PV – 007 - **Identification of ubiquitin-conjugating enzyme UBC7 from Leishmania infantum**

MANCIN, G.D.C.[1]; TORRES, C.[1]; FALCIONE, L.H.M.[2]; BRANDÃO, I.T.[1]; BAQUI, M.M.A.[2]; TEIXEIRA, F.R.[3]; GOMES, M.D.[1].

1. DEPARTMENT OF BIOCHEMISTRY AND IMMUNOLOGY, RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO, BRAZIL, RIBEIRÃO PRETO - SP - BRAZIL; 2. DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY AND PATHOGENIC BIOAGENTS, RIBEIRÃO PRETO MEDICAL SCOOL, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO, BRAZIL, RIBEIRÃO PRETO - SP - BRAZIL; 3. DEPARTMENT OF GENETICS AND EVOLUTION, FEDERAL UNIVERSITY OF SÃO CARLOS, SÃO CARLOS - SP - BRAZIL.

Introduction: *Leishmania infantum* is the etiological agent of visceral leishmaniasis, a disease that poses a serious public health problem if left untreated. The molecular characterization of the ubiquitination process may provide a better understanding of the molecular and cellular basis, thereby identifying potential targets for leishmaniasis treatment. **Objectives:** Molecularly characterize the LinfUbc7 of *L. infantum,* an ortholog of the *H. sapiens* UBC7 (UBE2G2) and *S. cerevisiae* Ubc7, as an ubiquitin-conjugating enzyme (E2-Ub). **Materials and methods:** The amino acid sequence of LinfUbc7 was aligned with equivalent sequences from *H. sapiens*, *L. mexicana*, *T. brucei*, and *S. cerevisiae*, and protein structures were retrieved from the AlphaFold database and superimposed for comparison. The synthetic LinfUbc7 cDNA fragment was subcloned into the pET28a vector for protein expression in the BL21 (DE3) strain of *E. coli* and purified using affinity chromatography. Mutants lacking the catalytic site or a lysine were expressed and purified similarly. An in vitro ubiquitination assay was performed to evaluate whether LinfUbc7 functions as a UB-E2 enzyme and how the mutations affect the enzyme's function. **Results:** The alignment of the orthologues revealed that L. infantum contains the conserved catalytic cysteine and the HPN motif, commonly found in E2-Ub enzymes, suggesting similar functions. Also, the structure of LinfUbc7 compared to UBC7 and yeast Ubc7 revealed high structural similarity. LinfUbc7 was able to transfer ubiquitin in cooperation ubiquitination in vitro assay, and undergoes autoubiquitination on lysine residues. **Conclusion:** LinfUbc7 is a functional E2 enzyme of *L. infantum*, structurally and functionally related to UBC7. These results represent the initial steps of understanding the function of the ubiquitination process in Leishmania.

Supported by: FAPESP - 2024/18743-4 **Keywords:** Leishmania infantum;Ubc7;autoubiquitination.

PV – 008 - **Epitranscriptomic modifications on the biology of *Leishmania***

LEITE, A.B.[1]; MARAN, S.R.[1]; ALVES, G.G.[1]; FERREIRA, T.R.[2]; RELIER, S.[3]; OBERDOERFFER, S.[3]; SACKS, D.L.[2]; BEZERRA, A.M.[4]; REZENDE, A.M.[4]; MORETTI, N.S.[1].

1. UNIVERSIDADE FEDERAL DE SÃO PAULO (UNIFESP), DEPARTAMENTO DE MICROBIOLOGIA E IMUNOLOGIA, LABORATÓRIO DE BIOLOGIA MOLECULAR DE PATÓGENOS (LBMP), SÃO PAULO-SP, SÃO PAULO - SP - BRAZIL; 2. INTRACELLULAR PARASITE BIOLOGY SECTION, LABORATORY OF PARASITIC DISEASES, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NIH, BETHESDA, MD 20892, USA, BETHESDA - UNITED STATES OF AMERICA; 3. LABORATORY OF RECEPTOR BIOLOGY AND GENE EXPRESSION, NATIONAL CANCER INSTITUTE, NIH, BETHESDA, MD 20892, USA, BETHESDA - UNITED STATES OF AMERICA; 4. INSTITUTO AGGEU MAGALHÃES, DEPARTAMENTO DE MICROBIOLOGIA, FIOCRUZ PERNAMBUCO, RECIFE – PE, BRAZIL, RECIFE - PE - BRAZIL.

Leishmania spp. are protozoan parasites that cause leishmaniasis in humans and animals. Throughout their life cycle, they alternate between invertebrate vectors and vertebrate hosts, adapting to distinct environments by regulating gene expression and metabolism. RNA chemical modifications—such as N4-acetylcytidine (ac4C), and adenine or cytidine methylations (m6A, m1A, m5C)—have been implicated in post-transcriptional regulation and may influence parasite adaptation. Together, these modifications constitute what is known as the epitranscriptome, occurring in tRNAs, rRNAs, and mRNAs across diverse organisms. ac4C is catalyzed by NAT10 while TRMT61A and NSUN2 catalyze the addition of m1A and m5C, respectively. They can modulate RNA stability and/or translation depending on its position. Given the primarily post-transcriptional gene regulation in *Leishmania*, we hypothesize that such modifications are involved in parasite differentiation and survival. Bioinformatics tools identified the *Leishmania* NAT10 ortholog; CRISPR/Cas9 was used to generate fluorescent-tagged and knockout lines; Total RNA from different life stages was analyzed via immuno-Northern blot and mass spectrometry to assess ac4C levels; Phenotypic assays were performed on TRMT61A and NSUN2 mutants. We detected ac4C in multiple *Leishmania* species, including *L. braziliensis* and *L. tropica*, with stage-specific ac4C patterns. *L. tropica* NAT10 sKO promastigotes exhibited reduced growth. TRMT61A heterozygous and NSUN2 knockout strains of *L. mexicana* showed no promastigote growth defects but exhibited altered differentiation into amastigotes and metacyclics. These results support a role for epitranscriptomic modifications in Leishmania biology and contribute to the broader understanding of RNA regulation in protozoan parasites.

Supported by: FAPESP 2023/16672-0
Keywords: epitranscriptome;Leishmania;RNA modifications.

PV – 009 - **Variation in Aquaglyceroporin 1 Localization Among Leishmania Species: Implications for Antimony Susceptibility**

DANIEL, N.S.; BOY, R.L.; ZAMPIERI, R.A.; FLOETER-WINTER, L.; LARANJEIRA-SILVA, M.F..
USP, SÃO PAULO - SP - BRAZIL.

Aquaglyceroporin 1 (AQP1) forms a transmembrane channel in *Leishmania* and remains the only characterized protein able of antimony (Sb) uptake, a metalloid that has been used as a first-line treatment for both visceral and cutaneous leishmaniasis since the early 20th century. Notably, species associated with visceral leishmaniasis tend to be less sensitive to Sb than those causing cutaneous disease. Although the mechanisms underlying this variation remain unclear, AQP1 has been implicated as a key factor influencing Sb susceptibility. Previous studies by our group showed that in *L. amazonensis*, AQP1 is localized in glycosomes (peroxisome-like organelles). This contrasts with the flagellar localization reported for AQP1 in *L. major*. Additionally, sequence analysis of *L. amazonensis* AQP1 revealed a C-terminal peroxisomal targeting signal 1 (PTS1) motif (Pro-Asn-Phe, “PNF”). When we examined AQP1 homolog sequences in other species, we found this PTS1 signal conserved in *L. mexicana, L. donovani* and *L. infantum*, but not in *L. major* or several other species, suggesting that the subcellular localization of AQP1 may differ across *Leishmania*. To investigate whether AQP1 localization correlates with Sb susceptibility, we have generated transgenic *Leishmania* lines overexpressing AQP1 fused to green fluorescent protein (GFP) in four species: *L. braziliensis, L. donovani, L. infantum*, and *L. major*. We also generated an *L. amazonensis* line overexpressing AQP1-GFP lacking the PTS1 signal sequence. We will perform assays to evaluate the Sb sensitivity of these overexpresing lines in order to determine whether AQP1’s subcellular localization influences Sb susceptibility in *Leishmania.*

Supported by: FAPESP 2024/14336-5
Keywords: AQP1;Antimony;Glycosome.

PV – 010 - **Beyond Histones: The role of YEATS domains in the cell cycle of *Leishmania mexicana***

GARCIA, M.A.D.N.[1]; MARINO, A.G.[1]; FRESQUI, S.D.F.M.[1]; MACHADO, F.C.[1]; TEIXEIRA, T.L.[2]; DA SILVA, M.S.[2]; DE MEDEIROS, L.C.A.S.[3]; RODRIGUES, A.J.C.[3]; MORETTI, N.S.[1].
1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 3. FUNDAÇÃO OSWALDO CRUZ, CURITIBA - PR - BRAZIL.

Protozoan parasites of the genus *Leishmania* are the etiological agents of leishmaniasis, a neglected tropical disease that afflicts millions globally. The parasite’s remarkable ability to adapt throughout its digenetic life cycle may rely on post-translational modifications such as lysine crotonylation. Proteins bearing the YEATS domain, function as 'readers' of crotonylation and are implicated in chromatin remodeling and gene expression. regulation. Although crotonylation has been associated with DNA repair and cell cycle control in mammalian cells, its functional role in *Leishmania* remains to be elucidated. This study aims to characterize the function of the YEATS proteins (ENL and Yaf9) in *Leishmania mexicana*, with a specific focus on their involvement in DNA repair and cell cycle regulation. *L. mexicana* strains engineered to overexpress ENL or Yaf9 were evaluated for their sensitivity to various genotoxic agents (HU, MMS, H_2O_2, UV-C, and SbIII), the subcellular localization of the YEATS proteins under stress conditions, chromatin architecture via electron microscopy, crotonylation levels in response to DNA damage, and cell cycle progression by flow cytometry. Overexpression of the YEATS proteins increases parasite resistance to genotoxic stress and influences chromatin organization, suggesting their participation in DNA repair pathways. Furthermore, cell cycle analysis demonstrated alterations in phase distribution, indicative of a regulatory function in cell cycle progression. Our findings demonstrate a functional association between YEATS domains and the processes of DNA repair and cell cycle regulation in *Leishmania*, thereby contributing to the broader understanding of crotonylation's epigenetic roles in protozoan parasites**.**

Supported by: FAPESP (2022/03075-0; 2025/03898-5)
Keywords: Crotonylation;YEATS domains; DNA repair.

PV – 011 - **Translational Regulation in adaption: Dissecting the Initiation Codon Context Language in Leishmania**

SANTI, A.M.M.[1]; JENKINS, P.[2]; COKELAER, T.[2]; DA FONSECA, J.P.[2]; TIKHONOVA, E.[3]; ALMONACID, C.R.[3]; KARAMYSHEV, A.[3]; KARAMYSHEVA, Z.[3]; SPÄTH, G.F.[2].
1. FIOCRUZ PARANÁ (ICC) AND INSTITUT PASTEUR (PARIS), CURITIBA - PR - BRAZIL; 2. INSTITUT PASTEUR, PARIS - FRANCE; 3. TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER, LUBBOCK - UNITED STATES OF AMERICA.

Leishmania parasites exhibit great phenotypic variability and adaptive capacity despite the absence of classical, promoter-driven gene regulation. Using an experimental evolution approach to study *Leishmania* fitness gain, we have discovered various alternative regulatory mechanisms based on gene dosage variation, or post-transcriptional and translational control. To investigate the role of translation initiation in gene expression, we generated transgenic *L. donovani* parasites expressing GFP under the control of different Kozak sequences. Our results demonstrate a direct effect of the nucleotide environment surrounding the initiation codon on both protein expression levels and mRNA stability, which correlated with mRNA recruitment to heavy polysomes. Currently, our focus lies on investigating the presence of regulons governed by the translation initiation sites.
Supported by: European Research Council (ERC) Synergy grant for the project 'DECOLeishRN – Decoding epistatic genome/RNome interactions in eukaryotic fitness gain using Leishmania parasites as a unique model system'
Keywords: Leishmania;Translational Regulation;Kozak sequences.

PV – 012 - **Genetic introgression and transcriptomic plasticity are associated with enhanced Leishmania infantum pathogenicity causing human cutaneous leishmaniasis in Tunisia**

SANTI, A.M.M.[1]; PIEL, L.[2]; LI, B.[2]; DA FONSECA, J.P.[2]; COKELAER, T.[2]; AOUN, K.[3]; BOURATBINE, A.[3]; SPÄTH, G.F.[2]. 1. FIOCRUZ PARANÁ (ICC) AND INSTITUT PASTEUR (PARIS), CURITIBA - PR - BRAZIL; 2. INSTITUT PASTEUR, PARIS - FRANCE; 3. INSTITUT PASTEUR DE TUNIS, TÚNIS - TUNISIA.

The protozoan parasite *Leishmania infantum* exhibits significant genetic variability among isolates, influencing disease manifestation and treatment response. Although *L. infantum* is classically described as the causative agent of Visceral Leishmaniasis (VL) – often associated with immune deficiency, cases of Cutaneous Leishmaniasis (CL) caused by this species in immunocompetent individuals have been reported in different countries. With the aim to gain new insight underlying this unusual change in *L. infantum* tropism and pathogenicity, we applied comparative genomic and transcriptomic approaches on two canine isolates (*Lcan*) and two human isolates associated with *CL* in Tunisia. Using DNA-seq analysis, we assessed aneuploidy, gene copy number variations (CNVs), and single-nucleotide polymorphisms (SNPs) between strains. While the *Lcan* isolates showed close genetic similarity to the *L. infantum* reference strain (JPCM5), the *CL* isolates formed a separate, highly divergent cluster based on SNP localization and frequency, differing not only from JPCM5 but also from each other. Utilizing the metagenomics sequence classification tool Kraken, we revealed a complex hybrid nature of the *LC* isolates, indicating genetic introgression from both *L. donovani* and *L. tropica*. Integration of RNA-seq and DNA-seq data showed that expression differences within the *LC* and *Lcan* sample groups are largely attributable to gene dosage effects. In contrast, significant expression changes independent of gene dosage were observed in-between the *LC* and *Lcan* sample groups, indicating post-transcriptional regulation as an additional regulatory mechanism for parasite adaptation in the field. This work highlights hybridization as a potential source of phenotypic variation in *L. infantum* and investigates how genome instability and transcriptomic plasticity contribute together to parasite evolvability and fitness gain.
Supported by: This project was supported by the Prix TREMPLIN de coopérationbilatéraleen recherche – Afrique and European Research Council (ERC) Synergy grant for the project 'DECOLeishRN – Decoding epistatic genome/RNome interactions in eukaryotic fitness gain using Leishmania parasites as a unique model system'
Keywords: Leishmania infantum;Genomics;Transcriptomics.

PV – 013 - **Exploring the extrachromosomal rDNA in testate amoebas (Amoebozoa: Arcellinida)**

BIM, M.A.[1]; BROWN, M.[2]; BARBOSA, J.P.A.[1]; LAHR, D.J.G.[1].
1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. MISSISSIPPI STATE UNIVERSITY, STARKVILLE - UNITED STATES OF AMERICA.

The established model of ribosomal RNA (rRNA) gene organization in eukaryotes is primarily based on animal genomes. In this model, the genes for the 18S, 5.8S, and 28S rRNAs are coded in a single transcription unit (rDNA), interspersed by two internal transcribed spacers (ITS1 and ITS2), which are typically arranged in tandem repeats in one or multiple chromosomes. However, significant diversity exists in other eukaryotic lineages: for example, in many Amoebozoa, these genes are found on extrachromosomal elements. Here, we investigate the rDNA structure of two testate amoeba species (Amoebozoa: Arcellinida), *Cryptodifflugia operculata* and *Arcella uspiensis*, using data from whole genome sequencing (Oxford Nanopore and Illumina) and transcriptome cDNA sequencing (Illumina). Genome assembly revealed that the rDNA in both species is located on circular extrachromosomal molecules, measuring approximately 10.1 kb in *C. operculata* and 16.2 kb in *A. uspiensis*. Despite these two species having diverged over 720 million years ago, the organization of their extrachromosomal elements is similar. Both feature an rDNA unit with the canonical 18S−ITS1−5.8S−ITS2 structure and contain a region of short repeats: a ~700 bp region of ~20 bp in tandem repeats in *C. operculata*, and a ~1590 bp region of ~23 bp in tandem repeats in *A. uspiensis*. The *A. uspiensis* extrachromosome also carries three 494 bp tandem repeats and two transcribed non-rDNA fragments of yet unknown function.
Supported by: CAPES - 88887.986366/2024-00
Keywords: Arcellinida;Extrachromosomal rDNA;Genome organization.

PV – 014 - **Metabolic Modeling of *T. brucei*: Insights into Histidine-Glutamate Pathway**

MONTANARO, G.T.; PINTO, B.R.; ALENCAR, M.B.; NASCIMENTO, J.D.F.; SILBER, A.M..
ICB/USP, SÃO PAULO - SP - BRAZIL.

