PV – 005 - **Effects of Conspecific Association on Feeding Parameters of *Rhodnius prolixus* Nymphs (Hemiptera: Reduviidae)**

AZEREDO, L.S.[1]; PEREIRA, M.H.[2]; GUARNERI, A.A.[1].
1. INSTITUTO RENÉ RACHOU, BELO HORIZONTE - MG - BRAZIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

Triatomines are hematophagous insects that live in colonies associated with host shelters, from which they obtain blood meals, making simultaneous feeding among conspecifics possible. Thus, group feeding, the release of large amounts of saliva during blood feeding and its anti-hemostatic activity, may potentially benefit other individuals feeding on the same host. Therefore, this study aimed to evaluate whether group feeding among conspecifics improves the feeding efficiency of *Rhodnius prolixus* nymphs across all nymphal instars. To achieve this, feeding parameters, including feeding efficiency (mg/min), blood intake (mg), and feeding time (min), were compared between nymphs feeding in groups (3 to 5 individuals) and nymphs feeding individually. The results demonstrated that feeding efficiency and blood intake did not differ significantly between grouped and isolated nymphs. However, nymphs feeding simultaneously showed reduced feeding times. Subsequently, feeding parameters were evaluated in nymphs that performed prior and subsequent feeding at the same feeding site. Nymphs feeding at sites previously exposed to conspecific blood feeding exhibited higher feeding efficiency across all evaluated nymphal instars. In addition, 1st, 2nd, and 4th instar nymphs showed greater blood intake compared to those that fed first at the site. Finally, using intravital microscopy of the host ear, the vasomotricity of peripheral blood vessels in mice subjected to one or two feeding events by 4th instar nymphs was evaluated. Mice exposed to bites from two nymphs showed a progressive reduction in the relative area of blood vessels within the first minutes after feeding, suggesting the occurrence of a vasoconstriction induced by the insect bite. Together, these results contribute to the understanding vector-host interactions, as feeding efficiency is associated with increased population success, in addition to being an important determinant of *Trypanosoma cruzi* vector competence. **Supported by:** FAPEMIG **Keywords:** Insect physiology; host-vector interaction; Ingestion rate.

PV – 007 - **Exploiting Genomic Instability: R-loop Homeostasis and Replication-Transcription Collisions Drive Evolutionary Plasticity in *Trypanosoma cruzi* and Trypanosoma brucei**

AZEVEDO, L.H.C.[1]; MIRANDA JUNIOR, A.S.[1]; DOS SANTOS, W.V.[1]; SILVA, B.M.M.[1]; FERREIRA, W.R.R.[1]; SOUZA, D.D.L.[1]; DA SILVA, V.G.[1]; MCCULLOCH, R.[2]; SABBAGA, M.C.Q.B.E.[3]; FRANCO, G.R.[1]; MACHADO, C.R.[1].
1. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL; 2. UNIVERSITY OF GLASGOW, GLASGOW - SCOTLAND; 3. BUTANTAN, SÃO PAULO - SP - BRAZIL.

Trypanosoma cruzi has a compartmentalized genome, balancing structural stability with antigenic diversity via genomic instability, compensating for the lack of canonical regulation of its polycistronic transcription. *T. brucei* shows a more stable genome, with variation concentrated in subtelomeric VSG-bearing regions. To understand how this stability is regulated, we induced genotoxic stress and investigated R-loop homeostasis and repair pathways, integrating RNA-Seq, DNA-Seq, ChIP-seq and DNAscent. Transcriptionally, TcCSB+/- and TcRH1+++ show a striking compensatory cross-talk, with nearly identical profiles repressing multigene families; the sole divergent feature was RPA, more associated with CSB. These adjustments reflect real DNA instability: sequencing of TcRH1+++ and TcRad51+++ revealed massive loss of heterozygosity through biased gene conversion toward the Non-Esmeraldo-like haplotype, with copy-number contractions and the DGF-1 family bearing the highest mutational burden. Conserved regions lacked replication origins, and DNA dosage correlated negatively with differential expression. Although CSB lines grow like WT, UV revealed species-specific responses: at 1500 J, *T. cruzi* is sensitive and *T. brucei* resistant, with TcCSB+/- more sensitive than WT and TbCSB-/- only stalling ~24 h before resuming growth. In *T. cruzi* WT, 24 h post-UV, ATM and ATR were repressed in survivors, suggesting signaling shutdown and energy reallocation (induced translation, ribosome biogenesis, protein catabolism; repressed endocytosis, cell cycle, DNA replication). In *T. brucei* WT, the response was temporally dynamic, dominated by symbiont-mediated immune evasion and rising protein-phosphorylation repression, with only ATM repressed; the CSB double-knockout amplified this from a more "stressed" basal state, though TbCSB-/- alone showed minimal impact (5 DEGs). This antigenic axis reflects in the VSG monoallelic system: dominant VSG Tb427.BES40.22, best-organized BES at ESAG6 (Tb427.BES122.2), with a rapid post-UV VSG burst and increased secondary-VSG leakage in CSB. Collectively, *T. cruzi* exploits localized replication-transcription collisions in the GPDR compartment to drive evolutionary plasticity and haplotype homogenization while safeguarding its core genome, whereas *T. brucei* meets the same stress with antigenic modulation and greater resilience. **Keywords:** Trypanosomatids; R-loop homeostasis; Genomic Instability.

