto. For GS, 253 individuals distributed among 16 local varieties and 11 landraces from both countries were analyzed by flow cytometry. In cytogenetic characterization, the knob sequence was physically mapped in two accessions from each country by fluorescent in situ hybridization using the 180 bp knob repeat probe labeled with Cy3. GS data were subjected to analysis of variance and Bonferroni test at $P > 0.01$. The GS ranged between 2C = 4.8 pg and 2C = 5.6 pg and significant differences were found between local varieties, landraces and countries. Comparing the two countries, the mean GS of natural maize populations in Brazil was higher than in Uruguay. The same result was also observed for the comparison of landraces and local varieties, with significant differences emerging between landraces and local varieties from both countries. The intragenomic analysis within the Cateto's landrace showed variation in the number of heterochromatic knobs between local varieties ranging between 13 to 24 knobs. This variability was observed not only for knob numbers, but also for the position in the chromosomes and knob sizes. Another interesting result was the presence of the B chromosome exclusively in the local variety from Uruguay. Analyzing the GS within the Cateto's landrace, it was noticed that there are no significant differences between the local varieties, however, the varieties had a different number of knobs and B chromosomes. These results corroborate previous studies indicating that there is no direct relationship between the GS and the composition of knobs, and that the intraspecific GS variation may be associated either with fluctuations in the repetitive content of the DNA or structural rearrangements. This basic research is the first step towards knowing the intragenomic variability of local varieties from the lowlands of South America. Understanding maize diversity allows inferring the evolutionary history of the species, in addition to benefiting the planning of public policies aimed at the conservation of this genetic resource and avoiding the loss of variability.
Key-words: Landraces; genome size; heterochromatic knobs; genetic resource; Lowlands of South America

Acknowledgement

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ID – 206
PHYLOGENETIC COMPARATIVE METHODS IN KARYOTYPE TRAITS EVOLUTION

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Abstract:
Phylogenetic comparative methods allow inferences about species' biological adaptation, given the taxon and character diversity relationship. However, studies based on ancestral state reconstruction and evaluation of major chromosomal rearrangements rarely consider the evaluation of phylogenetic signal (Pagel's λ), mode (Kappa - κ), and tempo (delta - δ) of character evolution, as well as the evolutionary model (BM, OU, EB, and White-noise). Ignoring how characters respond throughout evolution can weaken conclusions about the karyotype changes impact. Here, we aim to perform all these analyses based on 13 tribes of the subfamily Epidendroideae (Orchidaceae). Chromosome number (CN) and genome size (GS) data were organised into an online database (http://orchidcountsdb.epizy.com/). The 13 tribes were organised into five groups, and the phylogenetic trees were constructed based on four markers: ITS, matK, ycf1, and atpB. The CN varied from 2*n*=12 in *Gastrodia* (Gastrodieae) to 2*n*=162 in *Catasetum* (Cymbideae), while GS varied 45.5x, from 1C=0.58pg in *Polystachya* (Vandeae) to 26.4pg in *Nervilia* (Nervilieae). The ancestral base chromosome number estimated for the basal tribes and Malaxidinae was x=20, with a reduction in Arethusae and Vandae (x=19) and an increase in Cymbidieae (x=27). GS was estimated at 1C=12.33pg in the basal tribes, with a reduction in the other tribes (1C=c.3pg), especially in Malaxidinae (1C=1.96pg). CN presented a low phylogenetic signal (λ between 0 and 0.39), except for the basal tribes (λ = 0.63) and Cymbidae (λ = 0.89). On the other hand, GS presented a high phylogenetic signal (λ between 0.82 and 1), except for Arethusae (λ = 0). The mode and tempo of evolution suggest that both CN and GS show punctual evolution (κ < 1), with the long branches contributing more to the evolution of both characters (δ>1). The exception is Cymbidieae, which shows adaptive radiation for CN and GS (κ = 0 and 0 / δ = 0.46 and 0.31, respectively). The OU evolutionary model, which suggests a response to stabilising selection, was considered the best model for CN in all groups, while BM was the best for GS. Again, the exception is Cymbidieae, which had EB as the best model for both characters, reinforcing the suggestion of adaptive radiation. However, when considering the White-noise model, it proved to be the best model for most groups in both characters. The White-noise model makes sense, given the CN's conservation throughout the groups' phylogenies. For the basal tribes, the best model for CN was OU, which also makes sense given the phylogenetic signal (λ=0.63). However, in Cymbidieae, White-noise seems to be an incompatible result. Similarly, White-noise (despite its low AIC value) is not the most suitable model for GS, given its high phylogenetic signal in different groups. The second-best model (BM) for all groups and EB for Cymbidieae seem to describe changes in GS more adequately. It is concluded that describing the evolutionary model along with character evolution and both added to the reconstruction of ancestral states can enrich conclusions, suggesting whether such detected changes are adaptive or merely reflect chromosomal alterations generated at random.
Key-words: chromosome number evolution; genome size evolution; Orchidaceae; orchid; Epidendroideae