The life cycles of *Trypanosoma cruzi* and *Trypanosoma brucei* demand adaptation to diverse environments, including nutrient changes in their respective hosts. *T. cruzi* can metabolize histidine (His) to glutamate (Glu) in a four-step pathway, which is absent in *T. brucei*. To investigate both *T. cruzi's* metabolism and the pathway itself, we engineered *T. brucei* procyclic (PCF) cell lines expressing *T. cruzi*'s His degradation pathway. Three cell lines were developed, and we observed significant phenotypical differences, probably caused by intermediary metabolites' flux variability when the parasite expresses two, three or the four enzymes of the pathway. Complementing this experimental approach, we developed Genome-Scale Metabolic Models (GEMs) for PCF of *T. brucei* using COBRApy to systematically analyze its metabolic network with and without this pathway. The PCF model containing the partial or complete pathway shows what happens to metabolic fluxes when this pathway is present. For this, a comparison between the phenotypical results of cell lines and the flux of reactions in the model was made. *In vitro* results such as proliferation rate, O_2 consumption and CO_2 production were used to set the model to behave similarly with the generated cell lines. Cell lines expressing only two or three of the pathway enzymes produce less O_2 when incubated with proline, one of the main amino acids for ATP generation in this parasite. Our results indicate that the whole pathway is needed to maintain fluxes through other pathways producing Glu. Integrating genetic engineering with computational modeling provides a powerful framework to understand trypanosome metabolism, revealing functional implications of pathway differences and adaptive strategies in distinct environments.
Supported by: FAPESP 2020/16569-6
Keywords: histidine;Genome-scale model;Genetic engineering.

PV – 015 - **Plasmogamy in Testate Amoebae: Identifying Nuclear Fate Through Fluorescence Microscopy**

DE OLIVEIRA, T.E.; LAHR, D.J.G..
IB-USP, SÃO PAULO - SP - BRAZIL.

The evolution of sex and meiosis in unicellular eukaryotes is an ongoing area of research, and one of the least understood phenomena is plasmogamy (cytoplasmic fusion) in free living lineages. In the literature, plasmogamy is considered indirect evidence of sex/meiosis. Here we show that that photoperiod (length of "daylight") is one of the environmental triggers for plasmogamy phenomenon in *Arcella uspiensis,* a species of testate amoeba. Testate amoebae are eukaryotic single-celled organisms that produce an external shell (test), which serves as an indicator that plasmogamy has taken place (a cytoplasmic mass carrying multiple shells). In this study, we were able to induce the occurrence of plasmogamy in *A. uspiensis* cultures, and have characterized plasmogamy at the cellular level using multiple microscopy techniques. Our objective is to obtain direct evidence of sex (karyogamy and meiosis) using fluorescence microscopy with stains such as DAPI (4′,6-diamidino-2-phenylindole) and propidium iodide, as well as using classical HE histological staining techniques. This will allow us to determine whether plasmogamy in *A. uspiensis* is related to the existence of a sexual cycle in these organisms. We managed to produce several images that indicate to us what happens with the nucleus during plasmogamy and whether plasmogamy is or isn't related with sexual reproduction in *A. uspiensis.*

Keywords: PLASMOGAMY;SEX;AMOEBOZOA.

PV – 016 - **Diversity of Hypotrichs (Ciliophora: Hypotricha) in soil samples from the Atlantic Forest of Rio de Janeiro**

FERREIRA, L.D.A.; PAIVA, T.D.S..
INSTITUTO DE BIOLOGIA, UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

The Brazilian Atlantic Forest a Neotropical hotspot of biodiversity, featuring a wide range of habitats such as mangroves, rivers, streams, and lakes, in addition to the forest itself. However, its original area of approximately 1,300,000 km² has now been reduced to 7% of its former size due to human activity. Consequently, the impacts caused by habitat degradation result in potentially irreparable losses of biological diversity—effects that remain largely unknown when it comes to microorganisms. Among microorganisms, hypotrich ciliates are perhaps the most structurally complex microeukaryotes known, exhibiting compound locomotory cilia (cirri) on the ventral side of the body and sensory bristles on the dorsal side, as well as a characteristic polyhymenophore adoral zone. These organisms are considered pre-adapted to life in the micropores of sediments and occur in terrestrial, freshwater, brackish, or marine environments, where they act as omnivorous consumers. This study aims to provide a taxonomic inventory of the diversity of hypotrichs from the Atlantic Forest of Rio de Janeiro, and is part of an ongoing initiative to catalogue the ciliate diversity in this biome. The presented results are thus based on (i) voucher material deposited in the Collection of Ciliates and Other Protists (Instituto de Biologia, UFRJ), over the past three decades; (ii) new environmental sampling at georeferenced points; and (iii) a review of the relevant literature. For this purpose, photo documentation and analysis of specimens on permanent slides is being performed, along with curation of digital micrographs from the collection's image bank. Material from the new samplings will be used to establish cultures in Petri dishes to facilitate the isolation and identification of additional specimens, based on live observations and preparation of optical microscopy techniques (e.g., protargol impregnation) and electron microscopy. In addition, aliquots of soil and water samples will be set aside for environmental DNA extraction, complementing the inventory through metabarcoding analysis. As preliminary results, the following hypotrichs have been identified:
Apoamphisiella vernalis, Australocirrus cf. aspoecki., Cyrtohymena sp., Diaxonella sp., Fragmocirrus espeletiae, Gonostomum sp., Pattersoniella vitiphila, and Uroleptus lepisma. Upon completion, the study is expected to contribute to the taxonomic knowledge of hypotrichs in Brazil and enhance the understanding of their diversity.

Supported by: CNPQ Universal 421766/2021-2
Keywords: Ciliates;Free-living;Taxonomy.

PV – 017 - **Interstitial ciliates from sand shores of Ilha do Fundão (Guanabara Bay), Rio de Janeiro, with a critical assessment of genera Lacrymaria and Phialina (Ciliophora, Litostomatea)**

FERNANDES, M.L.S.; PAIVA, T.D.S..
INSTITUTO DE BIOLOGIA, UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

The Guanabara Bay is a eutrophic coastal bay in the city of Rio de Janeiro which is heavily impacted by anthropic activity. Among the organisms inhabiting Guanabara Bay, ciliates are fundamental elements in microscopic food webs and are considered reliable water quality indicators of organic pollution and the presence of heavy metals, but also sensitive to climate changes. Ciliates form a monophyletic group within the supergroup Alveolata, characterized by specialized ciliary structures and nuclear dualism—its main morphological synapomorphy. In this study, we present preliminary results of an ongoing inventory of ciliate diversity present in samples from sand shores of Guanabara Bay, collected from georeferenced sites at Ilha do Fundão. After collected and brought to the laboratory, samples of ca. 500 mL of water with sediments were aliquoted in Petri dishes, where cultures were established with addition of crushed rice grains. The ciliates were identified after live observations, protargol impregnation and scanning electron microscopy. Hitherto, we documented 16 species distributed among the classes Heterotrichea, Karyorelictea, Litostomata, Protostomatea, Protocruziea, and Spirotrichea. Of the recorded species, we found representatives tentatively assigned to *Lacrymaria* and *Phialina.* The specimens display overlapping taxonomic features, strengthening the present concerns on the ambiguous delineation of both genera, normally separated by the presence/absence of an extensible neck used to probe the surrounding environment for prey. The results prompted us to conduct a critical evaluation of both genera, based on morphology and molecular data.

Supported by: CNPq Universal 421766/2021-2
Keywords: Free-living;Microeukaryote;Taxonomy.

PV – 018 - **Coevolution in action: how the endosymbiont regulates the energy metabolism of *Angomonas deanei***

BARRINHA, A.[1]; MACHADO, A.C.L.[2]; SANTOS, M.D.M.[3]; CARVALHO, P.C.[3]; DE SOUZA, W.[4]; VALENTE, A.P.[5]; GALINA FILHO, A.[5]; MOTTA, M.C.M.[4].
1. IBCCF - UFRJ, BELO HORIZONTE - RJ - BRAZIL; 2. YALE UNIVERSITY SCHOOL OF MEDICINE, NEW HAVEN - UNITED STATES OF AMERICA; 3. FIOCRUZ-PARANÁ, CURITIBA - PR - BRAZIL; 4. IBCCF-UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 5. IBQM - UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Endosymbiosis in trypanosomatids involves a mutualistic association between a symbiotic bacterium and a host protozoan, and it is an excellent model for studying metabolic coevolution and the origin of organelles. This work investigated the influence of the symbionts on the metabolism of *Angomonas deanei* by comparing wild-type and aposymbiotic strains under different nutritional conditions. The presence of the symbiont enhanced cell proliferation in medium containing a single carbon source and increased O_2 consumption. Wild-type cells utilized oxidative phosphorylation to produce ATP, whereas aposymbiotic cells relied on substrate-level glycolysis, resulting in the excretion of greater amounts of fermentative products, such as acetate, succinate, and ethanol. Proteomic analysis revealed increased expression of glycolytic and fermentative enzymes by the aposymbiotic strain and oxidative phosphorylation enzymes by symbiont-harboring cells. These findings highlight the role of the symbiotic bacterium in optimizing host metabolism and provide insights into the evolution of parasitism in trypanosomatids when *A. deanei* is compared with pathogenic species.

Supported by: CNPq – 305299/2022-0; FAPERJ – E-26/201.011/2021
Keywords: oxidative phosphorylation;fermentation;symbiosis.

PV – 019 - **Endosymbiosis in trypanosomatids: the influence of the symbiotic bacteria on the glycolytic metabolism of Angomonas deanei**

DA SILVA, B.N.M.[1]; BARRINHA, A.[1]; GALINA FILHO, A.[2]; MOTTA, M.C.M.[1].
1. IBCCF - UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. IBQM - UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Endosymbiosis in trypanosomatids is a mutualistic association between a β-proteobacterium and its host protozoan that constitutes an excellent biological model for studying the origin of organelles in eukaryotic cells. This coevolutionary process involves intense metabolic exchanges: the bacterium completes essential biosynthetic pathways of the host, benefiting from its production of ATP and phospholipids. In *Angomonas deanei*, the symbiont (wild-type strain - AdWt) optimizes the host oxidative metabolism, while its absence (aposymbiotic strain - AdApo) makes the protozoan more fermentative. This work investigates the symbiont's influence on the glucose metabolism and the fate of glycolytic pyruvate in *A. deanei*. For this purpose, biochemical and ultrastructural analyses compared the effects of 5, 10, and 20 mM 2-Deoxy-D-glucose (2-DG) and 10 and 50 mM dichloroacetate (DCA) on both strains. 2-DG is an inhibitor of the glycolytic pathway, which binds to the hexokinase, thus preventing glucose phosphorylation; whereas DCA, inhibits pyruvate dehydrogenase kinase (PDK), leading to increased ATP production through mitochondrial oxidative phosphorylation. Growth curves, transmission electron microscopy (TEM) analyses, high-resolution respirometry and hexokinase enzymatic activity assays were performed. Preliminary results showed that 2-DG caused a dose-dependent growth reduction up to 14.7% in AdWt and 25.2% in AdApo, after 96h of treatment and decreased hexokinase activity (25% in AdWt, 21% in AdApo) with 20mM for 24h. In AdWt, 10 mM DCA for 24h increased O2 consumption, while 50 mM impacted mitochondrial physiology. Conversely, the AdApo strain was not affected by this inhibitor. These results indicate possible ultrastructural changes in *A. deanei*, especially in the mitochondrion. As this research progresses, it will be possible to clarify whether the symbiotic bacterium participate in glucose catabolism, thus optimizing the oxidative metabolism of the host cell.
Supported by: CNPQ - 305299/2022-0; FAPERJ - E-26/201.011/2021
Keywords: Endosymbiosis;trypanosomatids;glycolytic metabolism.

PV – 020 - **Effects of Kinesore, a kinesin-1 modulator, on the organization of the microtubule cytoskeleton of *A. deanei* and on the division of the symbiotic bacterium.**

DE LIMA, L.S.G.[1]; DE SOUZA, W.[2]; MOTTA, M.C.M.[1].
1. IBCCF - UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Angomonas deanei is a monoxenous trypanosomatid that coevolves with a symbiotic bacterium, representing an important model for studying the origin of organelles in eukaryotic cells. This mutualistic symbiosis is characterized by intense metabolic exchanges and the coordinated division of the bacterium with other structures, such as the nucleus and the kinetoplast. The suppression of tubulin expression by RNAi leads to the emergence of filamentous symbionts, indicating impaired bacterial division, and also the appearance of fat-cell protozoa. Similar results were obtained using trichostatin A, a histone deacetylase inhibitor, demonstrating the importance of the microtubule cytoskeleton and its dynamic instability for symbiont division. Motor proteins, such as kinesins, participate in intracellular transport and cytoskeletal organization. In view of the above, we investigated the effect of kinesore, a kinesin-1 modulator, on the organization of the microtubule cytoskeleton of *A. deanei* and on the division of the symbiotic bacterium. This modulator prevents kinesin-1 from binding to cargo, but maintains its motor activity, promoting microtubule remodeling. Based on the literature, concentrations of 25, 50, and 100 µM of kinesore were used in the growth and reversibility curves, cell viability tests, and light and electron microscopy analyses. Preliminary results showed changes in cell growth and morphology, since treatment with kinesore produced rounded cells with loss of the flagellum and prevented the division of the symbiotic bacterium. However, cell viability was maintained at all concentrations tested. The data obtained indicate the involvement of motor proteins in microtubule organization and in the division of the *A. deanei* symbiont.
Supported by: CNPq and FAPERJ
Keywords: Endosymbiosis in trypanosomatids;Microtubule cytoskeleton;Kinesore.

PV – 021 - **Biochemical characterization of F-box-like proteins (LinfFlps) from Leishmania infantum**

REGATIERI, W.K.P.[1]; DE CORREIA, C.R.S.T.B.[2]; TORRES, C.[2]; DE CASTRO, T.D.O.[1]; TEIXEIRA, F.R.[1].
1. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SÃO CARLOS - SP - BRAZIL; 2. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, SÃO CARLOS - SP - BRAZIL.

The Ubiquitin-Proteasome System (UPS) is the main pathway of intracellular proteolysis in eukaryotes and is essential for host alternation and parasitism. It plays a central role in protein degradation and maintenance of cellular homeostasis. Cullin-RING ligases (CRLs), the largest and most studied class of E3 ubiquitin ligases in mammals, are part of the UPS and regulate cell cycle and proliferation. The CRL1 complex is composed of SKP1, Cullin 1, RBX1, and an F-box protein that interacts with SKP1 through the F-box domain and recruits substrates for ubiquitination. Despite the importance of the UPS, little is known about this pathway in *Leishmania spp.*. Our group has characterized the *LinfCRL1* complex in *L. infantum*, which is associated with cell proliferation and cell cycle regulation. *In silico* analyses identified 11 potential proteins with an F-box motif, and the *LinfSkp1* interactome revealed 6 proteins containing this domain, termed F-box-like proteins (*LinfFlps*). In this study, we biochemically characterized *LinfFlp2* (LINF_150014400) and *LinfFlp3* (LINF_140018100) by identifying their interactomes. We detected 10 proteins exclusively associated with *LinfFlp2*, 40 exclusively associated with *LinfFlp3*, and 55 shared proteins involved in processes such as DNA maintenance, nucleic acid binding, metabolism, ribosomal function, protein folding, and intracellular transport. The genes LINF_280024500 and LINF_020012000 were identified in the *LinfFlp2* interactome; LINF_340011100, LINF_250005800, and LINF_220021200 were identified to the *LinfFlp3* interactome; and LINF_020008700, LINF_180007200, and LINF_270018900 were detected in both *LinfFlp2* and *LinfFlp3* interactomes respectively have been investigated. Our results suggested that *LinfFlps* likely act in multiple cellular pathways, either coordinately or complementarily, thereby contributing to parasite homeostasis.

Supported by: FAPESP - 2023/12058-5
Keywords: Leishmania infantum;Ubiquitin-Proteasome System;F-box-like proteins.

PV – 022 - **The Evolutionary Odyssey of the Methionine Pathway in Kinetoplastids: Unraveling a Tale of Dynamic Adaptation**

BUITRON, K.P.B.; VERGARA, P.T.; SILBER, A.M..
USP – UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

Early evolutionary transitions in Kinetoplastids involved extensive metabolic remodeling associated with their shift from free-living ancestors to obligate parasitism. Among these adaptations, sulphur amino acid metabolism underwent significant changes. Methionine (Met) and cysteine (Cys) are not only essential for protein synthesis but also serve as precursors for trypanothione biosynthesis, the central thiol involved in maintaining redox balance and protection against oxidative stress in these organisms. While *de novo* Met biosynthesis pathways have been well characterized in bacteria, archaea, and plants, much less is known about the presence and evolutionary trajectories of Met salvage pathways in early-diverging eukaryotes such as Kinetoplastids. This pathway involves: (i) conversion of Met to S-adenosylmethionine (SAM) by SAM synthetase (ADEMETS), (ii) transformation into S-adenosylhomocysteine (SAH) and its hydrolysis by SAH hydrolase (ADEHCYSH), and (iii) remethylation of homocysteine to Met by homocysteine S-methyltransferase (HMT). We conducted a comprehensive evolutionary analysis of these three core enzymes. Phylogenetic reconstructions revealed strong conservation of ADEMETS and HMT across both monoxenous and dixenous taxa, whereas ADEHCYSH exhibited greater variability across tree topology. Microsynteny analyses confirmed gene duplication events for ADEMETS. Notably, phylogenetic and codon usage analyses suggest a potential horizontal gene transfer event involving ADEHCYSH, which forms a well-supported clade with Leptospira spp. Additionally, selective pressure analysis revealed signatures of episodic positive selection in specific regions. Altogether, our results provide the first comprehensive characterization of the Met salvage pathway in Kinetoplastids, supporting its role as an ancestral and conserved metabolic trait shaped by lineage-specific evolutionary adaptations.

Supported by: FAPESP 2023/07669-5
Keywords: Methionine;Evolution;Adaptation.