PV – 008 - **The role of CaMKII and MPX proteins in signaling and response to genotoxic stress in *Trypanosoma cruzi***

FERREIRA, W.R.R.[1]; MIRANDA JUNIOR, A.S.[1]; GREIF, G.[2]; CHIRIBAO, M.L.[2]; ROBELLO, C.A.[2]; MACHADO, C.R.[3].
1. UFMG, BELO HORIZONTE - MG - BRAZIL; 2. INSTITUT PASTEUR DE MONTEVIDEO, MONTEVIDEO - URUGUAY; 3. UFMG, MONTEVIDEO - MG - BRAZIL.

Trypanosoma cruzi is the causative agent of Chagas disease, a neglected tropical illness affecting millions, for which vaccines are unavailable and current treatments have significant side effects. Understanding its biology is essential for developing therapeutic strategies, particularly how the parasite adapts to stress through interconnected pathways like the DNA Damage Response (DDR) and the oxidative stress response. This study investigated these interconnections using CaMKII (protein kinase) knockout and MPX (mitochondrial peroxidase) hemiknockout parasites. Under genotoxic stress (UV-C and cisplatin), MPX-depleted cells were more sensitive, with cell death being reversed by caffeine, suggesting a signaling pathway mediated by ATM/ATR kinases. Conversely, the CaMKII knockout exhibited greater resistance to both genotoxic and oxidative stress (H2O2 and tBOOH) compared to control cells. However, infection assays revealed that while CaMKII-deficient parasites are more resilient to stress, their infectivity in Vero cells was halved (40% vs. 80% in the control). Additionally, immunofluorescence showed that MPX undergoes changes in intracellular localization after DNA damage, distributing throughout the cytoplasm within 24 hours. These findings indicate that the CaMKII protein modulates cellular signaling to balance stress resistance and infective capacity, while the mitochondrial protein MPX plays a key role in the stress response signaling network. Collectively, the data point to a refined signaling system between the nucleus and the mitochondria in *T. cruzi*, involving protein kinases and repair proteins that are critical for parasite survival and pathogenesis. **Keywords:** *Trypanosoma cruzi*; DNA repair; DDR.

PV – 009 - **Development of a dormant *Trypanosoma cruzi* model using gamma radiation: insights into DNA damage response, dormancy, and benzonidazole resistance**

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

Chagas disease, caused by *Trypanosoma cruzi*, affects 6–7 million people worldwide, being a leading neglected tropical disease. The drugs Benznidazole (BZ) and Nifurtimox reduce parasitemia but are not fully effective. Recent studies have identified non-replicative, dormant-like parasite forms that resist current treatments and sustain infection. These forms can resume replication and are associated with DNA recombination, with signaling dependent on ATM kinase for cell cycle arrest. Our group uses gamma radiation to induce DNA breaks without killing the parasite, preserving infectivity. These irradiated parasites infect cells but do not replicate, resembling observed dormant forms. The goal is to standardize a drug-testing platform targeting irradiated dormant forms using the CFSE marker (CellTrace) to track replication in amastigote and epimastigote forms. For this, trypomastigote and epimastigote forms of *T. cruzi* CL Brener and DM28c strains are irradiated at 100–500 Gy and labeled with CFSE. Trypomastigotes are placed in contact with LLC-MK2 cells for 1 hour, and infection is monitored for 72 hours with or without 10 µM BZ, using microscopy and cytometry. In epimastigotes, CFSE-labeled irradiated cells are treated with 10 µM BZ for 120 hours; every 24 hours, samples are taken for PI cell cycle cytometry, CFSE decay, and growth monitoring. Immunofluorescence against γH2AX quantifies DNA damage. Results show irradiated parasites resist BZ in both forms, whereas in non-irradiated parasites only those with strong CFSE labeling (natural dormant forms) show resistance. γH2AX intensity in epimastigotes is dose- and strain-dependent. We now intend to monitor transcription levels of irradiated parasites by tracking newborn RNAs with labeled ribonucleotides. These data will help assess the full DNA metabolism profile of these irradiated dormant cells and their use as a drug-testing platform against *T. cruzi* dormant forms. **Supported by:** APQ-05883-24 **Keywords:** *Trypanosoma cruzi*; Dormancy; Drug-resistence.

PV – 010 - **XPBz deficiency compromises bulky DNA lesion repair and reduces the infectivity of *Trypanosoma cruzi* in vitro**

FERREIRA, W.R.R.[1]; MOREIRA, J.T.[1]; MIRANDA JUNIOR, A.S.[1]; ROBELLO, C.A.[2]; GREIF, G.[3]; CHIRIBAO, M.L.[3]; FRANCO, G.R.[1]; MACHADO, C.R.[4].
1. UFMG, BELO HORIZONTE - MG - BRAZIL; 2. INSTITUT PASTEUR DE MONTEVIDEO, MONTEVIDEO - URUGUAY; 3. INSTITUTO PASTEUR, MONTEVIDEO - URUGUAY; 4. UFMG, DIVINÓPOLIS - MG - BRAZIL.