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ID – 12

THE FIRST STEPS OF A HYBRID: INFERENCES FROM CYTOGENETICS AND MORPHOLOGY.

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Abstract:

Hybridisation plays a prominent role in the evolutionary history of plants. In this sense, the supposed hybrid zone formed by the South African species of the genus Schizochilus, S. bulbinella and S. flexuosus, offers the opportunity to follow the first steps of hybrid species, if the hybridisation event is confirmed. We employed genome size analyses (C-value), linear morphology of floral structures (bract, ovary and bract/ovary ratio) and pollen viability to test this hypothesis. Furthermore, the supposed hybrids were divided into three classes based on the colour of their sepals: white, cream and yellow. The genome size estimated by flow cytometry indicated that S. bulbinella (1C=4.40pg) has a smaller genome than S. flexuosus (1C=5.00pg, p=0), with the supposed hybrids presenting intermediate C-values: the white class differs from both parentals (1C=3.82pg, p<0.28), but presents a high internal variance (3.25x); the cream class does not differ from S. flexuosus (p=0.98), differing only from S. bulbinella (p=0), while the yellow class does not differ from S. bulbinella (p=0.92), differing only from S. flexuosus (p=0). However, there is a high variance between individuals of the cream class (7.88x), allowing them to be organised into three subgroups. The analysis of these subgroups indicates that only one of them differs from S. flexuosus (1C=5.80pg, p=0). The analysis of the linear morphology of the bract (length and width) and the ovary (length) indicated a difference between S. bulbinella and S. flexuosus in all measures (p=0). Considering the supposed hybrids, the white and cream classes present intermediate means between the two parental species, not differing from S. flexuosus only in ovary length (p>0.05). In contrast, the yellow class does not differ from S. bulbinella (p>0.05). In the bract/ovary ratio, S. flexuosus is different from S. bulbinella (p=0), and all classes of supposed hybrids and S. bulbinella form a single group (p>0.69). Pollen viability was equally high for both parental species and the supposed hybrids (99.96% of the pollens were viable in S. bulbinella, 99.91% in S. flexuosus and 98.5% in the supposed hybrids; p=0.81). The intermediate C-value and linear morphology results support the hybridisation hypothesis between S. flexuosus and S. bulbinella, in agreement with our previous results of floral geometrical morphometry (labellum, dorsal and lateral sepal). The whole set of evidence supports the hypothesis of hybridisation, especially for white and cream classes but less evident for the yellow class, which was quite similar to S. bulbinella in the different analyses. The high variability observed in the C-value data of the hybrids indicates a recent origin of these individuals. However, this result goes against the high pollen viability of the hybrids - something uncommon in recent hybrids. However, a meiotic analysis will clarify the regularity of this process and indicate, or not, the occurrence of typical meiotic hybrid irregularities.