PV – 023 - **The role of KAP6 and mitochondrial Topoisomerase II in the topology and replication of *Trypanosoma cruzi* mitochondrial DNA**

CAMELO, T.M.; MELO, N.S.; DE SOUZA, W.; MOTTA, M.C.M..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

The kinetoplast is an enlarged region of the single, branched mitochondria of trypanosomatids that houses mitochondrial DNA (kDNA), which is composed of maxicircles and minicircles, forming an extensive network. The shape of the kinetoplast and the arrangement of mitochondrial DNA vary according to the genera and developmental stages of the protozoan. KAPs (Kinetoplast Associated Proteins) are proteins associated with the kinetoplast that are involved in kDNA topology, replication, transcription, and repair. In addition to KAPs, mitochondrial topoisomerase II (TopoIImt) plays an important role in the kinetoplast, releasing the concatenated minicircles from the network to initiate the kDNA replication process. The objective of this work is to investigate the role of KAP6 and TopoIImt in the arrangement and replication of *T. cruzi* kDNA. Cells with deletion of one of the KAP6 or TopoIImt alleles were obtained by CRISPR-Cas9 and then the proliferation, viability, cell cycle and ultrastructure of these mutants were evaluated. No significant changes in proliferation or cell viability were observed, however cell cycle arrest and kDNA replication delay were observed in both types of mutants. Morphological changes were noted in the parasites, as well as modifications in kinetoplast shape and mitochondrial DNA arrangement, after deletion of these genes. The data obtained indicate that KAP6 and TopoIImt are essential proteins, since we were unable to obtain null mutants. We conclude that both proteins play important roles in the organization and replication of the kDNA network in *T. cruzi*.
Supported by: CAPES 88887.139802/2025-00
Keywords: kinetoplast;kinetoplast associated proteins (KAPs);mitochondrion.

PV – 024 - **Differential Expression of Peptidases in *Strigomonas culicis*: From Proteomic Data to Proteolytic Activity**

DOS SANTOS, J.F.B.[1]; BOMBAÇA, A.C.S.[1]; VITORIO, B.D.S.[1]; LOPES, G.D.[1]; GOMES, A.D.S.G.[2]; MENNA-BARRETO, R.F.S.[1]; D'AVILALEVY, C.M.[1]; VIDAL, V.E.[1].
1. INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL; 2. INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA, RIO DE JANEIRO - RJ - BRAZIL.

The monoxenous trypanosomatid *Strigomonas culicis* harbors an endosymbiotic bacterium with intermediate features between bacteria and organelles. This symbiont influences several metabolic pathways in the parasite, potentially including those concerning peptidases. *S. culicis* protein profile includes homologs of pathogenic trypanosomatids' peptidases which can act as virulence factors. Considering the availability of *S. culicis* genome and proteomes, we explored differences in peptidase expression between the wild-type (WT) and the aposymbiotic (APO) strains. We conducted a genome survey from GenBank and MEROPs to assess peptidase diversity, followed by proteomic analyses of WT and APO strains. Gene ontology was applied to identify the functions and characteristics of the enzymes, and functional analyses validated the modulation of selected peptidases. Our genomic survey revealed 334 peptidase sequences in GenBank and 114 in MEROPs. Proteomic analyses identified 90 peptidases, of which 43 displayed significant modulation between WT and APO strains. Cysteine and metallopeptidases were predominant, with calpains and GP63 standing out as the main peptidases. The APO strain exhibited increased gene and protein expression, along with higher GP63 activity, suggesting down regulation by the endosymbiont. In contrast, cysteine peptidase activity was reduced in APO cells. These findings improve our understanding of peptidase regulation in *S. culicis* and highlight the influence of endosymbiosis on parasite biology, providing insights towards future approaches to target parasite–host interactions.
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisado Estado do Rio de Janeiro - E-26/210.976/2024; Coordenação de Aperfeiçoamento de Pessoal Nível Superior - Financial Code 001
Keywords: trypanosomatid;cysteine peptidase;metallopeptidase.

PV – 025 - **CALPAIN *KNOCKOUTS* BY CRISPR/CAS9 IN *TRYPANOSOMA CRUZI***

BANDEIRA, K.V.; BOMBAÇA, A.C.S.; MOREIRA, C.J.D.C.; MENNA-BARRETO, R.F.S.; D'AVILALEVY, C.M.; VIDAL, V.E..
INSTITUTO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Calpains comprise a diverse family of calcium-dependent cysteine peptidases. Originally discovered in mammals, homologues have been found in other organisms, including a large number of calpain-coding genes in the Trypanosomatidae family. The etiological agent of Chagas disease, *Trypanosoma cruzi*, has 63 calpain sequences with differential gene expression across the parasite's life cycle. Considering the need for new chemotherapeutic compounds, calpain inhibitors could represent promising candidates. This study aims to elucidate the specific functions of selected calpains in *T. cruzi* using CRISPR/Cas9 system. We first analyzed the CRISPR/Cas9 *knockout* of calpains TcCLB.508999.200 (CALP1), previously reported as upregulated during metacyclogenesis, and TcCLB.506563.200 (CALP2), which has an ortholog localized at the tip of the *T. brucei* flagellum and displayed an increased expression in *T. cruzi* epimastigotes. Transfections were performed by electroporation of sgRNAs and DNA donor constructs corresponding to each sequence and confirmed by PCR, followed by clonal selection. Preliminary data indicate that CALP1$^{-/-}$ parasites exhibit arrested growth, with a significant decrease in proliferation from the fourth day compared to wild type epimastigotes, warranting further investigation. In addition, both CALP1 and CALP2 *knockouts* presented a significant reduction in cell body size. Functional analysis of calpains will enable the elucidation of their specific roles, contributing to the identification of more effective therapeutic targets for Chagas disease.
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - E-26/210.976/2024 **Keywords:** body size;growth;Chagas disease.

PV – 026 - **Small Heat Shock Proteins and their Influence on the Intestinal Physiology of Rhodnius prolixus, Vector of Chagas Disease.**

DA SILVA, M.R.D.F.; DE OLIVEIRA, P.L.; GUEDES, T..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Rhodnius prolixus, one major vector of *Trypanosoma cruzi,* is an obligate hematophagous insect whose infection is restricted to the digestive tract. The intestinal epithelial cells are key interaction sites between the parasite, the microbiota and the host. Transcriptomic analysis of the midgut of first-instar *R. prolixus* nymphs revealed that genes encoding *Small Heat Shock Proteins* (sHSPs) have their expression modulated upon infection with the trypomastigote form of *T. cruzi*. These proteins are positively regulated by blood-feeding but are downregulated when the parasite is present in the blood meal. Previous studies from our group demonstrated that silencing these sHSPs lead to disorganization of the intestinal cytoskeleton. Considering the role of the cytoskeleton in peristaltic movements, we investigated whether disorganization of actin filaments would affect gut motility. To address this, we developed a methodology for analyzing peristaltic contractions in the midgut, evaluating the motility pattern during digestion under different physiological conditions. We observed that sHSPs silencing alters the intestinal contractility, producing incomplete peristaltic waves that are interrupted somewhere along their usual way from the anterior to posterior end of the anterior midgut. Furthermore, *T. cruzi* infection recapitulates the effect of sHSP silencing. These findings suggest that sHSPs play a crucial role in maintaining the intestinal physiology of the vector and indicate that both sHSP regulation and parasite infection influence digestive tract motility, potentially impacting parasite-vector interactions.
Supported by: Capes e CNPq
Keywords: Rhodnius prolixus;Gut motility;Trypanosoma cruzi.

PV – 027 - **Analysis of the resistance of *Trypanosoma cruzi* to ionizing radiation and its effects on cellular viability and cell cycle**

SILVA, F.D.J.[1]; ABUCHERY, B.E.[2]; DA SILVA, M.S.[1].
1. INSTITUTE OF CHEMISTRY, UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL; 2. INSTITUTE OF CHEMISTRY, UNIVERSITY OF SÃO PAULO (USP), INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL.

Parasitic infections are one of the main causes of morbidity and mortality worldwide. A large proportion of these infections in humans can be attributed to trypanosomes, including *Trypanosoma cruzi* (the causative agent of Chagas disease) and *Trypanosoma brucei* (the causative agent of Sleeping Sickness). Treatment for these diseases relies on drugs that have side effects and allow the selection of resistant parasites that deal better with DNA damage. Studies have shown that trypanosomes present high resistance to ionizing radiation (IR) relative to other eukaryotes. However, the molecular mechanisms that orchestrate this unusual resistance to IR remain elusive. Here, we investigate the behavior of trypanosomes in response to IR. The results of our proliferation and viability assays suggest that *T. cruzi* can recover to low IR doses (100 Gy), but not to higher IR doses (300 and 500 Gy). To confirm these assays and further investigate the behavior of *T. cruzi* cells after IR, we performed viability assays and DNA content analysis using PI by flow cytometry. Our preliminary data suggest that a significant part of *T. cruzi* cells indeed die after being exposed to 300-500Gy of IR, and an accumulation of cells at the S phase of the cell cycle was observed 192 h after IR, mainly in the 300-500 Gy treated groups. We also performed IFA to detect γH2A (a DNA damage biomarker), which indicated the presence of DNA damage (after 192 h after IR) in 300-500Gy treated groups. Our results suggest that most of the *T. cruzi* population cannot repair the DNA damage caused by high doses of IR (300-500 Gy). Also, there is an apparent selection of parasites that better cope with the DNA damage caused by low doses of IR (100 Gy). We are investigating the behavior of *T. brucei* under the same IR conditions to determine if the findings in *T. cruzi* are common to all trypanosomatids. The insights gained from this project will help us to understand better the DDR caused by IR in trypanosomes.

Supported by: FAPESP 2024/21950-1
Keywords: DNA damage response, DNA double-strand break;Chagas disease;Trypanosomes.

PV – 028 - **Molecular detection of *Trypanosoma cruzi* DNA in domestic and production animals in a Chagas disease–endemic area under deforestation in the Amazon.**

ABREU, W.U.[1]; BRILHANTE, A.F.[2]; SANTANA, C.M.[3]; MALAVAZI, P.F.N.D.S.[4]; RECKZIEGEL, G.H.[2]; MELCHIOR, L.A.K.[2]; DA SILVA, T.I.B.[2]; HAERDRICH, S.[2]; DOS SANTOS, T.C.[5]; JORDÃO, F.T.[5]; SPERANCA, M.A.[5].
1. UNIVERSIDADE FEDERAL DO ABC, SÃO PAULO - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DO ACRE, RIO BRANCO - AC - BRAZIL; 3. UNIVERSIDADE FEDERAL DO ABC, SÃO BERNANRDO DO CAMPO - SP - BRAZIL; 4. UNIVERSIDADE FEDERAL DO ACRE,, RIO BRANCO - AC - BRAZIL; 5. UNIVERSIDADE FEDERAL DO ABC, SÃO BERNARDO DO CAMPO - SP - BRAZIL.

Introduction: Animal reservoirs contribute significantly to the spread of *Trypanosoma cruzi*, the etiological agent of Chagas disease (CD), in endemic areas through various zoonotic routes. Domestic dogs and cats are the main domestic reservoir hosts of *T. cruzi* and exhibit much greater infectivity to triatomines than seropositive humans. **Objective:** Given the epidemiological importance of animals in determining CD dynamics, this study aimed to detect *T. cruzi* in animals within the Amazônia+10 project, focusing on a survey in Acre, Brazil. **Materials and Methods:** Blood samples from 57 animals (18 cattle, 31 dogs, 3 horses, 3 cats, and 2 pigs) were collected in January and February 2024 in the municipality of Cruzeiro do Sul, Acre. DNA was extracted and subjected to conventional PCR targeting the 18S rRNA gene of *Trypanosoma* spp. The amplified products were sequenced (forward strand) using the Sanger method. **Results and Conclusion:** Nine samples (6 canine, 2 bovine, and 1 equine) were confirmed as *T. cruzi* by Sanger sequencing, based on percentage identity and e-value analysis using the BLAST (Basic Local Alignment Search Tool) platform. Among the PCR-positive samples, three (2 dogs and 1 horse) were also seroreactive in the Indirect Hemagglutination Assay (IHA) using the Chagastest HAI kit (Wiener Laboratories, Rosario, Argentina). These findings provide evidence of the susceptibility of multiple animal species in the region to CD. The results reinforce the need to include animal groups as sentinels and to combine molecular diagnostics with epidemiological surveys to improve surveillance and understanding of CD dynamics in endemic regions and critical border areas at risk for this protozoan infection.

Supported by: FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PÁULO - FAPESP - PROCESSO 2024/00176-6
Keywords: reservoirs;molecular detection;Chagas disease.

PV – 029 - **TcVps5 function and its impact on *Trypanosoma cruzi* biology**

DA SILVA, G.R.[1]; LEMOS, M.[2]; BANGS, J.D.[3]; HEISE, N.[1]; KOELLER, C.M.[1].
1. UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. CENABIO, RIO DE JANEIRO - RJ - BRAZIL; 3. UNIVERSITY AT BUFFALO, BUFFALO - UNITED STATES OF AMERICA.

Trypanosoma cruzi is a protozoan that causes Chagas disease, a neglected tropical disease that requires new therapeutic approaches. To develop effective treatments, it is essential to understand fundamental biological processes, such as the machinery that regulates protein trafficking in the secretory/endocytic pathway. In eukaryotes, protein retrograde transport from endosomes/lysosome to the Golgi is mediated by the retromer complex (RC). Previous studies have shown that the RC is important for viability, protein trafficking and autophagy in ***T. brucei***. However, the role of the RC in ***T. cruzi*** remains unexplored. This work aims to investigate the role of the RC in the secretory/endocytic pathways of ***T. cruzi*** focusing on Vps5, the only component of the RC involved in vesicle formation. TcVps5 gene was identified by comparative genomics using orthologous genes from ***Saccharomyces cerevisiae, Homo sapiens*** and ***T. brucei***. The relative expression of the gene across the life cycle stages of ***T. cruzi*** Dm28c strain was analyzed by RT-qPCR, which revealed that TcVps5 is predominantly expressed in epimastigotes when compared to amastigotes and tissue culture trypomastigotes (TCTs). TcVps5 knockout parasites (∆TcVps5) were generated using CRISPR/Cas9 and showed a decreased proliferation capacity when compared to the control parasites expressing Cas9. ∆TcVps5 epimastigotes presented reduced activity and processing of cruzipain and cathepsin B, a slight increase in the Golgi size observed by transmission electron microscopy, and increased surface labeling using fluorescent WGA, GSIB4 and tomato lectins by Flow Citometry. Also, ∆TcVps5 epimastigotes had a reduced capacity to differentiate into metacyclic trypomastigotes and infection kinetics showed a lower infection index and delayed release of TCTs. The results indicate that TcVps5 affects protein trafficking in the secretory/endocytic pathways, thereby influencing *T. cruzi* proliferation, differentiation, and infection processes.

Supported by: FAPERJ
Keywords: Vps5 retromer complex;Cruzipain;Trypanosoma cruzi.

PV – 030 - ***Trypanosoma cruzi* TcPAQR4 Receptor: *In Vitro* and *In Vivo* Screening of Specific Inhibitors**

PEREIRA, J.V.D.R.P.[1]; MONACHESI, M.C.E.[1]; CARVALHO, M.C.[1]; NICO/FAPERJ, D.[1]; TORRES, P.H.M.[2]; LOPES, A.H.D.C.S.[1].
1. INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and is usually transmitted by triatomine insects. The parasites undergo a complex process of cellular differentiation when transitioning between invertebrate and vertebrate hosts and vice versa. The lipid mediators platelet-activating factor (PAF) and lysophosphatidylcholine (LPC) play important pathophysiological roles in Chagas disease. Several LPC species, including C18:1 LPC, which mimics PAF effects, are synthesized by *T. cruzi*. Recently, we identified a receptor in *T. cruzi*, homologous to members of the progestin and adiponectin receptor (PAQR) family, that binds PAF and LPC. Furthermore, our group knocked out the *T. cruzi* PAQR gene (TcPAQR) and confirmed the identity of the expressed protein through immunoblotting and immunofluorescence assays using an anti-human PAQR antibody. TcPAQR knockout parasites were unable to respond to PAF or LPC in terms of *in vitro* cell differentiation and interactions with mouse peritoneal macrophages. Our hypothesis is that TcPAQR is a highly promising target for experimental chemotherapy. Based on a three-dimensional model of TcPAQR and molecular docking, which we previously performed to predict the interactions between the TcPAQR model and PAF or LPC, we conducted in silico screening of thousands of compounds from two Enamine ligand libraries to identify those capable of binding TcPAQR. This screening assessed the number of interactions between each molecule and the receptor using PLIP (Protein–Ligand Interaction Profiler) and calculated binding energies using AutoDock Vina, leading to the selection of eighteen molecules. From these compounds, *in vitro* viability assays will be performed on wild-type and TcPAQR knockout parasites, treated and untreated with the selected molecules. In addition, RT-qPCR will be used to compare mRNA levels of several genes involved in cell signaling in both wild-type and KO parasites, with and without treatment. Subsequently, *in vivo* screening will be carried out using the *Galleria mellonella* model with wild-type and TcPAQR knockout parasites, treated and untreated with these compounds.

Supported by: CNPq, CAPES, FAPERJ, INCTEM
Keywords: Trypanosoma cruzi;platelet-activating factor;lysophosphatidylcholine.