Trypanosoma cruzi, the etiological agent of Chagas disease, is characterized by having a unique and complex mitochondrion, making it a singular model for the study of eukaryotic biology. Among the essential mechanisms for its survival and adaptation to hosts, the nucleotide excision repair (NER) pathway stands out, being responsible for processing bulky DNA lesions. The present study investigated the functional role of the XPBz protein, a central component of the NER pathway, in the response to different genotoxic and oxidative stresses. To this end, XPBz knockout lineages were generated using the CRISPR/Cas9 technique and subjected to challenges with UV-C radiation, cisplatin, hydrogen peroxide, and tBOOH. The results demonstrated that the absence of XPBz confers increased sensitivity to UV-C radiation and cisplatin one hour after treatment, when compared to the Dm28c control lineage. Interestingly, this early cell death was reversed by caffeine pretreatment, which suggests a regulation mediated by the activation of ATM/ATR kinases in the DNA damage response (DDR) pathway. In contrast, the XPBz knockout did not significantly alter parasite survival under oxidative stress, indicating a functional specialization of the protein for bulky genomic lesions. Additionally, it was observed that the deletion of XPBz compromises the infective capacity in Vero cells, reducing it from 80% (control) to 60%. Taken together, the data indicate that the XPBz protein plays a fundamental role not only in the repair of critical genome damage but also in the modulation of cell death signaling and the efficacy of the *T. cruzi* biological cycle, consolidating the interconnection between repair systems and the pathogenesis of the parasite. **Supported by:** Apto. 202 **Keywords:** *Trypanosoma cruzi*; NER; XBPz.

PV – 011 - **R-loops, RNaseH1, RAD51, and genomic stability in *Trypanosoma cruzi***

MIRANDA JUNIOR, A.S.; BERTOLDO, W.R.; AZEVEDO, L.H.C.; RESENDE, B.C.; FERREIRA, W.R.R.; DOS SANTOS, W.V.; MOREIRA, J.T.; FRANCO, G.R.; MACEDO, A.M.; MACHADO, C.R.
UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

Trypanosoma cruzi faces different stress conditions throughout its life cycle. The parasite's ability to adapt to such adverse circumstances depends, at least in part, on the balance between genetic variability and DNA stability. The epigenetic factors regulating these processes remain poorly understood. Throughout the genome, structures composed of a DNA:RNA hybrid and a displaced single-stranded DNA molecule may form. These three-stranded nucleic acids, termed R-loops, participate in pathways that safeguard DNA, whereas an imbalance in their levels may lead to genomic instability. Given the polycistronic transcription pattern in *T. cruzi*, R-loops may be abundant in this parasite and influence genome stability. To evaluate this, we generated constitutive RNaseH1-overexpressing parasites (TcRH1+), since RNaseH1 is a ribonuclease that resolves R-loops. R-loop levels were measured by immunofluorescence using the S9.6 antibody, which detects DNA:RNA hybrids, while changes in DNA content and genome composition were analyzed by flow cytometry and short-read DNA sequencing. Approximately 70–80% of wild-type parasites (CL Brener) displayed focal and punctate R-loop labeling overlapping nuclear DNA, whereas TcRH1+ parasites showed only 9–10% labeling. After several culture passages, TcRH1+ parasites exhibited reduced relative DNA content (~0.86). A similar pattern was observed in parasites overexpressing the homologous recombination recombinase RAD51 (TcRAD51+), which showed a relative DNA content of 0.78 and only ~20% of S9.6-positive parasites, suggesting a possible non-canonical role of RAD51 in R-loop resolution. DNA sequencing of TcRH1+ and TcRAD51+ parasites confirmed the observed DNA loss, mainly affecting multigene family regions. Interestingly, such losses occurred in the same genomic regions in both lineages ($R^2 = 0.95$). Together, these data suggest that RNaseH1 and RAD51 are involved in controlling R-loop levels, with implications for *T. cruzi* genome stability. **Supported by:** CAPES, CNPq, FAPEMIG **Keywords:** R-loops; RNaseH1; RAD51.

PV – 012 - **Functional characterization of ATR and ATM kinases in the response to DNA damage in *Trypanosoma cruzi***

DOS SANTOS, W.V.; NETO, A.D.M.R.R.; MIRANDA JUNIOR, A.S.; RESENDE, B.C.; AZEVEDO, L.H.C.; FRANCO, G.R.; MACEDO, A.M.; MACHADO, C.R.
UFMG, BELO HORIZONTE - MG - BRAZIL.

The DNA damage response (DDR) is essential for maintaining genomic stability in *Trypanosoma cruzi*. Among the main regulators of this response are the kinases ATR (ATM and Rad3-related) and ATM (Ataxia Telangiectasia Mutated), involved in signaling replicative stress, transcriptional stress, and DNA double-strand breaks. In this study, hemiknockout cells were generated for the TcATR and TcATM genes. Cassette integration was confirmed by PCR, RT-qPCR, and next-generation sequencing (Illumina), demonstrating reduced transcript levels and altered responses to genotoxic agents. ATR hemiknockout cells showed increased resistance to UV radiation, suggesting that ATR is mainly associated with signaling cell death induced by transcriptional blockage. In contrast, ATM hemiknockout cells did not show significant alterations after UV exposure. Since UV-induced lesions can be partially tolerated through translesion synthesis, the results obtained with cisplatin, which also blocks transcription but generates more complex lesions, suggest that the remaining ATR allele may partially compensate for gene loss. In ATM hemiknockout cells, the increased sensitivity may be associated with failure to recruit RAD51 for R-loop resolution, favoring cell death signaling. After gamma radiation treatment (500 Gy), ATR hemiknockout cells exhibited early cell death between 48–96 h, corresponding to the period of global transcriptional shutdown previously described for the parasite. In contrast, ATM hemiknockout cells remained active for longer but started to die between 96–120 h, suggesting failure in DNA repair and persistent damage. Together, these results indicate that ATR and ATM play distinct and complementary roles in the adaptation of *T. cruzi* to genotoxic stress. **Supported by:** FAPEMIG **Keywords:** *Trypanosoma cruzi*; ATR (ATM and Rad3-related); ATM (Ataxia Telangiectasia Mutated).