Key-words: genome size; pollen viability; linear morphology; *Schizochilus*;

Acknowledgement

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ID – 189

TRANSLOCATIONS AND INVERSIONS: MAJOR CHROMOSOMAL REARRANGEMENTS RELATED TO *VIGNA* SAVI (LEGUMINOSAE) EVOLUTION

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Abstract:

The development of oligo-fluorescence *in situ* hybridization-based chromosome identification techniques has optimized time and provided detailed information for understanding chromosomal evolution in plants. Aiming to understand the chromosomal diversity, macrosynteny and karyotypic evolution of beans from the five *Vigna* subgenera (*V.* subg. *Vigna*, *V.* subg. *Haydonia*, *V.* subg. *Plectrotropis*, *V.* subg. *Lasiospron* and *V.* subg. *Ceratotropis*), we constructed cytogenetic maps for eight taxa, using oligo-FISH barcode and oligopainting probes. We used specific oligopainting probes from *P. vulgaris* chromosomes 2 and 3 (*Pv*2 in green and *Pv*3 in red) and two barcode probes of oligo libraries designed from *V. unguiculata* 'IT97K-499-35' reference genome, with 16 (red) and 14 (green) FISH signals, that cover the 11 chromosome pairs. The oligopainting and barcode probes were indirectly labeled with biotin or digoxigenin and were detected using Alexa Fluor 488 Streptavidin and rhodamine sheep anti-DIG, respectively. Additionally, genomic blocks were analyzed among the ancestral karyotype of the Phaseoleae tribe (APK) and two subspecies of *V.* subg. *Vigna* and one species from *V.* subg. *Ceratotropis*. The 11 orthologous pairs of *Vigna* analyzed species were identified using *V. unguiculata* (L.) Walp as model. We observed macrosynteny for chromosomes 2, 3, 4, 6, 7, 8, 9 and 10 in all investigated taxa, except for *V. vexillata* (L.) A. Rich, in which only chromosomes 4, 7 and 9 were unambiguously identified. Collinearity breaks of chromosomes 2 and 3 were revealed. We observed minor differences of the painting pattern among the subgenera, in addition to multiple intra and interblock inversions, and intrachromosomal translocations. Other chromosome rearrangements included a pericentric inversion in chromosome 4 (*V.* subg. *Vigna*), reciprocal translocation between chromosomes 1 and 5 (*V.* subg. *Ceratotropis*), possible deletion in chromosome 11 of *V. radiata*, in addition to multiple intrablock inversions and centromere repositioning via genomic blocks. The present study allowed the visualization of karyotypic patterns in each subgenus, bringing important information for the understanding of intrageneric karyotypic evolution, suggesting *V. vexillata* as the most karyotypically divergent species.

Key-words: Genomic blocks; Legumes; Oligo-FISH;

Acknowledgement

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ID – 209

SYNTHETIC POLIPLOIDY IN *ONCIDIUM CRISPUM* L. (ORCHIDACEAE): AN ORCHID OF ORNAMENTAL INTEREST

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Abstract:
Polyploidy is considered one of the major evolutionary forces in plants. The understanding of the polyploidization process is fundamental to address the effects of the genome enlargement. An interesting strategy to produce working models that allow the direct investigation about the consequences of polyploidy is the production of synthetic polyploids. At the same time, the induction of polyploids can produce genotypes with desirable commercial traits, considering that polyploids, in general, present an increase of vegetative organs. The species *Oncidium crispum* stands out for its ornamental interest, with large brown flowers with yellow spots. In addition, it has great potential for use in genetic improvement programs, since it is widely used in interspecific crosses. Despite the importance of the species, information about its biology is scarce. The present work aimed, in addition to chromosomal characterization of the species, to induce polyploids that could serve as a working model and eventually be used as a source of new materials. *Oncidium crispum* protocorms each one originated from one seed were in vitro treated with three colchicine concentrations (0%, 0.05% and 0.1%) during 4 and 7 days in each concentration. Eight protocorms per treatment were used as the starting point of the cultures. After development, the seedlings were initially subjected to chromosome counting and estimation of the DNA amount by means of flow cytometry. The induction of polyploidy was efficiently verified, since the diploids had a DNA amount of 3.58 pg and the artificial polyploids, 6.76pg. Chromosomal counts indicate 58 chromosomes for diploids (5 individuals), and 116 (ca) for synthetic tetraploids (28 individuals), confirming the genome duplication. At the end, it was observed the formation of 66 healthy tetraploids (23% on average) considering all treatments with greater efficiency for the concentration of 0.1%. The protocol seems to be efficient for obtaining synthetic polyploids that will now be detailed investigated at morphological, chromosomal, and molecular levels. Financial support: FAPEMIG, CNPq
Key-words: polyploidy; Oncidium; orchids; colchicine; flow cytomery