PV – 031 - **Evaluation of the Effects of *Leptomonas wallacei* Infection on the Morphology, Physiology, Behavior, and Microbiome of the Insect *Oncopeltus fasciatus***

PEREIRA, J.V.D.R.P.; DOS SANTOS, L.B.; GAMA, A.J.D.S.; HILL, L.J.; DA SILVEIRA, F.D.A.M.; BARRETO, C.P.; GONÇALVES, I.C.; DO CARMO, F.L.; LOPES, A.H.D.C.S..
INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Oncopeltus fasciatus (Hemiptera, Lygaeidae) is a phytophagous hemipteran and natural host of the monoxenous trypanosomatid *Leptomonas wallacei*. Although restricted to the digestive tract and initially considered commensal, infection has been linked to reduced lifespan, morphological deformities, reproductive impairment, and decreased offspring viability. This study investigates the effects of *L. wallacei* on host morphology, physiology, behavior, and gut microbiota, with emphasis on the reproductive system. Testes morphology was compared between insects from infected and cured colonies. Dissections revealed that infection was associated with more frequent and severe structural abnormalities. Female mate choice assays showed that infected females more often remated with the same male, whereas uninfected females preferred novel males. Egg viability was assessed across four mating combinations, revealing a general decline in viability with infection, particularly when infected males mated with uninfected females. Gut microbiome composition was examined by 16S rRNA metabarcoding from dissected intestines. Infected insects displayed markedly reduced microbial diversity, dominated by a few genera, especially *Enterococcus.* These findings demonstrate that *L. wallacei* infection, despite being localized to the gut, induces systemic effects on morphology, reproductive physiology, mating behavior, and microbial communities of *O. fasciatus*. The results suggest a complex host–parasite interaction in which gut-restricted pathogens can modulate distant physiological systems, potentially through indirect or immune-mediated pathways. Elucidating these mechanisms will advance understanding of parasite–host–microbiome dynamics in insects.
Supported by: CNPq, CAPES, FAPERJ, INCTEM
Keywords: morphophysiological alterations;sexual selection;microbiome;.

PV – 032 - **Beyond translation: How amino acid metabolism shapes *Trypanosoma cruzi*'s life cycle and virulence**

NASCIMENTO, J.D.F.[1]; BRAGA, M.S.[1]; FRANCISCO, A.F.[2]; JAYAWARDHANA, S.[2]; MENGARDA, A.C.A.[1]; SOUZA, H.F.S.[1]; BALLARI, M.S.[1]; ALENCAR, V.J.B.[1]; AGRIPINO, J.D.M.[1]; GARCIA, M.C.[1]; DURAN, J.J.R.[1]; GENTA, F.A.[3]; TAYLOR, M.[2]; KELLY, J.M.[2]; SILBER, A.M.[1].
1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDRES - UNITED KINGDOM; 3. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Amino acids play diverse roles in the biology of *Trypanosoma cruzi*, the causative agent of Chagas disease. Beyond protein synthesis, they support cell differentiation, osmoregulation, and stress responses. In this context, this study aims to identify the key steps within the amino acid metabolic network that are essential for *T. cruzi* to complete its life cycle. Using bar-seq CRISPR-Cas9 genome editing, we generated 44 mutant knockout cell lines targeting enzymes putatively involved in amino acid metabolism, comprising 16 homozygous and 28 heterozygous lines. Each cell line was assigned a unique barcode sequence to enable parallel phenotyping via next-generation sequencing. The mutant cell lines, along with the cell line expressing the thermostable red-shifted firefly luciferase PpyRE9h, were pooled in equal numbers to assess epimastigote proliferation and differentiation into metacyclic trypomastigotes *in vitro*. These infective forms were then purified and used to infect BALB/c and immunodeficient SCID mice. Parasite load was monitored by blood counting of parasitemia and bioluminescence. About half of the infected mice showed positive bioluminescence signal over the course of infection. At 25 days post-infection, all mice were euthanised, and bioluminescence analysis confirmed the presence of parasites in multiple tissues, particularly in the spleen, visceral fat, and other locations within the intestines. Samples for gDNA isolation and library preparation were collected throughout the proliferation, differentiation, and *in vivo* infection experiments. This approach will help elucidate the metabolic pathways that *T. cruzi* uses to adapt to diverse environments and may reveal novel targets for future drug design.
Supported by: Wellcome Trust - 222986/Z/21/Z
Keywords: CRISPR-Cas9 bar-seq;Amino acids metabolism;Trypanosoma cruzi.

PV – 033 -**MALDI-ToF/MS AS AN INNOVATIVE DIAGNOSTIC TOOL FOR DETECTION OF *TRYPANOSOMA CRUZI***

SILVA, V.A.; DE ANDRADE, B.A.; LIMA, J.M.G.; TEMPONE, A.G..
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

The diagnostic of Chagas disease represents a challenge for the public health system. Detection limitations and frequent asymptomatic presentation highlight the need for improved diagnostic strategies. This project aimed to assess Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-ToF/MS) as a rapid diagnostic tool for direct *T. cruzi* detection and to develop an in-house spectral library of the parasite. Epimastigote and trypomastigote forms of different *T. cruzi* strains (Y, DM28c, and Colombian) were *in vitro* cultivated with supplemented mediums. Protein extraction was performed by ethanol lysis, followed by precipitation with 70% formic acid and acetonitrile. Samples were applied to MALDI target plate with HCCA matrix and analyzed using a Bruker Microflex MALDI-ToF/MS in the 2,000–20,000 m/z range. A minimum of twenty spectra per strain were acquired to compose the spectral library. Initially, 24 spectra were obtained from both direct and tube-based extraction methods applied to trypomastigote and epimastigote forms of the Y strain, and epimastigotes from DM28c and Colombian strains. The equipment distinguished between developmental forms (epimastigotes and trypomastigotes), with different spectral data of the extraction methods, demonstrating sensitivity and specificity in parasite specie detection. The tube-based method yielded a higher quality spectral data for epimastigotes, while both methods were adequate for the trypomastigotes. MALDI-ToF/MS can be considered a promising and rapid tool for *T. cruzi* detection, with potential applications in clinical diagnostic and epidemiological studies.
Supported by: FAPESP 2024/16243-4, CAPES e FESIMA (Secretary of Health of São Paulo State).
Keywords: Trypanosoma cruzi;clinical diagnostic;MALDI-ToF/MS.

PV – 034 - **Functional investigation of the acetylornithine deacetylase gene in paromomycin resistance in Leishmania amazonensis**

BIRAL, G.M.; COELHO, A.C..
UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRAZIL.

Leishmaniasis is a parasitic disease considered neglected by the World Health Organization and caused by the protozoa of the genus Leishmania. In Brazil, more than 20,000 cases of the disease are reported annually, mainly caused by *Leishmania amazonensis* and *L. braziliensis*, whose species are causative of cutaneous leishmaniasis. The drugs available for the treatment of the disease are limited, relatively toxic and the treatment failures have been reported, which may be associated with factors such as the immune response of the infected patient, co-infection with other pathogens, such as the HIV, and factors directly related to the parasite, in which drug resistance is the main factor. Paromomycin is an alternative drug used in other regions of the world for the treatment of leishmaniasis, although it has not yet been approved for the treatment in Brazil. In addition, the mechanism of action and resistance of paromomycin is not completely understood in Leishmania, particularly in species endemic in Brazil. In this study, we propose to characterize the acetylornithine deacetylase (AOD) gene, possibly associated in resistance to paromomycin in L. amazonensis. This gene was identified mutated through bioinformatics analysis of the complete genome of paromomycin-resistant lines of L. amazonensis. For investigating the role of AOD in paromomycin resistance, we are using reverse genetic techniques, such as overexpression and gene knockout, in this species of the parasite. This gene has been amplified and cloned in an expression vector to investigate its role in drug resistance. Additionally, using CRISPR/Cas9 technology, mutant knockout lines for AOD gene have been also selected and generated to evaluate whether this enzyme plays a role in the resistance phenotype. Once transgenic lines were genetically validated, they were evaluated through of in vitro paromomycin susceptibility assays at promastigote and intracellular amastigote stages. These findings will contribute for a possible role of AOD in the mechanism of resistance to paromomycin in Leishmania.
Supported by: FAPESP 2024/10987-1
Keywords: Leishmania amazonensis;cutaneous leishmaniasis;drug resistance.

PV – 035 - **Molecular Signal of Undescribed *Leishmania-Like* Trypanosomatids in Culicidae and Ceratopogonidae Insects in a Lingering Atlantic Forest Park Restricted in a Southeast Brazilian Region**

JORDÃO, F.T.[1]; DOS SANTOS, T.C.[1]; DE ANDRADE, L.A.[1]; PEREIRA, G.L.[1]; MOLINA, J.S.T.D.O.[1]; DE OLIVEIRA, L.P.R.[1]; SUZUKI, R.B.[2]; CABRAL, A.D.[3]; SPERANCA, M.A.[1].
1. UNIVERSIDADE FEDERAL DO ABC, SÃO BERNARDO DO CAMPO - SP - BRAZIL; 2. UNIVERSIDADE DE MARÍLIA, MARÍLIA - SP - BRAZIL; 3. FACULDADE DE CIÊNCIAS MÉDICAS DA SANTA CASA DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

Leishmaniasis, caused by the genus *Leishmania* protozoa, is a disease characterized by the interaction of parasites, invertebrate vectors, and human and vertebrate hosts. Species of the Phlebotominae subfamily are the main *Leishmania* vector and Ceratopogonidae species are associated with the transmission of *Leishmania* from subgenus *Mundinia*. The city of Marília - SP, is known to be an endemic region for both leishmaniasis and Chaga's disease and also has a predominant urban Atlantic Forest biome. These characteristics are conducive to the occurrence of a great diversity of arthropods and animals typical of the biome, which coexist with the human population, thus making it a potential hotspot for both trypanosomatid and vector biodiversity. This study aims to investigate the diversity of *Leishmania* parasites in Marília - SP, by surveillance of hematophagous insects with the gene encoding the GH18 chitinase from *Leishmania*. Diptera insects were collected in the Rangel Pietraroia Forest Park in Marília - SP. DNA of collected samples were extracted and submitted to molecular identification of *Leishmania* by amplification of a 953 bp fragment corresponding to the GH18 chitinase encoding gene specific to the *Leishmania* genus. The positive PCR fragments were cloned, sequenced and submitted to BLAST analysis and phylogenetic reconstruction. From a diverse group of dipterans, specimens of Ceratopogonidae and Culicidae were positive for a 953 bp GH18 chitinase encoding gene fragment with 97 to 98% similarity with *Leishmania* species of the subgenus *Viannia* and *Leishmania* from new world. These results were confirmed by phylogenetic reconstruction, indicating the circulation of *Leishmania-like* parasites and a putative role of Ceratopogonidae and Culicidae Families species of insects as its hosts.
Supported by: FAPESP (2016/14514-4) e CAPES (88887.721976/2022-00)
Keywords: Leishmania;Ceratopogonidae;Culicidae.

PV – 036 - **Molecular Insights into the Binding of Dinuclear Fe(III) Complexes to Crucial *Trypanosoma cruzi* Proteins: An *In Silico* Approach**

ZOCATELI, P.I.A.; MOREIRA, F.F.; DAMATTA, R.A.; SEABRA, S.H..
UENF, CAMPOS DOS GOYTACAZES - RJ - BRAZIL.

Chagas disease, caused by *Trypanosoma cruzi*, remains a neglected tropical disease with limited treatments. Current drugs, nifurtimox and benznidazole, have severe side effects and variable efficacy, highlighting the need for new alternatives. Coordination compounds, especially metal complexes, have shown promise. Two novel dinuclear Fe(III) μ-oxo complexes, Fe1 and Fe2, exhibited nanomolar anti-trypanosomal activity and low host cell toxicity, making them attractive prototypes. To clarify the mechanism of action of Fe1 and Fe2 their molecular interactions with key *T. cruzi* proteins via docking simulations was executed. Docking was performed using AutoDock Vina against six targets (P1–P6), including five oxidoreductases and the hydrolase cruzain. Protein structures were sourced from RCSB PDB, prepared, and ligand poses optimised. The best poses were chosen by lowest binding free energy (ΔG). Interactions were visualized with PyMOL and analyzed with LigPlot+. Both Fe1 and Fe2 showed strong affinity for oxidoreductases (P1, P2, P3, P4, P6), with highly negative ΔG values, while cruzain (P5) had lower binding affinity. Fe1 formed stable interactions with multiple hydrogen bonds and hydrophobic contacts across all oxidoreductases. Fe2 also bound strongly but with a distinct interaction pattern, likely due to its isomeric structure. The most negative ΔG values were observed for P2 and P6. The preferential binding to oxidoreductases suggests these enzymes were the primary targets, potentially disrupting the parasite's redox balance and antioxidant defenses. Lower affinity for cruzain supported a redox-centred mechanism. Structural differences between Fe1 and Fe2 influence binding modes, impacting selectivity and activity. These results offer key molecular insights into the action mechanism of these metal complexes against *T. cruzi.*
Supported by: UENF, FAPERJ, CAPES.
Keywords: Oxidoreductase inhibition;Redox metabolism;Metal-based drugs.

PV – 037 - **Persistence of non-proliferative amastigotes after benznidazole treatment**

SILVA, T.D.S.[1]; LOCH, L.[2]; PARMEGIANI, G.N.[3]; SABBAGA, M.C.Q.B.E.[3]; SCHENKMAN, S.[3]; DE ALBUQUERQUE, S.[1].
1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 3. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Background: Chagas disease manifests differently in genetically distinct strains of *Trypanosoma cruzi*, referred to as Discrete Typing Units (DTUs), contributing to a complex immunological profile and distinct pathogenicity. The impact on the transient phenomenon of persistence may be associated with the genotypic and phenotypic dynamics of DTUs. The ineffectiveness of trypanocidal drugs in treatment may also stem from this complexity. **Aim:** This study aimed to identify and quantify dormant amastigote forms using monoclonal antibodies targeting various stage-specific surface antigens and replication proteins, and to analyze the presence of a dormancy profile in distinct strains with different DTUs. **Methods:** Preliminarily, we utilized two different strains with distinct DTUs (Dm28c – TcI and Y strain - TcII) and conducted an exploratory assay employing monoclonal antibodies (mAb 3C9, mAb 2C2, mAb 5E7, mAb 3C9, mAb 3F6 and mAb RPA-2) along with the fluorescent marker EdU (5ethynyl-2′-deoxyuridine), to quantify dormant amastigotes using the High-Content Screening (HCS) technique. **Results:** Our results indicated the existence of monoclonal antibodies that identify molecules in both proliferative and non-proliferative amastigotes. Variations were noted in the infection rates and the occurrence of non-proliferative amastigote forms in infected cultures subjected to benznidazole treatment. **Conclusion:** The findings suggest that the Y strain might contain a greater population of non-proliferative amastigotes compared to the Dm28c strain when exposed to benznidazole. Upon analysis, the RPA-2 target was found by attenuated fluorescence in the nucleus, serving as an additional marker for persistent cells, which may help us to elucidate the characteristics of this parasitic stage, significant for the comprehension of *T. cruzi* biology. This may elucidate and/or point out important molecules to aid in the development of better therapeutic strategies against Chagas disease.

Supported by: Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, CNPq - Processo: 140536/2021-3
Keywords: Non-proliferative;Dormancy;DTUs.

PV – 038 - **The influence of Ionic Iron on energy and glycosomal metabolism in Trypanosoma cruzi**

CAMPOS, D.B.; BARRINHA, A.; CRISTOVAM, J.B.D.B.; FRECHIANI, G.D.J.; MOTTA, M.C.M.; DICK, C.F..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosoma cruzi, the etiological agent of Chagas disease, is a trypanosomatid that continues to be a concern for global health organizations due to its current prevalence, which has led to an increase in the number of cases of this disease in non-endemic countries. *T. cruzi* has the key ability to alter its energy metabolism in response to varying nutritional availability throughout its life cycle, a characteristic demonstrated primarily by the modulation of its organelles involved in energy metabolism: mitochondria and glycosomes. Furthermore, iron (Fe) has been described as essential for *T. cruzi* homeostasis, since it can affect its proliferation, viability, and survival. Thus, the objective of this study is to investigate how ionic Fe modulates the redox state and metabolic pathways of *T. cruzi*, focusing on mitochondrial and glycosomal functionality. To achieve these objectives, we analyzed the mitochondrial oxygen consumption rate and glycosome localization in *T. cruzi* epimastigotes grown in medium depleted or supplemented with exogenous Fe. We observed that the concentration of exogenous Fe induces a loss of mitochondrial viability compared to the control condition, regardless of the carbon sources used, glucose or proline, reflected in impaired respiratory capacity. Furthermore, the glycosomes of these parasites appear to be modulated under these nutritional stress conditions, both in quantity and localization, as well as in protein profile within the organelle. These data, taken together, suggest that *T. cruzi* exhibits alternative and adaptive mechanisms when there is variation in exogenous Fe concentrations.

Keywords: energy metabolism;mitochondrial function;glycolysis.

PV – 039 - **Early changes at the skin interface during triatomine salivary proteins inoculation**

FONTOURA, G.D.R.S.[1]; SANTIAGO, P.B.D.M.[1]; SANTOS, L.D.M.[1]; CAVALCANTE, I.D.F.[1]; AOYAMA, M.M.M.B.[1]; BASTOS, I.M.D.[1]; CHARNEAU, S.O.[1]; DE SANTANA, J.M.[1]; FERCOQ, F.[2]; MOURAY, E.[2]; MARTIN, C.[2]; DE ARAÚJO, C.N.[1].
1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRAZIL; 2. MUSEUM NATIONAL D'HISTOIRE NATURELLE, PARIS - FRANCE.