PV – 013 - **The use of recombinant DNA technology for the heterologous expression of TcRH1: creating tools for the study of *Trypanosoma cruzi***

COSTA, P.H.D.C.; RESENDE, B.C.; MACHADO, C.R.
UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas disease, a neglected tropical disease that affects over 7 million people worldwide, primarily in Latin America (WHO, 2025). This parasite relies on a sophisticated DNA repair machinery and has been described as having a high rate of genomic integrity recovery after exposure to genotoxic agents, although such mechanisms have not been fully elucidated. In this sense, RNase H1 (RH1) is a ribonuclease that degrades the RNA component in R-loops, i.e., RNA:DNA hybrids, which can be generated by RNA polymerase stalling and whose accumulation causes genomic instability, mainly through dsDNA breaks, and can lead to the cell death of the protozoan. Therefore, this work aims, through recombinant DNA technology, to obtain pure TcRH1 for antibody production and in vitro functional assays, which will allow a better understanding of its functions in the parasite's DNA repair pathways, especially in TC-NER. To design the primer pair used in the PCR, the genomic sequence was retrieved from the TriTrypDB database, and primer quality analysis was performed using the OligoAnalyzer program. The PCR used the genome extracted from the epimastigote form of the CL Brener strain as the DNA template for the amplification of TcRH1, followed by amplicon purification. Then, the insert and the pET-28a-TEV plasmid vector were subjected to a double digestion step, using the 5' NcoI and 3' XhoI restriction enzymes, and, subsequently, in the vector:insert ligation, the T4 DNA Ligase enzyme was used according to the manufacturer's protocol, along with an additional 18 h (overnight) of incubation to ensure ligation. Future steps include confirming the formation of the pET-28a-TcRH1 constructs and expressing the proteins in the most suitable bacterial system. **Supported by:** CAPES, CNPq, FAPEMIG **Keywords:** RNase H1; *Trypanosoma cruzi*; Recombinant protein.

PV – 014 - **Fourier Transform Infrared Spectroscopy enables differentiation of diverse Trypanosomatids**

BUENO, G.C.[1]; MORAES, G.L.P.[1]; MACHADO, F.C.[1]; REIS, A.H.[1]; PELAES, L.A.G.[2]; MORETTI, N.S.[1]; GRISARD, E.C.[3]; SCHENKMAN, S.[1].

1. UNIVERSIDADE FEDERAL DO ESTADO DE SÃO PAULO, SAO PAULO - SP - BRAZIL; 2. BRUKER DALTONICS GMBH & CO. KG, BREMEN - GERMANY; 3. UNIVERSIDADE FEDERAL DE SANTA CATARINA, FLORIANOPOLIS - SC - BRAZIL.

Trypanosomatids comprise a diverse group of flagellated protozoa that includes species of major medical importance. Despite significant advances in molecular tools, understanding the biochemical differences among species and closely related phylogenetic groups remains a major challenge. In this context, Fourier-transform infrared (FTIR) spectroscopy has emerged as a promising approach for cellular characterization, enabling the acquisition of biochemical fingerprints associated with the molecular composition of organisms. FTIR spectroscopy provides a comprehensive analysis of cellular components, generating spectral profiles capable of revealing phenotypic differences among phylogenetically related organisms. Therefore, the present study aimed to investigate the potential of FTIR spectroscopy for the characterization and discrimination of different trypanosomatid species. To achieve this, several trypanosomatid species were cultivated and analyzed by FTIR spectroscopy. Metabolic variations were evaluated under different growth conditions, including differences in growth dynamics, the presence or absence of fetal bovine serum, and distinct culture media compositions. Spectral data were subsequently subjected to multivariate analysis to assess interspecies variability among *Trypanosoma cruzi*, Trypanosoma rangeli, Trypanosoma brucei, and Leishmania spp. Linear discriminant analysis (LDA) was performed using the lipid spectral region. FTIR analysis revealed distinct spectral signatures and the formation of well-defined clusters in the multivariate analysis. Notably, Leishmania spp. and T. rangeli formed clusters clearly separated from T. cruzi strains, demonstrating detectable biochemical differences among these organisms. These findings highlight the strong discriminatory power of FTIR spectroscopy and indicate the presence of species-specific structural and biochemical characteristics. **Supported by:** CNPq - Processo: 132288/2026-5 **Keywords:** Trypanosomatids; Fourier-transform infrared spectroscopy; Species discrimination.

PV – 015 - **How do *Leishmania* cells fuse? A functional and mechanistic dissection of HAP2-1 and HAP2-2.**

VOIGT, F.H.[1]; LAHR, D.J.G.[2]; REY, F.[3]; CATTA-PRETA, C.M.C.[1].

1. DEPARTMENT OF PARASITOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. DEPARTMENT OF ZOOLOGY, INSTITUTE OF BIOSCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO - SP - BRAZIL; 3. INSTITUT PASTEUR, UNIVERSITÉ PARIS CITÉ, STRUCTURAL VIROLOGY UNIT, CNRS UMR3569, PARIS - FRANCE.