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Universidade Federal de Juiz de Fora

ID – 143

HIGH-QUALITY CHROMOSOME-SCALE ASSEMBLY REVEALS THE COMPLETE GENOMIC RESHUFFLING OF THE DYSPLOID *PHASEOLUS LEPTOSTACHYUS* BEAN GENOME

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Abstract:

Phaseolus L. beans are well-known for their nutritional and economical importance, with at least five domesticated species and two centres of diversification, the Mesoamerican and the Andean. *Phaseolus leptostachyus* belongs to the Leptostachyus clade, a group of three wild species that underwent a recent dysploid event (~ 1 mya), reducing their chromosome number from *2n* = 22 to *2n* = 20 through a nested chromosome fusion. Oligo-FISH results indicated high rates of chromosomal evolution (number of structural rearrangements per million years) in *P. leptostachyus*, even when compared to its sister species (*P. macvaughii*) or to the rates known so far for mammals and other plants, making this a model species to investigate the mechanisms behind a rapid genome reshuffling. The aim of this work was to investigate the genome organization of *P. leptostachyus* integrating genomic and cytogenetics approaches. For this purpose, high molecular weight DNA was extract from young leaves of *P. leptostachyus* and sequenced through Pac-Bio HiFi and Omni-C (for single chromatin-capture) platforms. The high-quality HiFi reads were assembled into contigs, and after plastid contamination filtration, scaffolded into pseudomolecules using the generated Hi-C contact maps. After assembly quality check and annotation, syntenic blocks were identified and compared to other *Phaseolus* genomes available. The pseudochromosomes were ordered according to their synteny to *Phaseolus vulgaris* reference genome. Our data shows that all chromosome pairs of *P. leptostachyus* underwent translocation events, showing that chromosomal evolution rate is even higher than previously described, with at least 28 breaks of synteny in comparison to *P. vulgaris* reference genome. The whole genome assembly of *P. leptostachyus* helped us to elucidate its unprecedent genome organization and the degree of chromosomal rearrangements, giving us resources to investigate breaking points in order to understand if repetitive sequences may have a role in driving this rapid genome reshuffling.

Key-words: disploidy; karyotype evolution; structural rearrangements; synteny; translocations

Acknowledgement

FACEPE and CNPq

ID – 66

KARYOTYPE VARIABILITY IN SWEET POTATO ACCESSIONS (*IPOMOEA BATATAS* (L.) LAM., CONVOLVULACEAE)

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Abstract:
The sweet potato (*Ipomoea batatas* (L.) Lam.) is a hexaploid species (2n=6x=90) which produces a tuberous root widely used for human and animal food. It is among the six most important food crops in the world. Genetic breeding has been essential to increase productivity in different environments and root quality. The success of breeding programs relies on a wide genetic variability for traits of agronomic interest that conserved in germplasm banks. Considering the importance of the cytogenetic characterization of these genotypes, especially because its hexaploid condition and vegetative propagation, the objective of this work was to characterize the karyotype of sweet potato accessions from the Germplasm Bank of Federal University of Lavras (UFLA). Four accessions were analyzed: Ligeirinha (commercial cultivar) UFVJM-61 (producer selection), 666 and 1153 genotypes obtained after two cycles of recurrent selection. The first three has white/yellow pulp, while 1153 has purple. Branches were collected in the experimental fields of the Center for Development and Transfer of Technology (CDTT) of UFLA and cultivated to obtain roots. Root meristems were pre-treated with 2mM 8-hydroxyquinoline for 4h at room temperature and fixed in methanol: acetic acid (3: 1). To prepare slides, the cell wall was submitted to enzymatic digestion (pectinase 100 U: cellulase 200 U, pH 4.8), for 1h and 40min at 37°C. Chromosome preparation was obtained by flame-drying technique for fluorescent in situ hybridization (FISH) experiments to locate the 5S and 35S rDNA loci. Three accessions presented 90 chromosomes and the genotype 1153 presented aneuploidy (2n=6x=90-1). Six 5S rDNA signals were observed for all accessions, showing variation in chromosome location (proximal and some in the entire arm). The 35S signals were located in the terminal region of the chromosomes in all accessions, but in different numbers (12, 14 and 16). The karyotypic variability observed in the four accessions of *I. batatas* indicates that it is relevant to expand the detailed description of the karyotype of the accessions of the sweet potato germplasm bank at UFLA, to guide conservation and the choice of parents aiming at controlled crossings.
Key-words: Germplasm; FISH; ribosomal genes;