The salivary glands of triatomines, vectors of the Chagas disease protozoan *Trypanosoma cruzi*, express proteins and peptides that target the hemostatic, immune, and inflammatory systems of their mammalian hosts. Past transcriptomic and proteomic analyses of salivary glands revealed the diversity and abundance of molecules present in this tissue. Here, we produced recombinant His-tagged salivary proteins Antigen-5 and OBP from *Rhodnius neglectus,* as well as a hemolymph Defensin from *Rhodnius prolixus,* in *Escherichia coli* BL21(DE3) to investigate their individual effects on innate immunological mechanisms in the vertebrate host. The effects of the recombinant proteins and the salivary gland extract (SGE) inoculated in the skin of mice were evaluated using flow cytometry and histology. We show that SGE induced the recruitment of neutrophils, eosinophils, macrophages, lymphocytes, and Langerhans cells, except monocytes, in the mouse group injected with this extract. Surprisingly, among the recombinant proteins, *R. neglectus* OBP recruited approximately three times more cells than the SGE group. Neutrophils were the most prevalent cell type in both SGE and OBP mice groups, with S100A9 staining supporting these findings. There was a tendency toward an increase in the number of eosinophils in the mouse group injected with Antigen-5, confirmed by Congo Red, and a trend toward a decrease in the Langerhans cell counts in the mouse group injected with Defensin. There was no difference in mast cell counts for any of the conditions. Transcriptomic analysis of skin biopsies collected from the inoculation sites is currently under investigation. Overall, these findings deepen our understanding of triatomine saliva and provide valuable insights into the vector-host interactions that may facilitate the transmission of *T. cruzi*. Understanding the mechanisms involved in this interface is promising for the development of effective anti-triatomine vaccines and drug therapeutics.
Supported by: FAP-DF 00193-00002600/2022-70
Keywords: Arthropod;Vector;Chagas disease.

PV – 040 - **Targeting Dormant *Trypanosoma cruzi:* A Novel Drug-Screening Platform Using Gamma-Irradiated Parasites and CFSE Tracking**

RESENDE, B.C.; GARCIA, M.E.D.L.P.; ANDRADE, L.D.O.; MACEDO, A.M.; FRANCO, G.R.; MACHADO, C.R..
UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

Chagas disease, caused by *Trypanosoma cruzi*, affects an estimated 6 to 7 million people worldwide, making it one of the leading neglected tropical diseases. Although there is no fully effective treatment, the drugs Benznidazole and Nifurtimox are used to reduce parasitemia. Recent studies have identified non-replicative forms of the parasite, in a dormant-like state, that resist current treatments and sustain infection. These forms can resume replication and are associated with DNA recombination processes, with signaling dependent on the ATM kinase for cell cycle arrest. Our research group used gamma radiation to induce DNA breaks in the parasite without killing it, preserving its infectivity. These irradiated parasites infect cells but do not replicate, even after 72 hours in culture. The goal of this study is to standardize a new drug-testing platform targeting dormant forms, using the CellTrace CFSE marker for replication tracking. For this purpose, DM28c strain trypomastigotes are irradiated at 500Gy, labeled with CFSE, a fluorescent dye that marks the parasite without killing it, ensuring that each division cycle dilutes CFSE among daughter cells. The parasites are then placed in contact with LLC-MK2 cells for 1 hour. After washing, the drug is added, and infection is monitored for 72 hours, using both microscopy and cytometry to assess intracellular CFSE-positive parasites. Flow cytometry strongly supported microscopy-based infection counts, but the cytometer's ability to analyze a large number of events provides greater statistical reliability than microscopy for our analytical method. Results showed that irradiated parasites resist Benznidazole, while only non-irradiated parasites with strong CFSE labeling persisted after 72 hours. The next steps involve testing peptides from the venom of the spider *Lycosa erythrognatha*—previously effective against Leishmania sp.—against dormant forms of *T. cruzi.*
Supported by: Fapemig - BPD00548-22
Keywords: Trypanosoma cruzi;Dormancy;Drug-resistence.

PV – 041 - **Expression of mutants of the TcRlp Ras family GTPase in *Trypanosoma cruzi* epimastigotes: effects in cell ultrastructure**

DE FARIAS, K.L.[1]; NEPOMUCENO-SILVA, J.L.[1]; DA SILVA, J.R.[1]; SEABRA, S.H.[2]; MOREIRA, F.F.[2].
1. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, MACAE - RJ - BRAZIL; 2. UNIVERSIDADE FEDERAL DO NORTE FLUMINENSE, CAMPOS DOS GOYTACAZES - RJ - BRAZIL.

Ras family GTPases act as molecular regulators of essential cellular processes such as cell proliferation, growth, and differentiation, alternating between active (GTP-bound) and inactive (GDP-bound) states. These GTPases are also modified by isoprenylation (geranylgeranylation [20C] or farnesylation [15C]) at a C-terminal CaaX motif that allows membrane association, which is essential for their function. The parasite *Trypanosoma cruzi* has only one Ras family gene, encoding the protein TcRlp (*Trypanosoma cruzi* Ras-like protein). TcRlp has the CaaX motif CVLL, which is modified by geranylgeranylation. We previously demonstrated that the expression of a dominant positive mutant of TcRlp (TcRlp-Q61K) reduces epimastigote proliferation and increases metacyclogenesis *in vitro*, and that interference with C-terminal geranylgeranylation of such mutants completely abolish these effects. Here we investigate how the expression of mutant versions of TcRlp affects the ultrastructure of epimastigotes. Therefore, we performed routine transmission electron microscopy (TEM) to compare epimastigotes lineages expressing the following mutant versions of this GTPase: GFP-TcRlp-WT (wild-type TcRlp), GFP-TcRlp-Q61K (dominant positive), GFP-TcRlp-S17N (dominant negative), GFP-TcRlp-dCaaX (non-prenylated), as well as the double mutant GFP-TcRlp-Q61K-dCaaX. TEM analyses revealed clear ultrastructural defects in the mitochondria of the TcRlp-Q61K mutant, with many sites of mitochondrial membranes disorganization. Importantly, these mitochondrial alterations are absent in the double mutants, indicating that geranylgeranylation of TcRlp CaaX motif is essential to these effects. These structural anomalies in the mitochondria of lineages expressing the dominant positive version of TcRlp will probably have an impact the energy metabolism of the parasite and may be linked to the decreased proliferation capacity observed for this lineage. Further investigation is needed to explore this hypothesis and to elucidate the cellular function of this Ras-related GTPase in *T. cruzi.*

Keywords: Mitochondria;GTPase;Chagas disease.

PV – 042 - **Exploring the Machinery Associated with tRNA Transcription in *Trypanosoma cruzi***

LOTERIO-SILVA, M.[1]; SILVA, H.G.D.S.[2]; DA CUNHA, J.P.C.[3].
1. INSTITUTO DE CIÊNCIAS BIOMÉDICAS - USP, BELO HORIZONTE - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 3. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Trypanosoma cruzi is the etiological agent of Chagas disease. Unlike most eukaryotes, regulation of gene expression in *T. cruzi* occurs predominantly at the post-transcriptional level, due to the absence of conventional RNA polymerase II promoters and the presence of polycistronic transcription units. Despite this peculiarity, the parasite possesses the three classical eukaryotic RNA polymerases (RNAPs), each specialized in transcribing distinct RNA classes. RNA polymerase III (RNAP III), for instance, synthesizes small RNAs, tRNAs, and 5S rRNAs, using known promoters and transcription factors such as the multi-subunit complexes TFIIIB and TFIIIC. In trypanosomatids, TFIIIC has not been fully characterized, limiting our understanding of RNAP III-mediated transcription in these parasites.Through in *silico analyses*, we identified the RPC4 subunit of RNAP III and the Tfc1 subunit of TFIIIC, both previously annotated as hypothetical proteins. This study aimed to investigate their subcellular localization, abundance across cell cycle phases, genomic distribution and the effects of their partial depletion on global proteomic profiles and and cellular differentiation rates. Using CRISPR/Cas9, we generated: (i) Ty1-tagged RPC4 and Tfc1 lineages; (ii) heterozygous knockout (HzKO) for each gene ($RPC4^{+/-}$ and $Tfc1^{+/-}$). All clones were validated by Western blot and/or PCR. Immunofluorescence of Ty1-RPC4 cells revealed RNAP III modulation during the cell cycle. HzKO $Tfc1^{+/-}$ exhibited a significant reduction in differentiation from epimastigotes to metacyclic trypomastigotes compared to control. Proteomic analyses showed that $RPC4^{+/-}$ and $Tfc1^{+/-}$ lines shared ~50% of differentially expressed proteins, many related to chromatin assembly and translation, suggesting their functional association. Collectively, these findings advance our knowledge of RNAP III transcription in T. cruzi and provide a basis for future studies on tRNA transcription regulation in trypanosomatids.

Supported by: Capes - 88887.826338/2023-00
Keywords: RNA Polymerases III;tRNA;Transcription.

PV – 043 - **IMPACT OF IRON OVERLOAD IN THE ENZYME FE REDUCTASE FROM *TRYPANOSOMA CRUZI***

DE PINHO, B.R.M.[1]; FRECHIANI, G.D.J.[2]; CAMPOS, D.B.[1]; CRISTOVAM, J.B.D.B.[1]; VIEYRA, A.[1]; DICK, C.F.[1].
1. UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. BIOTRANS-UNIGRANRIO, DUQUE DE CAXIAS - RJ - BRAZIL.

Trypanosoma cruzi is a protozoan that lives in a variety of environments, from an insect vector to a mammal. Therefore, this parasite must have fine control over its nutrient uptake, especially iron (Fe). Fe acts as a cofactor for several metabolic processes, including producing reactive oxygen species (ROS) that are beneficial at specific concentrations but can be harmful in excess. Fe is found in its poorly soluble ionic form (Fe^{3+}) especially associated with metalloproteins, or Fe^{2+} , which is soluble in a neutral pH medium and can be internalized in its ionic form and not conjugated to proteins. Fe-reductase (TcFR) is a protein in the cell membrane of *T. cruzi* that is involved in converting Fe^{3+} into Fe^{2+} . A Fe^{2+} transporter (TcIT) acts with TcFR, essential for the translocation of Fe^{2+}across the plasma membrane. When soluble Fe is not correctly stored, the ion accumulates in the cytosol and reacts with hydrogen peroxide, generating ROS, which, in excess, generates lipid peroxidation by Fenton reaction. Our objective is to describe the response of an exogenous Fe overload on TcFR activity and *T. cruzi* metabolism, focusing on the processes of proliferation, cell differentiation, and, consequently, on the virulence and energy metabolism of the parasite. Aspects such as cell viability, lipid peroxidation, activity, and expression of the TcFR enzyme were evaluated. We observed an increase in lipid peroxidation due to Fe imbalance in cells cultured in depleted media and with Fe overload. Immunofluorescence assays confirm the presence of TcFR, colocalized with the parasite's plasma membrane in both conditions. In addition, the activity of TcFR in different conditions can interfere with the parasite's metabolism in its different evolutionary forms, influencing proliferation, viability, and energy metabolism. Thus, the results suggest that the impacts in cell metabolism may be caused by variation in intracellular Fe concentration.

Keywords: Fe-reductase;Trypanosomatid;Oxidative Stress.

PV – 044 - **The Role of the Kynurenine Pathway in the Adaptation to Hematophagy in Rhodnius prolixus**

COELHO, J.P.F.; DE ALMEIDA, I.F.D.C.; DE OLIVEIRA, P.L..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

During feeding, like other hematophagous arthropods, kissing bugs like *Rhodnius prolixus* ingest an amount of blood significantly larger than its own body weight. Several metabolic adaptations contribute to a high-protein intake digestion and to handling suddenly increased concentrations of some metabolites. Previous results from our group revealed that the Kynurenine Pathway (KP), responsible for tryptophan catabolism, acts on gut homeostasis and hematophagy adaptation of *Aedes aegypti*, while recent studies showed its effect on *Drosophila melanogaster* zinc metabolism. Therefore, our goal is to identify KP metabolites in *R. prolixus,* in order to understand if the pathway has a role in the adaptation to blood feeding. Analysis of KP metabolites in midgut, hindgut and Malpighian tubules samples by HPLC revealed a 3-hydroxykynurenine (3-HK) accumulation after the blood meal. However, key KP metabolites, such as xanthurenic and kynurenic acid, were not found. Inhibition of KMO, the KP enzyme that converts L-kynurenine into 3-HK, accomplished by feeding N1 nymphs with blood supplemented with a selective inhibitor (Ro-61-8048), resulted in death and a hemolymph accumulation phenotype at the highest concentration (1 mg/mL), and, at sub-lethal doses, caused delayed molting and digestion. The mortality effect by Ro-61-8048 was attenuated when associated with a zinc chelator, while the body turgescence phenotype persisted. Current results indicate relevant diferences between the KP in *R. prolixus* and other insects. The lethal effect of KMO inhibition being attenuated by a zinc chelator suggests that, as seen in *D. melanogaster*, 3-HK could be acting sequestering the metal in intracellular granules in the Malpighian tubules. This leads us to propose that the KP, besides regulating water transport, influences zinc signaling through an unknown, yet essential, mechanism. Collectively, our data reveal a pleiotropic contribution by the KP on adaptation to hematophagy.

Supported by: CAPES, CNPq, FAPERJ, INCT-EM
Keywords: Triptofano;Rhodnius prolixus;Zinco.

PV – 045 - **Comparative analysis and experimental validation of the m6A RNA modification machinery in Plasmodium spp.**

LOPES, D.; BARROS, R.R.D.M..
UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

Malaria remains a major global health concern, particularly in tropical regions, with Plasmodium falciparum, P. vivax, and P. knowlesi being the main species associated with human infection. The survival of these parasites across different hosts and tissues depends on complex gene regulatory mechanisms. Among them, post-transcriptional modifications such as N6-methyladenosine (m6A) play a crucial role by modulating transcript stability, translation, and localization. Although enzymes related to the m⁶A machinery have been described in P. falciparum, comparative data across species and functional validation remain limited.
In this study, we performed in silico analyses using PlasmoDB database to identify candidate genes associated with RNA modification machinery in Plasmodium spp. By combining searches based on annotation terms and conserved domains, we retrieved 47 genes in three reference species: 15 in P. falciparum 3D7, 16 in P. knowlesi H, and 16 in P. vivax Sal-1, classified into 37 writers, 6 readers, and 4 undefined genes. No consistent candidates for erasers were identified. Among writers, pseudouridine synthases, methyltransferases, and dimethyladenosine transferases were prominent; readers were represented by YTH domain-containing proteins, found in two conserved copies across the species (60–75% amino acid identity). The absence of erasers may indicate structural divergence or a distinct regulatory mechanism in Plasmodium.
Comparative analysis revealed that P. falciparum and P. knowlesi share closer similarity in gene repertoire and chromosomal location, while P. vivax exhibited distinctive features, including exclusive genes (dimethyladenosine transferases) and four conserved hypothetical proteins. These differences may relate to unique biological traits of P. vivax, such as hypnozoite formation and clinical relapses.
Our findings highlight the conservation of the core m6A machinery (writers MT-A70 and YTH readers) in Plasmodium spp., while uncovering evolutionary plasticity in accessory modifiers. The next step of this work will involve experimental validation in synchronized P. falciparum cultures, with transcriptional analysis by qPCR under basal and stress conditions. Overall, this study contributes to understanding epitranscriptomic regulation in malaria parasites and may reveal new therapeutic targets.
Keywords: Plasmodium falciparum;m⁶A modification;epitranscriptomic regulation.

PV – 046 - **Iron Overload Induces Ferroptosis in *Trypanosoma cruzi***

FRECHIANI, G.D.J.; CRISTOVAM, J.B.D.B.; CAMPOS, D.B.; CAMELO, T.M.; BARRINHA, A.; VIEYRA, A.; MOTTA, M.C.M.; DICK, C.F..
UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Trypansoma cruzi life cycle is intricate, involving transitions between two hosts and various developmental stages, necessitating adaptations to diverse energy sources for survival, notably through ionic transport mechanisms. Iron (Fe) transport is a crucial process within this context, as Fe plays a vital role in the energy metabolism, ATP production, DNA synthesis, and generation of reactive oxygen species (ROS) in all living organisms due to its unique redox properties, serving as both a necessary element and a potential hazard contingent upon cellular conditions. Excessive Fe levels can trigger lipid peroxidation, leading to iron-induced cell death, known as Ferroptosis. Ferroptosis is a ROS-dependent cell death associated with two main biochemical characteristics: iron accumulation and lipid peroxidation. Furthermore, this type of cell death can be triggered by the extrinsic or intrinsic pathway, depending on the expression of transporters or antioxidative enzymes, unlike the classic programmed cell death. Our objective is to assess the impact of excessive exogenous Fe on the proliferation of *T. cruzi*, the regulation of ROS generation, the functionality of antioxidant enzymes and on the virulence of these parasites. Exogenous Fe overload inhibits cell proliferation, and the addition of Ferrostatin-1, a potent inhibitor of ferroptosis, recovers cellular proliferation. In addition, Fe overload leads to higher lipid peroxidation levels than control cells. To regulate the integrity of the cell, antioxidant catalysts, like Fe-SOD, help to regulate ROS production by converting them to less harmful molecules. Furthermore, we observed that Fe overload induces H_2O_2 production, consequently impacting cell viability, thereby altering the parasite's virulence compared to control counterparts. Altogether, these results suggest that Fe overload may trigger cell death in *T. cruzi*, possibly through ferroptosis.
Keywords: Ferroptosis;Oxidative stress;Lipid peroxidation.