Natural hybrid genotypes and experimental hybridization in sandflies show that *Leishmania* parasites undergo genetic exchange through fusion of parental cells. HAP2 is a conserved gamete fusogen that mediates membrane merger during sexual reproduction across eukaryotes. *Leishmania* encodes two HAP2 paralogs, HAP2-1 and HAP2-2, with asymmetric functions: HAP2-1 is required in only one parental cell, whereas HAP2-2 is required bilaterally. The molecular basis of this asymmetry remains unknown. To address this question, we combined comparative sequence and phylogenetic analyses across Euglenozoa and other eukaryotic and archaeal lineages. A maximum-likelihood tree (RAxML-NG, Q.pfam+R7, 200 bootstrap) resolved well-supported HAP2-1 and HAP2-2 clades restricted to *Leishmaniinae*, with faster divergence in HAP2-2; *Trypanosoma* retained a single ortholog, consistent with a *Leishmaniinae*-specific duplication. The AlphaFold3 server (AF3) predicted HAP2-1 in its characteristic post-fusion trimeric conformation, identifying the membrane-insertion loop (containing the triad L226/F283/L267), inter-protomer contacts stabilizing the post-fusion hairpin, and an ionic layer formed by intra-protomer salt bridges. For HAP2-2, in contrast, AF3 could not predict a post-fusion trimer but only a monomer in a pre-fusion conformation with sparse, partially exposed hydrophobic residues (F258/F260). This observation suggests that HAP2-2 may lack fusogenic activity and instead acts as an accessory partner to HAP2-1. To test this, we are mutating residues predicted to interact with the membrane. Mutants will be tested in vitro for fusion rescue in null-mutant backgrounds, then validated in vivo through sand-fly co-infection and selected by double drug resistance. Wild-type and mutant re-expression lines are under construction, and interaction profiling will test whether HAP2-2 binds HAP2-1. This work aims to understand how HAP2-1 and HAP2-2 cooperate to induce cell-cell fusion in *Leishmania*. **Supported by:** FAPESP 2026/15621-0 **Keywords:** HAP2; cell-cell fusion; Leishmania.

PV – 016 - **A telomere-to-telomere, haplotype-phased genome of *Leishmania donovani* Ld1S reveals the structural basis of genome plasticity**

COKELAER, T.; DA FONSECA, J.P.; LEGUEN, C.; SPÄTH, G.F..
INSTITUT PASTEUR, PARIS - FRANCE.

OBJECTIVES. Lacking classical transcriptional control, Leishmania depends on genome plasticity - aneuploidy, gene copy number variation, haplotype diversity - for dosage-dependent expression and adaptation. This unusual mode of regulation depends on genomic compartments difficult to assemble or detect - including tandem arrays, chromosome ends and centromeres - and on haplotypes usually merged into a single consensus. We aimed to build a gapless, telomere-to-telomere (T2T), haplotype-aware reference for the Sudanese strain Ld1S, and to use it to dissect the architecture underlying the instability of its genome. **RESULTS.** We generated a gapless 34.36 Mb genome with all 36 chromosomes and a complete maxicircle using Nanopore sequencing. Hi-C contact maps and uniform read coverage supported the integrity of all chromosomes, and 99.6% of the Euglenozoa core genome was recovered. The assembly resolves 72 chromosome ends, providing the first complete view of genomic compartments. This assembly revealed three architectural features underlying genome plasticity. First, we mapped the centromeres, using four independent methods (Hi-C, base J, sequence composition and deep learning). They are gene-free, usually at strand switches, and divergent between chromosomes. Their sequence composition correlated with chromosome ploidy and karyotypic instability. Second, the genome contains abundant G-quadruplexes. They exceed levels in T. brucei or yeast, are four-fold enriched in intergenic DNA, and accumulate at centromeres and within GP63, amastin and kinesin gene arrays. Their distribution suggests replication-associated copy number variation, providing a mechanism for dosage plasticity. Phasing resolved a mosaic of homozygous and heterozygous tracts, revealed multi-allelic loci within antigenic and drug-resistance gene families, and identified 66 kb of asymmetric copy number variation on chromosomes 12, 31 and 34 affecting K39 kinesin and trans-sialidase loci. Together these structural features broaden the parasite's adaptive landscape driving gene dosage-dependent fitness gain**s. CONCLUSIONS.** This first T2T genome of Ld1S links genome plasticity to a defined architecture. Centromere organization correlates with chromosome ploidy and stability, G4-rich regions highlight loci prone to copy number variation, and haplotype resolution reveals sequence and dosage differences at antigenic and drug-resistance genes. Together these features explain adaptation in Leishmania. **Supported by:** ERC Synergy project DecoLeishRN, Grant agreement ID: 101071613 **Keywords:** Leishmania donovani; telomere-to-telomere assembly; genome plasticity.