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ID 218 –
CHROMOSOME NUMBER IS DIRECTLY ASSOCIATED WITH REPRODUCTIVE PERFORMANCE IN *LIPPIA ALBA* (MILL.) N.E.BR. POLYPLOID COMPLEX

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Abstract:
Polyploidization (multiplication of the complete set of chromosomes) is considered one of the main mechanisms of plant speciation and evolution and it is now known that all existing seed plants have suffered one or more old polyploidization events. The underlying processes by which polyploids deal with reproductive disadvantages in the early stages after their formation are still poorly understood. The chromosome constitution of each ploidy level might contributes to better understand this process as well as for plant adaptation and reproduction. *Lippia alba* (Verbenaceae), native from Brazil, is an important medicinal species due to the presence of different chemical components being several biological activities attributed to the species. *L. alba* constitute a natural polyploid complex with five distinct chromosome numbers associated with five DNA contents. Here we describe the "fitness" for diploids (2n=30), triploids (2n=45) and tetraploids (2n=60) making possible the association between chromosome number (genome size) and reproductive traits of each cytotype. According to the available material, at least 29 diploids, 6 triploids and 5 tetraploids were evaluated regarding the average of seed production, seed germination, seed length, width, and seed weight. All traits were investigated under open pollination except for seed production that was evaluated under open and controlled pollination. To compare the performance of different cytotypes, the accession with the highest value was used as a reference (W=1, higher fitness), based on the formulae W=1-s, where "W" is the aptitude and "s" is the selection coefficient. The reference individual always had s=0. The fitness of the other accessions was calculated in relation to the reference individual. In general, the increase of genome size makes the polyploids more efficient than diploids under controlled pollination, especially tetraploids. On the other hand, under open pollination diploids showed higher performance than triploids and tetraploids. Considering the same ploidy level, it was observed that even within diploids the behavior of the accessions varied more than expected, suggesting that not only the chromosome number but also the chromosome constitution might influence the adaptation of the cytotype. Financial support: CNPq, CAPES, FAPEMIG
Key-words: polyploidy; Lippia alba; fitness;

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ID - 32

REPEAT-BASED PHYLOGENOMICS SUPPORTS MONOPHYLETIC SECTIONS WITHIN THE MONOCENTRIC GENUS *JUNCUS* L. (JUNCACEAE JUSS.)