PV – 047 - **CRISPR-Cas9-mediated single-knockout of Hexokinase reveals its crucial role in *Trypanosoma cruzi* epimastigotes cells by impairing parasite proliferation and bioenergetics**

AGOSTINHO, L.T.[1]; VIEIRA, C.S.D.[1]; MOREIRA, I.C.D.F.[1]; NOGUEIRA, N.P.D.A.[1]; DE CARVALHO, D.B.[2]; LIMA, A.P.C.D.A.[2]; PAES, M.C.[1].
1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosoma cruzi, the protozoan responsible for Chagas disease, undergoes several environmental and metabolic adaptations during its life cycle. In the proliferative epimastigote stage, glucose uptake and metabolism are critical for sustaining cell growth. Hexokinase, the enzyme that catalyzes the first step of glycolysis, plays a key role in this process by phosphorylating glucose and committing it to the energy-producing pathway or to the HMP shunt. Given its central role in carbohydrate metabolism, this study focuses on investigating the impact of *T. cruzi* hexokinase disruption through CRISPR/Cas9-mediated knockout, aiming to elucidate its importance to the energy metabolism and proliferation. The knockout was validated by PCR and only single-allele mutant parasites were obtained: TcHKg-sKO, indicating this gene may be essential for the parasite survival. The partial deletion resulted in nearly 50% reduction in hexokinase activity of the parasite which reflected in the proliferation exhibiting a huge reduction on the third, fifth and especially seventh day after incubation. Proliferation was also evaluated with glucose supplementation and showed that the addition of glucose does not increase parasite replication. After 24h of incubation the glucose uptake of TcHKg-sKO was measured showing an even uptake compared with the control parasite, although it did not reflect on proliferation, demonstrating the crucial role of hexokinase in cell growth. Finally, looking at the mitochondria, the mutant displayed a ruined mitochondrial physiology, exhibiting dysfunctions in cellular respiration, oxidative phosphorylation, and maximum respiratory capacity resulting in a poor ATP production. These findings reinforce the centrality of hexokinase as a key enzyme in *T. cruzi* and highlight its essential role in metabolic balance, growth, and survival. This work emphasizes not only the importance of the enzyme but also the potential of targeting it for therapeutic strategies.

Supported by: FAPERJ, CAPES, CNPq and INCT-EM
Keywords: Trypanosoma cruzi;CRISPR/Cas9;glucose metabolism.

PV – 048 - **Deferoxamine Impacts Osmoregulation in *Trypanosoma cruzi***

SILVA, A.D.S.; CRISTOVAM, J.B.D.B.; FRECHIANI, G.D.J.; CAMPOS, D.B.; VIEYRA, A.; DICK, C.F..
UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosoma cruzi, the etiological agent of Chagas disease, is a flagellate of the Trypanosomatidae family. Iron (Fe) is an essential micronutrient for most living organisms. However, due to its very low redox potential, Fe can become harmful by catalyzing the formation of reactive oxygen species (ROS). For this reason, many living systems have developed mechanisms to control the internalization, metabolism, and storage of this ion. Acidocalcisomes are acidic organelles rich in calcium (Ca^{2+}), polyphosphate, and pyrophosphate. They contain several other ions and help to regulate osmotic pressure. The identification of a vacuolar Fe transporter in *T. brucei* acidocalcisomes and the presence of Fe in *T. cruzi* acidocalcisomes suggest that this organelle may be responsible for storing this ion. Deferoxamine (DFO) is an iron chelating agent that helps with iron overload by forming a stable 1:1 complex with Fe. Therefore, the aim of this study is to analyze the role of Fe in the osmoregulation process. DFO treatment induces a reduction in intracellular Fe after 1 hour at a concentration of 100 µg/mL. With this treatment, cells exhibited a reduced capacity for cell shrinkage in response to hyperosmotic stress. Furthermore, it was shown that volume recovery was also altered with different concentrations of Fe and DFO. Furthermore, DFO treatment at different concentrations did not alter cell viability. The acidocalcisome profile was also evaluated by immunofluorescence in cells treated or not with DFO. Taken together, these data reinforce the idea that Fe is important for regulating the response to hyperosmotic stress in *T. cruzi*.

Keywords: acidocalcisomes;Deferoxamine;Osmoregulation.

PV – 049 - **Iron storage and osmoregulation in *Trypanosoma cruzi***

CRISTOVAM, J.B.D.B.; CAMPOS, D.B.; FRECHIANI, G.D.J.; SILVA, A.D.S.; CAMELO, T.M.; MOTTA, M.C.M.; AUGUSTO, I.; DE MIRANDA, K.R.; VIEYRA, A.; DICK, C.F..
UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosoma cruzi is the etiological agent of Chagas disease. Iron (Fe) is an essential micronutrient for several organisms. For this protozoan, its importance extends beyond participation in various metabolic processes, as it is highly necessary for survival, growth, and virulence. Recent studies by our group have demonstrated that variations in exogenous iron levels can lead to alterations in mitochondrial function and reactive oxygen species (ROS) levels, ultimately resulting in cell death. Acidocalcisomes are acidic organelles rich in calcium, polyphosphate, and pyrophosphate. These organelles are composed of several other ions and participate in the cellular osmoregulation of *T. cruzi*. The presence of a vacuolar iron transporter in *T. brucei* and Fe in the acidocalcisomes of *T. cruzi* epimastigotes, demonstrated in previous studies, suggest that this organelle is responsible for Fe storage. Because it requires highly regulated intracellular iron disposition, the objective of this work is to study the role and dynamics of acidocalcisomes in the cellular iron storage of *T. cruzi*. The addition of exogenous Fe to the culture medium did not affect viability, increased proliferation, and restored the cell cycle of the parasites; however, a tendency for intracellular Fe accumulation was observed. Furthermore, a difference in cellular regulatory capacity was observed under hyperosmotic stress, with cells cultured with Fe supplementation not demonstrating the same capacity for cell shrinkage. Although we observed no differences in mRNA levels for a putative transporter identified as acidocalcisome vacuolar iron transporter (TcVIT), an immunofluorescence assay revealed differences in acidocalcisome content, and transmission electron microscopy showed changes in the number of these organelles. These results lead us to hypothesize that acidocalcisomes are involved in the process of cellular Fe storage and cellular osmoregulation in *T. cruzi*.
Keywords: Acidocalcisomes;Osmoregulation;Iron storage.

PV – 050 - **Deletion of glycosomal fumarate reductase impacts the energy metabolism of *Trypanosoma cruzi* epimastigotes and proliferation**

LIMA, A.L.B.[1]; VIEIRA, C.S.D.[1]; WANG, W.[2]; TARLETON, R.[2]; NOGUEIRA, N.P.D.A.[1]; PAES, M.C.[1].
1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL; 2. , UNIVERSITY OF GEORGIA, GEOGIA - UNITED STATES OF AMERICA.

Glycosomal fumarate reductase (FRDg) catalyzes the conversion of fumarate to succinate, regenerating NAD^+ inside the glycosome. This reaction is the final step of succinic fermentation in *Trypanosoma cruzi*, a pathway maintained by trypanosomatids even in the presence of Oxygen and succinate is excreted extracellularly. As FRDg is absent in mammalian cells, it represents an attractive target for the development of new therapeutic strategies against Chagas disease. In this study, we evaluated the impact of FRDg knockout (KO) on the energy metabolism of *T. cruzi* epimastigotes using the CRISPR/Cas9 system. Considering that epimastigote proliferation strongly depends on glycolysis and succinic fermentation, FRDg deletion was performed, and proliferation under two different conditions was evaluated: high-glucose medium (LIT supplemented with 10 mM glucose) and low-glucose medium (LIT). Control cells increased proliferation by 76% when incubated in high-glucose LIT. FRDg-KO growth in high glucose was reduced by 43% compared to its control in the same condition. Genetic alterations in FRDg that led to differences in proliferation did not affect hexokinase activity. These results indicate that FRDg disruption negatively affects the final steps of glucose metabolism and the high proliferation sustained by glycolysis. Succinate levels of the mutant strain, compared to the control, remained unchanged in both intracellular and extracellular environments, FRDg-KO parasites showed intracellular alanine accumulation and increased excretion, possibly reflecting metabolic adaptation. Preliminary respirometry showed decreased respiration in 5-day KO cells, which returned to control levels by day 7, indicating that FRDg deletion affects respiration, possibly due to altered $NADH/NAD^+$ balance. In conclusion, FRDg deletion impairs glucose-dependent proliferation, alters alanine metabolism, and affects respiration, highlighting its key role in *T. cruzi* epimastigote energy metabolism.
Supported by: FAPERJ, CAPES, CNPq, Cetreina-UERJ
Keywords: Trypanosoma cruzi;Fumarate reductase;Energy metabolism.

PV – 051 - **Exploring Endoplasmic Reticulum-Associated Degradation (ERAD) in Leishmania infantum**

TORRES, C.[1]; MANCIN, G.D.C.[1]; DE CORREIA, C.R.S.T.B.[1]; GOMES, E.[2]; DE CASTRO, T.D.O.[2]; REGATIERI, W.K.P.[2]; GOMES, M.D.[1]; TEIXEIRA, F.R.[1].
1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DE SÃO CARLOS, SÃO CARLOS - SP - BRAZIL.

Endoplasmic Reticulum-Associated Degradation (ERAD) is a key eukaryotic pathway that removes misfolded proteins from the endoplasmic reticulum and directs them for degradation in the proteasome. It comprises ERAD-L, -M, and -C, which target luminal, transmembrane, and cytosolic substrates, respectively, each mediated by a distinct ubiquitin ligase complex. The central component of ERAD-L and -M is E3 ligase Hrd1. This E3 has a transmembrane domain that retrotranslocates substrates to be ubiquitinated by the RING finger domain on the citosolic side. ERAD pathway remains unexplored in Leishmania parasites that cause leishmaniasis. Owing to host alternation, ERAD is crucial for parasitism, mitigating ER stress caused by different environmental conditions during the life cycle. Thus, Leishmania ERAD machinery, comprising the E3 Hrd1 function, remains unexplored. Leishmania infantum encodes a Hrd1 ortholog (LINF_150022200, LinfHrd1) sharing respectively 20% and 26% amino acid identity with S. cerevisiae and H. sapiens Hrd1. LinfHrd1 is structurally similar to both orthologs showing RMSD values of 12 (human) and 8,5 Å (yeast). The transmembrane domain is highly conserved and the RING domain varies in each organism, explaining RMSD high values. To assess LinfHrd1 essentiality in L. infantum, the gene was knocked out (ΔLinfHrd1) using CRISPR-Cas9. ΔLinfHrd1 remained viable and exhibited slower proliferation compared to the wild type. ΔLinfHrd1 was unable to differentiate into amastigotes or proliferate at 37°C, which could affect macrophage infectivity. Current analyses include macrophage infection, cell cycle analysis by flow cytometry, and identification of ERAD components through cloning HA-tagged LinfHrd1 followed by immunoprecipitation and mass spectrometry. These results contribute with L. infantum ERAD characterization, essential for understanding the parasite biology.

Supported by: Processo FAPESP 2024/17930-5
Keywords: Endoplasmic Reticulum-Associated Degradation ERAD;Hrd1;Leishmania infantum.

PV – 052 - **Taxonomic revision of *Cyclopyxis Lobostoma*: an integrated view of morphological and molecular data in Amoebozoa.**

DOS SANTOS, M.O.; LAHR, D.J.G..
UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

This research project aims to clarify the taxonomy of the amoeba species Cyclopyxis lobostoma, native to the Cidade Universitária Forest Reserve. We intend to integrate morphological and molecular analyses to clarify the taxonomy of C. lobostoma. We have collected new samples of the species, and characterized them morphologically, using both light and scanning electron microscopy. We have finalized the morphometric analysis of 100 individuals for 5 morphometric variables, which showed: 1. Few variables could be explained simultaneously; only the relationship between shell diameter and external diameter of the aperture showed an R^2 closer to 1 (0.894); 2. There were inconsistencies in sample histograms, with at least two sample curves, indicating possible overlaps in the range of individuals analyzed; 3. PCA (Principal Component Analysis) indicated the presence of several possible distinct groups, also with incomplete representations. We are also expanding morphometric analyses and sequencing NAD9/NAD7 genic regions for phylogenetic reconstruction. The central objective of the project is to determine whether the observed morphological variation in C. lobostoma represents phenotypic plasticity within a single lineage or indicates the presence of cryptic species, that is, different lineages with similar morphologies. The hypotheses to be tested are: H0 (null hypothesis): The morphological variation is the result of phenotypic plasticity and does not justify the proposal of a new taxonomy; H1 (alternative hypothesis): The morphological variation indicates the presence of cryptic species and new taxonomic classifications should be proposed. So far, we conclude that the species description is inconsistent with nature, presenting morphotypes that can be configured as cryptic species, different genetic lineages with similar morphologies, or phenotypic plasticity, or even identical genetic lineages with different morphologies (mating types, life stages, etc.)

Supported by: PUB-USP 2024/2025
Keywords: Amoebozoa;taxonomic revision;molecular philogeny.

PV – 053 - **Unlocking the Antiparasitic Potential of Marine Microbiomes: Specialized Metabolites from Sponge-Associated Tenacibaculum mesophilum Against Trypanosoma cruzi**

LIMA, J.M.G.[1]; SILVA, V.A.[1]; OREFICE, D.P.[1]; LAGO, J.H.G.[2]; TEMPONE, A.G.[1].
1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DO ABC, SANTO ANDRÉ - SP - BRAZIL.

Marine environments, particularly those harboring bacteria associated with invertebrates, represent a promising yet underexplored reservoir of chemical diversity. These microorganisms are capable of producing structurally unique specialized metabolites with biological activities. This chemodiversity offers an attractive avenue for the discovery of novel drugs and could be explored for Chagas disease (CD). Affecting 7-8 million people, CD´s current therapy is limited in Brazil to the single drug benznidazole, underscoring the urgent need for new alternatives. The present study evaluated the anti-*T. cruzi* potential of specialized metabolites from a marine sponge-associated bacterium, isolated from the microbiota of *Amphimedon* spp., collected at a depth of 11m at Búzios Island (SP). Identification by 16S rRNA sequencing and MALDI-ToF/MS confirmed it as *Tenacibaculum mesophilum*, a Gram-negative, iridescent, rod-shaped bacterium, which was subsequently cultivated on Marine Agar and in Marine Broth. Crude extracts were obtained from solid (*Td*ExBra) and liquid (*Td*ExBrb) cultures using liquid–liquid partitioning with ethyl acetate, followed by further partitioning with hexane and water:methanol, yielding the fractions *Td*FrAcOEta and *Td*FrHexa. The *Td*FrAcOEta, *Td*FrHexa, and *Td*ExBrb, tested at 300 µg/mL against *T. cruzi* (Y) trypomastigotes, resulted in 100% parasite mortality. The EC_{50} was determined by resazurin assay and confirmed by optical microscopy, resulting in EC_{50} values of 3.9, 8.7, and 15.8 µg/mL, respectively. ^{1}H NMR analyses suggested the presence of aromatic compounds, fatty acids, and siderophores in *Td*ExBrc. This is the first report of antiparasitic activity in the genus *Tenacibaculum*, a pioneering discovery that opens new perspectives for identifying novel prototypes for CD.
Supported by: FAPESP 2024/07481-9 e FAPESP 2024/16224-3.
Keywords: Trypanosoma cruzi;Marine Bacteria;therapy.

PV – 054 - **Succinate Dehydrogenase subunit 10 deletion reveals a mitochondrial metabolic alteration, causes succinate accumulation and affects developmental progression in Trypanosoma cruzi**

DE CASTRO, G.A.C.[1]; MOREIRA, I.C.D.F.[1]; WANG, W.[2]; TARLETON, R.[2]; PAES, M.C.[1]; NOGUEIRA, N.P.D.A.[1].
1. UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL; 2. UNIVERSITY OF GEORGIA, ATHENS - UNITED STATES OF AMERICA.

Chagas disease, also known as American trypanosomiasis, is a tropical incidence zoonosis promoted by Trypanosoma cruzi. In the current scenario, the pharmacological treatments, despite their demonstrated efficacy in the acute phase, exhibit numerous adverse effects and have low efficacy in patients during the chronic phase of the disease. Therefore, understanding the molecular mechanisms that govern the peculiar energy metabolism of T. cruzi represents potentially exploitable processes for developing new therapies. Mitochondrial respiratory enzymes play a central role in energy production in aerobic organisms. Complex II, also known as succinate-dehydrogenase, is responsible for converting succinate into fumarate and often plays a pivotal role in adapting parasites in the hosts. Given its importance, we used the CRISPR/Cas9 system to produce null mutants for the subunit 10 of the T. cruzi succinate dehydrogenase (TcSDH10-KO) Brazil strain expressing Tdtomato. High-resolution respirometry assays were performed to assess whether TcSDH deletion could interfere with the parasite's bioenergetics. We observed the disruption of the SDH10 gene reduced oxygen consumption in 7-day cells, although 10-day cells did not have any statistical differences. We also analyzed intracellular and extracellular succinate to understand if the deletion may cause interference in its metabolism. TcSDH10-KO parasites showed a succinate buildup inside the parasite, suggesting less efficiency of the enzyme, which resulted in increased secretion of the succinate. Collectively, our results show that SDH10 deletion may have reduced succinate-dehydrogenase activity and impaired the mitochondrial respiration in epimastigotes.
Supported by: FAPERJ, PIBIC-UERJ, INCT-FAPERJ-CNPq
Keywords: Succinate-dehydrogenase;crispr cas9;phenotype screening.

PV – 055 - **FAZ10 at the FAZ: Structural Insights from Computational Prediction to Experimental Validation in *Trypanosoma brucei***

MOGOLLON, C.M.O.[1]; LEONARDO, D.A.[2]; IZUMI, C.[1]; ALVES, L.C.[1]; GARRAT, R.C.[2]; BAQUI, M.M.A.[1].
1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, USP, RIBEIRAO PRETO - SP - BRAZIL; 2. INSTITUTO DE FÍSICA DE SÃO CARLOS, USP, SAO CARLOS - SP - BRAZIL.