PV – 017 - **Plasmogamy in testate amoebae: Identifying nuclear fates through fluorescence microscopy**

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

Science classifies life into three domains: Archaea, Bacteria, and Eukarya. Testate amoebae belong to the domain Eukarya. Studies on the evolution of sex and meiosis in unicellular eukaryotes are an emerging field, and one of the least understood phenomena in these organisms is plasmogamy (cytoplasmic fusion), which remains poorly characterized in the literature. In a previous project, we were able to experimentally identify photoperiod as one of the environmental triggers for the occurrence of this phenomenon in *Arcella uspiensis*, a species of testate amoeba. At present, we are able to induce this phenomenon in our amoeba cultures, allowing us to investigate in greater detail what occurs in these organisms during plasmogamy. Plasmogamy is considered in the literature to be indirect evidence of sexual reproduction. Therefore, we aim to identify direct evidence of sexual reproduction, specifically karyogamy and meiosis, through fluorescence microscopy using dyes such as DRAQ5 . In this way, we will be able to determine whether plasmogamy in *Arcella uspiensis* is associated with the presence of a sexual life cycle in these organisms. Most individual amoebae analyzed so far possess two or three nuclei, whereas those with more than three generally show an aberrant morphology and increased size compared to typical *Arcella uspiensis*. All analyzed clusters contained amoebae with more than three nuclei, suggesting a possible association between aberrant morphology, increased size, and plasmogamy. DRAQ5 also strongly stained the nucleolus, which was unexpected since it is a DNA intercalating dye, while the nucleolus is generally described as being predominantly composed of ribosomal RNA. This project has the potential to provide new insights into the reproduction and life cycle of these organisms, while also contributing to the advancement of knowledge in evolutionary protistology. **Supported by:** USP - PUB – 5355 **Keywords:** Plasmogamy; Kariogamy; fluorescence microscopy.

PV – 018 - **Analyses of *Plasmodium falciparum* diacylglycerol acyltransferase (*Pf*DGAT) functions for parasite proliferation and virulence.**

YOSHIDA, M.[1]; FUKUMOTO, J.[2]; MIYAZAKI, S.[1]; TOKUOKA, S.M.[3]; OBINATA, H.[4]; HAYAKAWA, E.S.H.[5]; ONIZUKA, Y.[4]; INAOKA, D.K.[1]; SAKURA, T.[1]; KITA, K.[1]; YAHATA, K.[2]; TAKASHIMA, E.[2]; SHINDOU, H.[6]; TOKUMASU, F.[4].
1. NAGASAKI UNIVERSITY, NAGASAKI - JAPAN; 2. EHIME UNIVERSITY, MATSUYAMA - JAPAN; 3. THE UNIVERSITY OF TOKYO, TOKYO - JAPAN; 4. GUNMA UNIVERSITY, MAEBASHI - JAPAN; 5. JICHI MEDICAL UNIVERSITY, SHIMOTSUKE - JAPAN; 6. JAPAN INSTITUTE FOR HEALTH SECURITY, TOKYO - JAPAN.

Malaria parasites proliferate and produce numerous daughter cells in both liver and blood stages. This process is accompanied by marked increase in membrane area of parasites and rapid morphological changes, that require precise control of phospholipid synthesis and membrane generation. While parasites can uptake phospholipids from extracellular environment, *de novo* synthesis of phospholipids by parasites are also important to meet the transient increase in lipid demand. *Plasmodium falciparum* (*Pf*) has a similar phospholipid synthesis pathway and enzymes to those of mammals. However, detailed functions of these phospholipids *de novo* synthetic pathway have not been elucidated at molecular level. We focused on diacylglycerol acyltransferase (DGAT), which catalyzes conversion of DAG into triacylglycerol. DAG is an important intermediate molecule as it is used for most of all phospholipid species in *de novo* synthesis pathway and intracellular signaling. We hypothesized that disruption of *Pf*DGAT alter DAG metabolism and consequently affect lipid membrane genesis during blood stage. We generated *Pf*DGAT conditional knockout (cKO) parasites that showed reduced growth starting at 80-hour post invasion, indicating *Pf*DGAT is essential for parasite proliferation. In addition, deletion of *Pf*DGAT suppressed differentiation into gametocyte, transmissible form of mosquito. cKO also reduced knobs that are protrusion of protein and membrane complex appeared on the surface of parasitized erythrocytes. These knobs are responsible for adherence of parasitized erythrocytes that cause severe complications of *P. falciparum* including cerebral malaria. In addition, *Pf*DGAT disruption changed lipid profiles and reduced adhesion to vascular endothelial cells. These findings suggest that the membrane structure of parasitized erythrocytes was altered due to the loss of PfDGAT. *Pf*DGAT is suggested to play multiple roles, including parasite proliferation, regulation of transmissible stage formation and cytoadhearance.
Keywords: Malaria; Acyltransferase; cell adhesion.

PV – 019 - **The symbiotic bacterium modulates the metabolic plasticity of *Angomonas deanei* under glycolytic stress**