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Abstract:
The repetitive fraction of eukaryotic genomes is diverse and usually fast evolving and neutral. Repetitive DNA characterization based on low-coverage genomic data has helped to clarify evolutionary trends in phylogenetically complex plant lineages. The genus *Juncus* L. comprises ~315 species, traditionally recognized as holocentric. Phylogenetically, the two subgenera and ten sections of *Juncus* are shown to be non-monophyletic, and the most recent phylogeny yields inconclusive results in their delimitation, so further systematic studies should be performed before adopting any taxonomic changes. Recently, four species of *Juncus* have been described as monocentric, but a clear understanding of centromere type and genome evolution among their species is hindered by their unclear phylogenetic relationships. Here, we reassess the current systematic scenario in *Juncus* using low-coverage genome skimming data of 31 *Juncus* species to construct phylogenetic hypotheses based on complete assembled plastomes and repetitive sequences using Geneious software. Furthermore, we characterized the repetitive fraction of these genomes to understand the evolution of repetitive elements using the Repeat Explorer pipeline in Galaxy software. Genome sizes available in the literature were used to establish correlations with the repetitive fraction. In addition, we performed Fluorescent In Situ Hybridization (FISH) of the previously described 155 bp JeSat1 centromeric DNA satellite and immuno-FISH of this satellite combined with the CENH3 protein, on one species from each section recognized in this work, to explore the centromeric structure of these species from a cytogenomic point of view. Repeat-base and plastome phylogeny revealed highly congruent topologies, corroborating the monophyletism of five of the ten sections of the genus and the paraphyletism of both subgenera (*Agathyron* Raf. and *Juncus* L.). This phylogenomic analysis provides the first well-supported evolutionary evidence for the group. We found specific genomic trends for major clades, with higher abundances of transposable elements (TE) in the *Juncotypus*+*Tenageia*+*Juncus* sections (3 - 56%) than in the *Stygiopsis*+*Ozophyllum* sections (0.21 - 23%). Additionally, we observed a significant correlation between genome size and TE content within *Juncus* (r^2 = 0.96; p < 0.001). Remarkably, these clades showed opposite trends in satellite DNA and TE abundances, suggesting a 'genomic competition' among the repetitive elements community. Immunostaining with the CENH3 antibody confirmed the monocentricity of the genus and its combination with a probe for centromeric satellite DNA indicated a possible role of this sequence in centromere function. The results observed in this study show that genome size within monocentric *Juncus* species is influenced by the expansion/contraction of repeats. In *Juncus*, the abundance and diversification of repetitive elements appear to be leading the underlying mechanisms that have driven a differentiation in genome size and phylogenetic relationships. The dynamicity of the repetitive fractions of these genomes may be correlated with their ancient origin (54 Mya) and reveals the potential of comparative genomic analyses for understanding plant systematics and evolution.

Key-words: chromosome; high-throughput sequencing; cytogenomics;

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ID – 34

REPEAT-BASED HOLOCENTROMERES OF *LUZULA SYLVATICA* (HUDS.) GAUDIN SHED LIGHT ON THE EVOLUTIONARY TRANSITION FROM MONO- TO HOLOCENTRICITY IN JUNCACEAE JUSS.

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Abstract:

In general, two types of chromosome morphology exist with respect to the centromere organization. While monocentric chromosomes exhibit a single centromere-specific CENH3-containing domain in the primary constriction, holocentric species possess several CENH3 domains distributed along their entire chromosomes. The family Juncaceae Juss. exhibits both centromere types among its related genera *Juncus* L. (monocentric) and *Luzula* DC. (holocentric), providing an excellent opportunity to study the transition between the centromeric types in phylogenetically related species. In order to analyze the centromeric organization and genome evolution of *L. sylvatica* (Huds.) Gaudin, a holocentric species of the family, we present a comprehensive analysis based on a chromosome-level genome assembly and a comparative analysis of its repetitive fraction with other species of the family and their closest monocentric relative *J. effusus* L. For this, we assembled the *L. sylvatica* genome at the chromosome-scale using PacBio HiFi and Dovetail Omni-C (DNase-based Hi-C) sequencing plus SALSA scaffolding. Because centromeres tend to be repeat-based, we analyzed comparatively the genome repetitive fraction of six *Luzula* species using the Repeat Explorer pipeline and the retrotransposon finder DANTE_LTR tool in the Galaxy software Version 0.1.8.0. Enriched repeats in *L. sylvatica* centromeres were identified using a mapping approach based on the similarity of CENH3 ChIP-seq reads and the repetitive sequences. Additionally, immuno-FISH, in combination with super-resolution microscopy, was performed to explore the centromeric structure of *L. sylvatica* from a cytogenomic point of view. Genes, DNA methylation profiles, and most repeats are evenly distributed along the chromosomes. Centromeric regions, identified by CENH3-ChIP-seq are predominantly associated with the 124 bp-long monomers of the satellite repeat LsylSat_124. Other satellite repeats of *L. sylvatica* are CENH3-negative. Comparative analysis in a phylogenetic context revealed that LsylSat_124 is abundant in all genera except in *L. elegans*. Our genome characterization combined with immuno-FISH microscopy showed that the holocentromeres of *L. sylvatica* are composed of multiple repeat-based centromeric units. Comparison of syntenic blocks between *L. sylvatica* ($2n = 12$) and *J. effusus* ($2n = 42$) genomes revealed that the chromosome number reduction in *Luzula* was most likely caused by multiple fusions of entire chromosomes, with unusual conservation of the centromeric sequences of that region. This suggests that holocentromere formation could be linked to the expansion of the monocentromere into adjacent chromosomal regions. Our findings provide the first evidence for a dynamic repeat-based holocentromere evolution involved in the transition from mono- to holocentromeres in plants.