Trypanosoma brucei, the causative agent of African trypanosomiasis, relies on the flagellum attachment zone (FAZ) to maintain cell morphology and ensure proper coordination during cell division. Among the FAZ components, FAZ10 is a high-molecular-mass protein localized at the FAZ and is implicated in cleavage furrow positioning and cytoskeletal organization. Despite its recognized functional importance, the structural characteristics of FAZ10 remain largely unexplored. In this study, we focus on the FAZ10 central region as an entry point to uncover new insights into its structure and potential mechanisms of action. *In silico* analyses using AlphaFold2, MARCOIL2, and IUPRED2A revealed a low-disorder region consisting of a coiled-coil with dimeric conformation, flanked by two globular domains. To investigate this architecture, we expressed and purified the full central region (~67 kDa), the isolated coiled-coil domain (~33 kDa), and one globular domain (~11 kDa) using Ni-NTA affinity chromatography followed by size-exclusion chromatography (SEC). SEC-MALS confirmed dimer formation for both the central region and the coiled-coil domain, while the globular domain remained monomeric. Circular dichroism spectroscopy supported the predicted secondary structure, and electron microscopy revealed an elongated, filament-like structure of approximately 40nm in length. Altogether, our findings demonstrate that the central region of FAZ10 adopts a dimeric filamentous conformation driven by its coiled-coil architecture. This supports the notion that full-length FAZ10 exists as a dimer and may assemble into higher-order structures, potentially serving as a structural scaffold within the FAZ of *Trypanosoma brucei*. Such oligomeric organization is likely crucial for maintaining cytoskeletal integrity, cellular polarity, and morphogenesis. A deeper understanding of the biochemical and biophysical properties of FAZ10 is therefore essential to elucidate the architecture and function of the FAZ. Given that African trypanosomiasis remains a neglected tropical disease, targeting structural proteins like FAZ10 could also contribute to the development of novel therapeutic strategies.

Supported by: FAPESP, CAPES, FAEPA
Keywords: Flagellum attachment zone;Trypanosoma brucei;coiled coil proteins.

PV – 056 - **Is dissolved organic carbon a main factor affecting testate amoebae diversity in shallow-water restinga habitats?**

DE SOUZA, A.G.D.S.; MARTHA, T.M.R.; PAIVA, T.D.S..
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Planktonic protozoa have often been neglected as a pivotal group in energy flow in aquatic ecosystems. Due to resistant decay shells, testate amoebae are considered an important group to understand ecosystem changes. Since testate amoebae are often found in moist, organic-matter-rich environments, they can be used as tools to understand the carbon cycle as a whole. Especially in light of current climate change scenarios, unveiling the interaction of biodiversity with carbon may help planning and decision making. Dissolved organic carbon (DOC) is one of the forms in which carbon can directly or indirectly affect biodiversity in aquatic systems. Thus DOC can influence a variety of environmental parameters, changing the planktonic communities composition. Our objective was to observe the relationship of testate amoeba communities and environmental factors in aquatic ecosystems of the northern region of Rio de Janeiro, with emphasis in their relation with a broad DOC gradient found in these environments. Our initial hypothesis is that the testate amoebae community structure will respond due to its expected closer relation with organic matter concentrations and it will also respond negatively to salinity increase, due to oxidation stress. We applied multivariate statistical analyses to environmental variables (conductivity, DOC, pH, [O2]; salinity, temperature, turbidity) measured during environmental sampling campaigns conducted at Restinga de Jurubatiba (Macaé, RJ) during the year of 2017, in order to assess their influence on the diversity of testate amoebae. Our results show that DOC increase had no direct effects in community structure. On the other hand, the community composition and abundance was influenced by salinity in the shallow-water restinga habitats analyzed.

Supported by: CNPQ, CAPES
Keywords: environmental gradient;restinga;biological indicators.

PV – 057 - **lncRNA-DNA interaction and triple helix formation in *Trypanosoma rangeli***

KAWAGOE, E.K.[1]; FIGUEREDO, B.[1]; D'AGOSTINI, L.S.[1]; DE LIZ, L.V.[1]; MARTINS, C.L.[1]; GRISARD, E.C.[1]; VIEIRA, T.S.W.J.[2]; VIEIRA, R.F.D.C.[2]; WOLF, I.R.[2]; STOCO, P.H.[1]; WAGNER, G.[1].
1. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRAZIL; 2. UNIVERSITY OF NORTH CAROLINA AT CHARLOTTE, CHARLOTTE - UNITED STATES OF AMERICA.

Trypanosoma rangeli is a non-pathogenic hemoflagellate parasite that can infect mammals, including humans, as well as triatomine bugs. Like other trypanosomes, *T. rangeli* has constitutive transcription, and gene regulation occurs at a post-transcriptional level. In this context, non-coding RNAs become interesting candidates for regulating gene expression. lncRNAs are non-coding transcripts that are more than 200 nucleotides long and can regulate gene expression. In *Trypanosoma brucei*, the *Ksplice3137a* lncRNA is a key regulator of parasite growth and differentiation from slender to stumpy forms. Here, we aimed to identify lncRNAs in *T. rangeli*, examine how their expression differs between different life stages, and explore their potential to interact with DNA. For that, total RNA was obtained from culture-derived epimastigotes and *in-vitro* differentiated trypomastigotes. Raw data generated by Illumina HiSeq were processed with BWA-MEM2 and fastp, assembled with Trinity and MMseqs2, analysed by CPC2 for coding potential, quantified with Salmon, and analysed by DESeq2 for differential expression with a FDR <0.01. Differentially expressed lncRNAs were submitted to PATO to predict potential interactions and triple helix formation against the reference genome. The average number of assembled lncRNAs for epimastigotes and trypomastigotes was 22,991 and 21,367, respectively. Among these, a total of 5,833 were considered differentially expressed (55% up-regulated and 45% down-regulated). The average number of differentially expressed lncRNAs that can potentially interact with DNA to form a triple helix was 443 and 366 for epimastigotes and trypomastigotes, respectively. Furthermore, the presence of novel lncRNAs demonstrates the parasite's plasticity. The large number of unknown lncRNAs requires further analysis and represents a large data set to be explored.
Supported by: CAPES 88887.799203/2022-00, CAPES PrInt 88887.936829/2024-00
Keywords: Transcriptomics;Non-coding RNA;Trypanosomatids.

PV – 058 - **New standardized methodology for permanent slides of testate amoeba, with a focus on cell preservation.**

MARTHA, T.M.R.; DE SOUZA, A.G.D.S.; PAIVA, T.D.S..
INSTITUTO DE BIOLOGIA, UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Specimen preservation allows for the deposition of physical evidence of the taxonomic practice, in the form of voucher or types, and is widely acknowledged as mandatory for species descriptions – and likewise, are necessary for protist taxonomy too. Among protists, testate amoebae are a polyphyletic assembly of shelled amoeboid taxa distributed among the supergroups Amoebozoa, Stramenopiles and Rhizaria, of which taxonomy is often solely based on shell morphology. However, there is growing evidence that cell structures, such as nucleus, epipodia and pseudopod structure hold potentially useful, yet little explored, taxonomic information, albeit hitherto there is no standardized protocol for fixating specimens to consistently facilitate preservation and assessing of such features. Aiming to fill this gap, the present study consists of an attempt into a new methodology with focus on light microscopy. We tested, primarily, the preservation of specimens using either glutaraldehyde and Bouin fixative, with glycerin as storage medium on excavated glass slides, and sealed with Entellan. Preparations were performed with Netzellia lobostoma obtained from a freshwater pond in Rio de Janeiro. As result, we observed that the slides with glutaraldehyde better preserved the soft parts, retaining pseudopodia integrity. Further steps will include testing shell removal procedures and staining specific organellae and other cell structures.
Keywords: Preservation;Protist collection;Permanent slides.

PV – 059 - **An insight into the sialoproteome of *Triatoma costalimai* (Hemiptera, Reduviidae, Triatominae)**

SANTOS, L.D.M.[1]; DA COSTA, N.A.V.[1]; CAVALCANTE, I.D.F.[1]; DE OLIVEIRA, J.M.[1]; FONTOURA, G.D.R.S.[1]; BENTES, K.L.D.S.[1]; DE OLIVEIRA, L.S.[1]; CHARNEAU, S.O.[1]; DE GODOY, L.M.F.[2]; GURGEL-GONÇALVES, R.[1]; BASTOS, I.M.D.[1]; DE SANTANA, J.M.[1]; SANTIAGO, P.B.D.M.[1]; DE ARAÚJO, C.N.[1].
1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRAZIL; 2. FIOCRUZ, CURITIBA - PR - BRAZIL.

The triatomine bug *Triatoma costalimai* is a neglected vector capable of transmitting *Trypanosoma cruzi*, the causative agent of Chagas disease, in both domestic and peridomestic environments; yet it remains poorly studied. To feed, triatomines produce saliva rich in molecules that inhibit coagulation and modulate the host's immune response and inflammation. Proteomic analysis is a crucial tool for investigating the salivary composition of these insects. This study investigated protein expression in the salivary glands of *T. costalimai*, using wild-caught individuals maintained in a colony. The salivary extract was obtained by dissection of the glands and analyzed by mass spectrometry, with data processing performed using PEAKS software. A total of 841 proteins were identified, of which 475 were detected in multiple biological replicates. Of these, 25.2% were classified as secreted, and 74.8% as constitutive. The secreted protein category was further subdivided by function and included calicins (the most abundant superfamily), proteases (serine and metalloproteases), inositol phosphate phosphatases, and salivary apyrases. Other secreted enzymes included carboxylesterases, nucleases, ferritin, and transferrin. Several proteins of interest were identified, including protease inhibitors (Kazal, serpins, pacifistins, and a probable Kunitz), as well as proteins such as trialisin, MYS/hemolysin, antimicrobial peptides, antigen-5, odorant-binding proteins, and other secreted proteins like vitellogenin/apolipophorin, heat shock proteins, and calreticulin. This is the first study of the *T. costalimai* salivary gland proteome, providing a foundation for understanding the biology of this vector and deepening our understanding of the composition of triatomine salivary gland compounds, which could serve in the development of vector control strategies and the prevention of Chagas disease.
Supported by: FAP-DF 00193-00002600/2022-70
Keywords: Salivary gland;Vector;Mass spectrometry.

PV – 060 - **Identification of transcription start sites, cis-regulatory elements, and distinct epigenetic marks in *T. cruzi dSSRs***

LOPES, L.D.S.[1]; DE LIMA, P.L.C.[1]; PIRES, D.[2]; DA CUNHA, J.P.C.[3].
1. USP/ICB, SÃO PAULO - SP - BRAZIL; 2. FUNDAÇÃO BUTANTAN, SÃO PAULO - SP - BRAZIL; 3. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Trypanosomatids have a remarkable genomic structure: their protein-coding genes are organized into long polycistronic transcription units (PTUs) flanked by intergenic regions with divergent (d) or convergent (c) strand switch regions. The genome is partionated into distinct linear compartments harboring conserved/housekeeping genes (core) and non-syntenic and multigenic family of virulence-factors (disruptive). Recently, our group identified an asymmetric distribution of nascent transcription throughout the *Trypanosoma cruzi* genome, especially among PTUs enriched with conserved genes and virulence-associated genes. In trypanosomes, differences in mature transcript levels are largely driven by post-transcriptional mechanisms, in contrast to complex eukaryotes, where regulation predominantly occurs at the level of transcription initiation. Epigenetic marks, such as deposition of histone variant H2B.V, is enriched at dSSRs, likely marking transcription start sites (TSSs), which remain elusive in *T. cruzi*. To better understand the mechanisms associated with transcription initiation in *T. cruzi*, we aimed to identify the TSSs, as well as their possible cis-regulatory elements. To this end, we performed small RNA triphosphate sequencing (small 3pRNA-seq) to comprehensively map the distribution of nascent transcripts in the parasite. We observed an enrichment of nascent transcripts in dSSRs, supporting the notion that transcription of protein-coding genes preferentially initiates in these regions. To further explore this data, we generated *T. cruzi* lineages with tagged RNA Pol II for further ChIP-seq analysis. Using in silico tools, we identified the presence of epigenetic marks and possible cis-regulatory elements present in different classes of dSSRs. We observed that dSSRs located between disruptive PTUs differ significantly from those between core PTUs in terms of size, presence of 5-hydroxymethylcytosine, nucleosome density, and conserved motifs. These differences suggest a mechanism underlying the differential nascent activity observed between these genomic compartments. Taken together, the results obtained so far contribute to advancing our understanding of the transcriptional regulation in *T. cruzi* and provide a foundation for future studies exploring compartment-specific gene expression control in this parasite.
Supported by: FAPESP 2022/15610-8
Keywords: gene regulation;TSS;epigenetic marks .

PV – 061 - **The impact of the acetyltransferase NAT10 of *Leishmania mexicana* on gene expression and chromatin structure regulation**

ALVES, G.G.[1]; LEITE, A.B.[1]; MARAN, S.R.[1]; MONTEIRO, H.[2]; PANESSA, G.M.[3]; ALVES, C.E.G.[3]; MENESES, A.V.I.[4]; ANTUNES, L.B.[5]; FERREIRA, T.R.[6]; MASSIRER, K.[2]; PRADA, C.F.[4]; CESTARI, I.[5]; LANGLAIS, D.[7]; REZENDE, A.M.[8]; DE MEDEIROS, L.C.A.S.[9]; SACKS, D.L.[6]; CUSSIOL, J.R.[3]; MORETTI, N.S.[3].

1. UNIFESP, BELO HORIZONTE - SP - BRAZIL; 2. UNICAMP, CAMPINAS - SP - BRAZIL; 3. UNIFESP, SÃO PAULO - SP - BRAZIL; 4. UNIVERSITÉ DE MONTRÉAL, SAINT-HYACINTHE - CANADA; 5. MCGILL UNIVERSITY, MONTREAL - CANADA; 6. NIH, BETHESDA - UNITED STATES OF AMERICA; 7. MCGILL UNIVERSITY, MONTRÉAL - CANADA; 8. FIOCRUZ, BELO HORIZONTE - MG - BRAZIL; 9. FIOCRUZ, CURITIBA - PR - BRAZIL.

Leishmania spp. display a complex life cycle and rely on finely tuned gene regulatory mechanisms to survive in the distinct environments encountered in their vertebrate and invertebrate hosts. Although gene expression in these parasites is predominantly controlled post-transcriptionally, epigenetic factors and chromatin organization may also play a role. The N-acetyltransferase NAT10 catalyzes cytidine acetylation in RNA (ac^4C) and has also been implicated in modulating DNA accessibility through changes in chromatin structure, thereby influencing gene expression regulation. To elucidate the role of NAT10 in chromatin structure and cellular responses of *Leishmania mexicana* through functional and omics-based approaches we decided to do functional complementation; enzymatic activity assays; phenotyping in *L. mexicana* mutants; transmission electron microscopy (TEM); ATAC-seq and RNA-seq. We found that heterologous expression of NAT10-WT in *S. cerevisiae* partially complemented the phenotype, whereas NAT10-MUT failed to restore growth. *In vitro* assays confirmed acetyltransferase activity for the wild-type protein and residual activity for the mutant variant. In *L. mexicana*, NAT10-MUT overexpression led to reduced proliferation and marked morphological alterations compared to NAT10-WT and control lines. TEM analysis revealed increased nucleolar chromatin compaction in the control line relative to SgKO-NAT10. ATAC-seq data indicated higher chromatin accessibility in SSRs of the SgKO-NAT10 line compared to T7/Cas9. RNA-seq analysis showed that genes involved in post-transcriptional regulation, RNA processing, chromatin/epigenetic organization, DNA repair, autophagy, and protein degradation were the most affected by the mutant variant, with concomitant downregulation of metabolic and transport pathways. LmexNAT10 plays a multifaceted role in *L. mexicana*, modulating chromatin accessibility, gene expression, and cellular phenotypes. Loss or mutation of the enzyme compromises chromatin compaction, alters critical transcriptomic profiles, and reduces parasite growth, indicating that NAT10 integrates essential epigenetic and post-transcriptional pathways required for parasite adaptation.

Supported by: FAPESP (2023/02323-3)

Keywords: NAT10;Leishmania mexicana;Acetilação.

PV – 062 - **IDENTIFICATION AND CHARACTERIZATION OF A NEW CATHEPSIN-TYPE CYSTEINE PROTEASE IN *Trypanosoma cruzi***

SMELTZER, T.D.A.L.; DE CARVALHO, D.B.; LIMA, A.P.C.D.A..

UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

In mammalian cells, cysteine cathepsins are papain-like proteases that fulfill important functions in proteostasis, and are implicated in pathologies such as cancer and neurodegeneration. To date, two cysteine proteases (CPs) of the papain family have been identified in *Trypanosoma cruzi*, the cathepsin L-like protease, cruzipain, and a cathepsin B-like (TcCatB) protease. In this work, we have identified a new cathepsin-like single copy gene in the genome of *T. cruzi* Dm28, herein named TcCatT, that shares approximately 30 % similarity with mammalian cysteine cathepsins, cruzipain and TcCatB. The predicted protein sequence of TcCatT has conserved catalytic residues, as well as residues surrounding the active site which are common to most mammalian and protozoan cathepsins. In mammalian cathepsins L, B, H, S, F and K, residue 27, near the catalytic cysteine25, is Phe, while His27 was found solely in the carboxypeptidase cathepsin X and in TcCat. We have generated TcCatT null mutants in *T. cruzi* epimastigotes using CRISPR-Cas9, and generated a complemented line. The knockout mutants grow normally and complete the cycle in the mammalian cell. To characterize TcCaT, recombinant enzyme was expressed in *Leishmania tarentolae* as a fusion protein with 6X_His and affinity purified. Enzymatic assays using synthetic fluorogenic substrates showed that TcCatT accepts Arg at the P1 position, but not Phe or Val, and Phe, Gly or Arg in the P2 position. The *K*m values were 6.03 ±1.87 µM for Z-Phe-Arg-MCA and 13.5 µM for Z-Arg-Arg-MCA. Furthermore, TcCatT was well inhibited by leupeptin, but poorly inhibited by E-64, cystatin or chagasin. Our results suggests that TcCat has distinctive kinetic properties, with an inhibition profile distinct from previous characterized cathepsins.

Supported by: FAPERJ, CNPq

Keywords: cathepsin;protease;cruzi.