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

Endosymbiosis in trypanosomatids of the subfamily Strigomonadinae provides an excellent model for investigating cellular evolution and the origin of eukaryotic organelles. In *Angomonas deanei*, the mutualistic association with its endosymbiotic β-Proteobacterium involves extensive metabolic integration, in which the bacterium complements the host essential biosynthetic pathways, while receiving lipids and other essential metabolites. Here, we investigated the role of the symbiont in host glucose metabolism by inhibiting glycolysis with 2-deoxy-D-glucose (2-DG) and comparing wild-type (symbiont-containing) and aposymbiotic (symbiont-free) cells. Treatment with 2-DG reduced cell proliferation and delayed cell duplication in both strains, with a more pronounced effect in the aposymbiotic cells, indicating a greater dependence on glycolysis for growth. In contrast, mitochondrial dysfunction was observed exclusively in the wild-type strain, including reductions in maximal oxygen consumption, reserve respiratory capacity, mitochondrial membrane potential, and reactive oxygen species (ROS) production. Ultrastructural analyses revealed enlarged glycosomes with reduced electron density and the accumulation of lipid bodies close to the endosymbiont in wild-type cells, whereas Golgi disorganization occurred in both strains. These findings indicate a high bioenergetic integration within the glycosome-mitochondrion-symbiont triad, suggesting that in the wild-type strain, but not in the aposymbiotic one, the lack of glycolytic pathway products impacts the oxidative metabolism, directly impairing the mitochondrial physiology. Thus, our results demonstrate, through the induction of glycolytic stress, the influence of the endosymbiont on the metabolic coupling between glycolysis and oxidative metabolism in *A. deanei*.
Supported by: CNPq – 305299/2022-0; FAPERJ – E-26/201.011/2021 **Keywords:** 2-DG; glycolytic pathway; mitochondrial dysfunction.

PV – 020 - **An updated map of the trypanosome nuclear pore complex provides an architecture of the nuclear basket and mechanistic implications for nuclear export**

ZOLTNER, M.[1]; GABIATTI, B.[2]; ZAHEDIFARD, F.[3]; SHEN, S.[3]; KRENZER, J.[2]; BRAUNE, S.[2]; JEKIC, L.[2]; KRAMER, S.[2].

1. DEPARTMENT OF PARASITOLOGY, FACULTY OF SCIENCE, CHARLES UNIVERSITY IN PRAGUE, PRAGUE - CZECH REPUBLIC; 2. BIOCENTER, UNIVERSITY OF WÜRZBURG, WÜRZBURG - GERMANY; 3. DEPARTMENT OF PARASITOLOGY, FACULTY OF SCIENCE, PRAGUE - CZECH REPUBLIC.

We employed a combination of proximity labelling and expansion microscopy to identify nuclear- and cytoplasmic-site specific subcomplexes of the trypanosome nuclear pore complex (NPC). This approach revealed conserved, asymmetrical functional hubs supporting inherent roles in directed transport across the NPC. These asymmetric components could be leveraged as anchor points to predict the relative sub-pore localisation of known nucleoporins (NUPs) and other proteins with TrypTag-assigned pore localisation, as based on their labelling pattern as BioID baits. We discovered novel NPC associated components inclusive positional information, which we now interrogate for functional roles to explore trypanosome-specific adaptions of the nuclear basket, export control, pore membrane anchoring and RNP remodelling. We propose an architecture of the trypanosome nuclear basket and report functional data on basket associated TREX-2 homologs. Further, NUP BioID data contained information on transient interactions between NUPs and cargos undergoing nuclear import or export. As proof of concept, we analysed the interactions of pre-60S ribosomal particles, representing among the most abundant, largest and bulkiest export cargos, with NUPs by vice versa proximity labelling. **Supported by:** GACR / DFG **Keywords:** nuclear pore complex; nuclear export; Trypanosoma.

PV – 021 - **Impact of bifunctional sphingolipid ∆4-desaturase/C4-hydroxylase on sphingolipid biosynthesis and the viability of *Trypanosoma brucei* bloodstream forms**

RODRIGUEZ, M.F.[1]; DE BRITO, M.S.[1]; DA SILVA, G.R.[1]; KOELLER, C.M.[1]; BANGS, J.D.[2]; HEISE, N.[1].

1. INSTITUTO DE BIOFISICA CARLOS CHAGAS FILHO - UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 2. UNIVERSITY AT BUFFALO, NEW YORK - UNITED STATES OF AMERICA.

Trypanosoma brucei, the causative agent of human and animal African trypanosomiasis, remains a major neglected tropical pathogen requiring new therapeutic strategies. Enzymes involved in sphingolipid biosynthesis have emerged as promising drug targets because sphingolipids are essential for membrane integrity, cell signaling, growth, and differentiation. In eukaryotes, dihydrosphingosine formed during sphingolipid biosynthesis is converted into dihydroceramide by ceramide synthases, which is next desaturated by sphingolipid desaturase (DES) to generate ceramide, a precursor of complex neutral and acidic sphingolipids. *T. brucei* is the only known eukaryote capable of synthesizing the three main phosphosphingolipids: sphingomyelin (SM), inositol phosphorylceramide (IPC), and ethanolamine phosphorylceramide (EPC). In addition, the production of these phospholipids is essential and developmentally regulated. IPC predominates in procyclic forms (PCFs), EPC in bloodstream forms (BSFs), whereas SM is present in both stages. BSFs are enriched in d18:1/16:0 ceramide, while PCFs accumulate d18:0/16:0 dihydroceramide, suggesting differential expression and/or stage-specific regulation of the TbDES. To investigate TbDES function, we generated BSFs expressing a C-terminal HA-tagged TbDES and demonstrated by immunofluorescence that the enzyme localizes primarily to the ER and Golgi. RNA interference-mediated silencing of TbDES resulted in progressive growth arrest, complete inhibition of proliferation after 96 h, and a marked reduction in TbDES expression. Untargeted metabolomic analyses revealed the accumulation of d18:0 and a decrease in d18:1 and t18:0 sphingoid bases, confirming efficient enzyme depletion. These findings demonstrate that TbDES functions as a bifunctional sphingolipid Δ4-desaturase/C4-hydroxylase and plays a central role in regulating sphingolipid biosynthesis and parasite viability, supporting its potential as a target for new anti-trypanosomal therapies. **Supported by:** Faperj e CNPQ-UFRJ **Keywords:** sphingolipid desaturase; metabolomics; T; brucei.