Key-words: genome assembly; chip-seq; cytogenomics; repeat-based centromeres;

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Ensino e divulgação científica em Citogenética

ID – 151

CONTROVERSIAL TOPICS IN CYTOGENETICS: THE IMPORTANCE OF CONTINUING EDUCATION TRAINING FOR THE QUALITY IMPROVEMENT OF LABORATORY PRACTICE

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Abstract:
The need for continuing biomedical education is recognized and has always been on the agenda, as the health area gains new technologies and new scientific parameters every day. Laboratory staff evaluates the interest, relevance, convenience, individualization, and systematic of how turning it effective. That is the challenge. It is known that cytogenetics training requires extensive time and that the interest of trainees decreases due to this time and effort demanded. Furthermore, there are a limited number of cytogenetics training centers, although there is an increase in the demand for karyotyping tests and understanding the structural variations. The objective of the present study was to assess how much the workflow in cytogenetics analysis remains subjective and if there is an update of knowledge. In this context, a questionnaire was distributed to Brazilian cytogeneticists to evaluate specific topics that include updated conceptual terms. The questions were: Q1. In your daily work routine, do you indicate the use of Band C for; Q2. In AgNOR staining, do you use it to; Q3. Have you ever observed and reported the heteromorphism of chromosome 19 in your analysis routine; Q4. When you find in your analysis a chromosome with a fragile site region, e.g. fra(16)(q22), in some metaphases, do you report the result as mosaicism; Q5. Did you read and know the most specific ISCN 2020 updates in the area you work with; Q6. Do you know any method for measuring the resolution of chromosomal bands for G banding; Q7. Do you know the term Chromoanasynthesis; Q8. Are you familiar with the term monosomal karyotype; Q9. Were you taught to "measure" constitutive heterochromatin variants by any method or just by eye; Q10. What is your expertise in cytogenetics? Forty-six cytogeneticists participated and the answers indicated that for questions Q2 and Q4 the concepts are misunderstood. For Q3 the concept is unknown for 78% and Q5 indicated that the ISCN 2020 reading was for 91% of participants. Question Q6 showed that 80% know any method to measure chromosomal resolution. For the concepts of Q7 and Q8, the terms were not updated to 87% and 57%, respectively. Question Q9 indicated that although there is a method for measuring constitutive heterochromatin variants, the subjective "naked eye" method is used by 46%. Q10 demonstrated 22% have broadened their knowledge through cytogenomics. Most cytogenetics laboratories use the most recent ISCN, but not only this knowledge should be spread. The use of C banding and AgNOR staining should be clarified. The lack of high-quality cytogeneticists compromises the laboratories' quality and karyotype results. Continued training in analysis, cytogenetics concepts, and ISCN changes should be the main topics in clinical laboratories to help improve knowledge and good-quality reports. Cytogeneticists' development also emphasizes the encouragement of the laboratories to the team for learning and professional self-awareness combined with recycling developed in a context with multidisciplinary collaboration.
Key-words: Continuing education; Quality assurance; Cytogenetics learning; Quality Control; Karyotype

Acknowledgement

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