PV – 063 - **An insight into the hemolymph content of *Triatoma* and *Rhodnius* species (Hemiptera, Reduviidae, Triatominae)**

DE OLIVEIRA, J.M.[1]; DE OLIVEIRA, L.S.[1]; CHARNEAU, S.O.[1]; DE GODOY, L.M.F.[2]; FONTOURA, G.D.R.S.[1]; SANTOS, L.D.M.[1]; CAVALCANTE, I.D.F.[1]; DA COSTA, N.A.V.[1]; PIZUTTI, L.L.B.[1]; GURGEL-GONÇALVES, R.[1]; BASTOS, I.M.D.[1]; DE SANTANA, J.M.[1]; SANTIAGO, P.B.D.M.[1]; DE ARAÚJO, C.N.[1].
1. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRAZIL; 2. INSTITUTO CARLOS CHAGAS / FUNDAÇÃO OSWALDO CRUZ, CURITIBA - PR - BRAZIL.

Triatomines are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. The immune system plays a fundamental role in protecting the vector against parasite infection. Tissues involved in the immune response include the gut, the fat body, and the circulatory system. Hemolymph serves as a circulatory fluid that contains immune components. Despite the importance of this fluid, characterization of its molecules has been limited. To address this, we performed a proteomic analysis of the hemolymph of four triatomine species: *Rhodnius prolixus, Rhodnius neglectus, Triatoma infestans*, and *Triatoma costalimai*, and generated protein profiles for each to identify the primary protein components of the immune system and their potential roles in defense. The results revealed a diversity of proteins related to melanization, the primary defense mechanism of insects, as well as antimicrobial peptides, proteins involved in pathogen recognition and signaling pathway activation, antioxidant activity, iron homeostasis, and coagulation. Analysis of protein sequences among the species revealed significant differences in protein composition. The striking differences in protein sequences between the two genera limited the quantitative analysis of the study, highlighting the understudied nature of the hemolymph compositions of these organisms. Also noteworthy were proteins from the lipocalin family, which are known for their role in binding and transporting small hydrophobic molecules. These proteins have been extensively studied in salivary glands, but their function in hemolymph remains unknown. Here, we present the proteomic characterization of hemolymph from four species of triatomines and discuss their potential immunological importance. For two of them, *R. neglectus* and *T. costalimai*, this is the first proteomic analysis. Furthermore, hemolymph may represent a source of bioactive molecules, such as antimicrobial peptides, that could have biotechnological applications.

Supported by: FAPDF FAP-DF 00193-00002600/2022-70
Keywords: Proteome;Haematophagous arthropods;Trypanosoma cruzi.

PV – 064 - **STUDY OF DNA REPAIR MECHANISMS AND DORMANCY PHENOTYPE IN *Trypanosoma cruzi*: A PROTEOMIC APPROACH**

DE SOUZA, F.H.[1]; DE ALMEIDA, R.F.[1]; RESENDE, B.C.[2]; MORINI, F.S.[1]; MATIAS, L.H.[1]; BATISTA, M.[1]; PAVONI, D.[1]; RAMOS, A.S.P.[1]; FRAGOSO, S.P.[1]; MACHADO, C.R.[2]; MARCHINI, F.K.[1]; DE GODOY, L.M.F.[1].
1. INSTITUTO CARLOS CHAGAS-FIOCRUZ/PARANÁ, CURITIBA - PR - BRAZIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

Trypanosoma cruzi, the causative agent of Chagas disease, relies on DNA damage response (DDR) pathways to maintain genomic integrity, enabling survival within host cells, treatment resistance, entry into dormancy and progression to chronic infection. In eukaryotes, DDR is tightly regulated by signaling and epigenetic mechanisms, including post-translational modifications (PTMs) of histone (hPTMs) and non-histone proteins, with phosphorylation serving as a key regulatory element. Although DDR in *T. cruzi* has been documented, the specific molecular effectors and the roles of phosphorylation and hPTMs in this context remain poorly defined. In this study, we employed integrated proteome, phosphoproteome, and hPTM profiling to investigate *T. cruzi* responses to genotoxic stress and dormancy. High-resolution LC-MS/MS was performed on wild-type and mutant parasites deficient in homologous recombination/replication stress response, following exposure to different genotoxic agents. Preliminary results revealed numerous regulated proteins and hPTM sites differentiating wild-type from DDR-deficient parasites, as well as proteome modulation in dormant parasites post-gamma radiation. Phosphoproteome and cisplatin-response analyses are ongoing. These findings will provide a comprehensive view of DDR-associated and dormancy pathways, advancing the understanding of epigenetic regulation in *T. cruzi* and supporting the identification of molecular markers and therapeutic targets, including candidates for epigenetic drug development against Chagas disease.

Supported by: PEP ICC-008-FIO-21-2-38/INOVA DDS VPPCB-005-FIO-21-2-31 Financiador: Fiocruz
Keywords: DDR;Dormancy;Proteomics.

PV – 065 - **Functional characterization of the adaptor protein complex 4 (AP-4) of Trypanosoma cruzi**

MATIAS, L.H.; MORINI, F.S.; FRAGOSO, S.P.; RAMOS, A.S.P..
INSTITUTO CARLOS CHAGAS FIOCRUZ PARANÁ, CURITIBA - PR - BRAZIL.

Adaptor protein (AP) complexes are essential regulators of vesicle trafficking in eukaryotic cells, mediating cargo selection and the recruiting coat and accessory proteins during vesicle formation. Five AP complexes (AP-1 to AP-5) have been described, coordinating transport through the endocytic and secretory pathways. Among trypanosomatids of medical relevance, Trypanosoma cruzi, the etiological agent of Chagas disease, is unique in possessing AP-1 to AP-4, whereas AP-5 has not been identified in these parasites. Functional studies of AP-1 in T. cruzi by our group revealed its involvement in parasite differentiation, infectivity, and cruzipain trafficking to reservosomes, yet the roles of the other AP complexes remain largely unknown. Interestingly, unlike in higher eukaryotes, immunoprecipitation of T. cruzi clathrin-associated proteins indicated that AP-4 interacts with clathrin, indicating a divergent and potentially specialized function in vesicle trafficking. To functional characterize AP-4, we generated a T. cruzi null knockout of the AP4-ε subunit. Scanning and transmission electron microscopy revealed alterations in cell shape and ultrastructure, including partial Golgi disaggregation. Endocytosis rates of transferrin and BSA, measured by flow cytometry, were reduced in Tcap4-e (-/-) mutant cells. Ongoing experiments aim to determine the expression and subcellular localization of AP-4 across different stages of the parasite's life cycle and assess the impact of AP-4 loss on differentiation and host-cell infectivity. Notably, the absence of AP-1 does not interfere with the transport of mucins and trans-sialidases through the secretory pathway, raising the possibility that AP-4 may be responsible for mediating the intracellular transport of these key virulence factors, which is also under investigation.

Supported by: PEP ICC-008-FIO-21-2-38
Keywords: Adaptor protein complex 4 (AP-4);Trypanosoma cruzi;Vesicle trafficking.

PV – 066 - **Characterization of a Trypsin-Like Serine Peptidase from the Saliva of the Triatomine *Rhodnius neglectus***

CAVALCANTE, I.D.F.; SANTOS, L.D.M.; FONTOURA, G.D.R.S.; BENTES, K.L.D.S.; DE OLIVEIRA, J.M.; BASTOS, I.M.D.; DE SANTANA, J.M.; SANTIAGO, P.B.D.M.; DE ARAÚJO, C.N..
UNB, BRASILIA - DF - BRAZIL.

Triatomines, vectors of the protozoan *Trypanosoma cruzi*, obtain a blood meal by piercingsmall blood vessels and ingesting the blood that flows to the feeding site, which triggers various host responses. During this process, triatomines release a complex mixture of pharmacologically active compounds at the bite site, which overcome the host's hemostasis, inflammation, and immune response, facilitating blood intake. A salivary gland transcriptome study demonstrated that the transcripts of a putative trypsin-like serine peptidasegene from *Rhodnius neglectus* were present in the salivary glands of this species of triatomine bug. Here, we produce and characterize this salivary protein belonging to the trypsin-like serine peptidase family. The recombinant protein was obtained in a soluble bacterial fraction; SDS-PAGE and Western blotting confirmed its expression. Preliminary activity tests showed that rRnTryp was unable to hydrolyze the fluorogenic substrate Z-Phe-Arg-AMC, a substrate for kallikrein, which promotes vasodilation. Enzyme activity assays are ongoing. A serine peptidase with trypsin-like activity was also identified in the saliva of another species of triatomine, *Triatoma infestans*. The release of members of the serine peptidase family at the bite site of triatomines suggests that these proteins play a role in blood feeding and vector-host interactions.

Supported by: Agência financiadora – FAPDF Número do projeto - FAP-DF 00193-00002600/2022-70
Keywords: Vector;Salivary proteins;Chagas disease.

PV – 067 - **Natural coinfections of Leishmania infantum and other vector-borne pathogens in dogs**

MARTINS, C.L.[1]; FIGUEREDO, B.[1]; ROSAR, A.D.S.[1]; SILVA, A.C.D.S.[2]; PHILIPPE, E.J.W.[1]; WAGNER, G.[1]; QUARESMA, P.F.[1]; GRISARD, E.C.[1]; STEINDEL, M.[1]; VIEIRA, T.S.W.J.[2]; VIEIRA, R.F.D.C.[2]; STOCO, P.H.[1].
1. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANÓPOLIS - SC - BRAZIL; 2. UNIVERSITY OF NORTH CAROLINA AT CHARLOTTE, CHARLOTTE - UNITED STATES OF AMERICA.

Canine visceral leishmaniasis (CVL), caused by *Leishmania infantum,* is a severe zoonosis in southern Brazil, a region with diverse arthropod vectors and atypical transmission dynamics. Coinfections with other vector-borne pathogens (VBPs) are frequent and may affect CVL diagnosis, treatment, and disease progression. This study investigated coinfections in dogs naturally infected with *L. infantum* and assessed the possible association between parasite load and the presence of other pathogens. Ninety-six spleen samples from dogs seropositive for *Leishmania* spp. were collected during necropsy between 2010-2020 in Florianópolis and were tested for a panel of VBPs by PCR and qPCR, with species confirmation by amplicon sequencing of positive samples. *L. infantum* identification was confirmed by PCR-RFLP and ITS1 sequencing, and splenic parasite load was quantified by qPCR. Overall, 26% (25/96) of dogs were coinfected with at least another pathogen species. The most frequent coinfections were *Anaplasma* spp./*E. canis* (13.54%), followed by *E. canis* (5.21%), *Rangelia vitalli* (2.8%), *Mycoplasma* spp. (1.04%), and *Hepatozoon* spp. (1.04%). Three dogs had multiple coinfections. The mean parasite load was higher in dogs with CVL (908,316.4 parasites/1000 cells) than in coinfected CVL dogs (418,530.9), but without statistical association (Mann-Whitney test, p = 0.4002). No correlation was observed between parasite load and the number or species of coinfecting pathogens (Spearman correlation, p = 0.4282). Dogs evaluated in 2020 showed the highest coinfection frequency (9/30) and pathogen diversity (4/7); however, no significant temporal trend was observed (p = 0.292). These findings reveal the occurrence of multiple VBPs in *L. infantum*-infected dogs in Florianópolis and highlight the need for continuous molecular and epidemiological surveillance involving domestic and wild animals as well as vectors.
Supported by: Capes, FAPESC,Capes/PDSE 88881.982885/2024-01
Keywords: Canine visceral leishmaniasis;vector-borne pathogens;zoonotic diseases.

PV – 068 - **Clinical and epidemiological profile of malaria patients reported in PubMed case reports - a systematic review**

LOPES, D.; DE JESUS, T.S.; CHARLES, S.J.; PISA, I.T.; LOPES, L.R..
UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

This systematic review aimed to characterize the clinical, diagnostic, therapeutic, and epidemiological profiles of malaria patients described in case reports indexed in PubMed. Following PICOS and PRISMA guidelines, we included studies reporting individual cases with confirmed malaria diagnosis, excluding series with more than five patients, non-human studies, and reports lacking essential clinical information. Literature searches were performed in PubMed using predefined terms, and data extraction was supported by artificial intelligence tools (ChatGPT and Google Gemini) to identify signs, symptoms, diagnostic methods, treatments, and epidemiological information, with all outputs verified through manual review of the full texts. A total of 176 articles were analyzed, comprising 198 confirmed cases and 14 indirectly related individuals. Most patients were male (67.8%), with a wide age range, and 4.64% died. Infections were predominantly acquired in endemic countries, especially in Africa and Asia, although a substantial proportion were diagnosed in non-endemic regions, indicating the relevance of imported cases. Plasmodium falciparum was the most frequent species (62.3%), followed by P. vivax (25.1%), with occasional co-infections and rare species. Fever (99.4%), thrombocytopenia (58.5%), anemia (58.0%), headache (53.4%), and chills (45.5%) were the most common clinical findings. Peripheral blood smear was the primary diagnostic method (96.0%), complemented by complete blood counts, serology, rapid tests, and PCR. Artesunate (48.9%) was the most frequently prescribed antimalarial, often combined with other agents and supportive measures. Case reports, combined with AI-assisted data extraction, provided valuable insights into rare presentations, complex diagnoses, and individualized treatment strategies for malaria.
Keywords: Systematic review;Case reports;Malaria.

PV – 069 - **Do Polo-like Kinase and 9-1-1 Checkpoint complex modulate Genome Plasticity and the maintenance in Leishmania?**

GONZAGA, B.S.; BLACK, J.A.; TOSI, L.R.O..
UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL.

The protozoan parasite *Leishmania* has a remarkably plastic genome, exemplified by the routine detection of gene amplifications, aneuploidy, ploidy mosaicism and chromosomal rearrangements. These features are likely driven by an atypical replication program defined by few active origins, persistent subtelomeric replication and peculiar checkpoint factors that may orchestrate adaptations under stress. Thus, activation of cell cycle checkpoints is essential to both prevent replication collapse and moderate this inherent genome instability; despite their importance, we understand little of their functionality. To address this deficit, our laboratory studies cell cycle checkpoint factors in *Leishmania*, including the 9-1-1 complex, a structural sensor of damaged or misreplicated DNA, and Polo-like kinase (PLK), a functionally divergent kinase in trypanosomatids that acts during basal body duplication, flagellar biogenesis and cytokinesis. Recently, PLK has been shown as a moderator of 9-1-1 activities through the phosphorylation of Rad9, a 9-1-1 subunit. Altogether, these findings underscore the relevance of investigating factors such as 9-1-1 and PLK to understand how different pathways integrate to balance genome plasticity with cell cycle signalling. We have shown that the *Leishmania* 9-1-1 subunit Rad1 exhibits unique subcellular distribution dynamics, suggesting functions beyond those observed in model organisms (doi.org/10.1016/j.exppara.2023.108639). Therefore, to further investigate these factors in *L. major*, we generated parasite cell lines edited by CRISPR/Cas9 expressing Rad1 and PLK fused to an N-terminal 3xMyc epitope, enabling analysis of their expression and subcellular localisation after DNA damage or replication stress. A better understanding of these signalling factors can elucidate the regulatory axes in which checkpoint mechanisms not only preserve genome integrity but also sustain plasticity in *Leishmania*.

Supported by: FAPESP 2024/07364-2
Keywords: Replication checkpoint;Rad1;Polo-like Kinase.

PV – 070 - **Insights into the biochemical and functional role of *Trypanosoma cruzi* thimet oligopeptidase**

SILVA, J.L.B.E.; BOAVA, A.D.; CARVALHO, A.M.D.S.; CARDOSO, I.D.C.C.; DA SILVA, V.L.S.; DE MELO, F.D.S.M.; DE ARAÚJO, C.N.; DE SANTANA, J.M.; CHARNEAU, S.O.; MOTTA, F.N.; BASTOS, I.M.D..
UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRAZIL.

Proteases play a crucial role in the virulence and survival of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Thimet oligopeptidase (TOP) is a zinc-dependent metallopeptidase belonging to the M3 family that cleaves bradykinin, a vasoactive peptide that increases vascular permeability. In humans, TOP is one of the main intracellular oligopeptides processor, selecting antigens presented by the MHC-I in cases of viral infections and cancer. TOP of *T. cruzi* (TOPTc) has been identified in the secretome of the parasite in different phases of its life cycle, indicating a possible interaction with the host cell. In this context, the objective of this work was to express recombinant TOPTc and perform its biochemical and functional characterization. Among the main results, we observed that the enzyme is active towards the substrate Mca-Pro-Leu-Gly-Pro-D-Lys(Dnp) in 50mM phosphate buffer pH 7.0. TOPTc activity is dependent on zinc and the enzyme is thermophilic. Inhibition is not affected by EDTA, but the enzyme is inhibited by Cpp-Ala-Ala-Phe-pAb, with an IC50 of 54,29nM. TOPTc cleaves hormone and neuropeptides such as bradykinin, LHRH, angiotensin I and II. Western blot of parasite extracts demonstrated differential protein abundance across the three life-cycle stages, with the highest levels in epimastigotes, followed by trypomastigotes and amastigotes. A secretion assay performed on cultured epimastigotes, followed by Western blot, revealed a strong release of secreted protein into the medium. Immunofluorescence of epimastigotes showed a strong and localized concentration near the flagellar pocket region, suggesting the subcellular localization of TOPTc within this compartment. Our findings provide a characterization of TOPTc, highlighting its biochemical properties, subcellular localization, and expression pattern, offering a basis for understanding its functional role in *T. cruzi*.

Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Finance code 001, the CNPq (316892/2023-8), and Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF, Grants 00193-00002136/2023-01 and 00193-00000833/2021-57)
Keywords: Metalloprotease;Biochemical Characterization;Structural Analysis.