PV – 022 - **Structural insights into the interaction between RPA-1 and RPA-2 in *Leishmania major***

DE OLIVEIRA, I.P.; BARBOSA, P.D.S.; FERNANDES, C.A.H.; CANO, M.I.N.
UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU - SP - BRAZIL.

Leishmania major is the causative agent of tegumentary leishmaniasis, a neglected tropical disease affecting millions worldwide. Current treatments are limited by toxicity, reduced efficacy, and increased drug resistance, highlighting the need for new therapeutic targets. Telomere-associated proteins have emerged as promising candidates due to their essential roles in parasite survival. Among these proteins, Replication Protein A (RPA), a heterotrimeric complex involved in DNA replication, repair, and recombination, plays an important role in parasite telomere maintenance. However, the interaction between its subunits remains poorly characterized in *L. major*. This study investigates the interaction between LmRPA-1 and LmRPA-2 using computational and experimental approaches. The LmRPA-1/LmRPA-2 complex model predicted by AlphaFold3 was visually compared with experimentally determined RPA structures from *Homo sapiens* (cryo-EM, PDB 8RK2) and *Ustilago maydis* (X-ray crystallography, PDB 4GNX), supporting its use as the starting structure for molecular dynamics simulations. A 500 ns simulation showed structural stabilization after approximately 300 ns based on RMSD analysis. Twenty-one snapshots extracted every 10 ns from the equilibrated trajectory (300–500 ns) were analyzed by residue-contact mapping and MM/GBSA calculations using HawkDock. Contact map analysis revealed a stable interaction interface between the α-helices of the OBF3 domain of LmRPA-1 and the OBF1 domain of LmRPA-2. The complex exhibited a mean MM/GBSA binding free energy of −108.37 kcal·mol^{-1}, suggesting a favorable LmRPA-1/LmRPA-2 interaction. The predicted interaction was confirmed by biophysical assays using both proteins in the recombinant form. The importance of the mapped residues in LmRPA-1/LmRPA-2 interaction and stabilization will be evaluated by mutagenesis to provide a structural basis for future characterization of the RPA complex in *L. major*. **Supported by:** CAPES 88887.202416/2025-00; CNPq 304229/2022-9 **Keywords:** Leishmania major; Replication Protein A; Interaction interface.

PV – 023 - **Exploring *Leishmania donovani* transcriptomic instability applying complementary long- and short-read sequencing protocols**

DA FONSECA, J.P.; COKELAER, T.; SPÄTH, G.F.
INSTITUT PASTEUR, PARIS - FRANCE.

Leishmania controls differential gene expression mostly through post-transcriptional mechanisms via mRNA turnover, alternative trans-splicing and expression of regulatory RNAs. How these mechanisms integrate to generate fitness gains is unknown. Here, we dissect transcriptome instability by combining short-read Illumina RNA-seq (Total, mRNA, Splice Leader) with long-read PacBio Iso-seq and SL-seq to define how isoform switching, alternative 3′/5′ UTR usage, and non-coding RNA expression may enable phenotypic adaptation in *L. donovani*.

Our integrated RNAseq approach successfully captured previously unannotated UTRs, identifying 447 novel unannotated transcripts. We revealed important transcriptomic instability at both 5’ and 3’ UTRs, including extensive variability in the 3’ end position, with 50% of the transcripts exhibiting a variability exceeding 100 bp. We further identified a non-random pattern of deletions inside 3’ UTRs concentrated on secondary-structure-forming motifs (triplexes, direct/mirror repeats, G4, and STR). Data will be presented on how these processes of transcriptome plasticity drive adaptive phenotypes during parasite fitness gain in culture.

Our work suggests that post-transcriptional plasticity is an un underappreciated driver of parasite phenotypic adaptation. By mapping how isoform usage, UTR architecture and ncRNA expression change during culture adaptation, we will uncover the hidden complexity of the parasite's highly dynamic expression program. Combined with an experimental evolution approach, our system will reveal RNA-based mechanisms that promote fitness gains beyond simple gene dosage-dependent changes, advancing our fundamental understanding of *Leishmania* biology and its survival strategies. **Supported by:** ERC Synergy Project DecoLeishRN, Grant agreement ID : 101071613 **Keywords:** Leishmania donovani; transcriptome instability; adaptation.

PV – 024 - **A Tangled Mess - Investigating the proteins involved in the limited chromatin condensation and segregation of *Trypanosoma cruzi***

XAVIER, S.K.; AOKI, J.I.; SABBAGA, M.C.Q.B.E.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Trypanosoma cruzi is the etiological agent of Chagas disease. The typical chromosome condensation that allows for proper segregation during mitosis in model eukaryotes is not observed in *T. cruzi*. Identifying the proteins involved in such a limited degree of condensation or in chromosome segregation is fundamental in understanding mitosis in *T. cruzi*. Our objective is to generate a dataset of proteins associated with *T. cruzi* chromatin in different moments of the cell cycle. Protocols for *T. cruzi* cell cycle synchronization were developed using two different drugs: Hydroxyurea (HU), which inhibits ribonucleotide reductase leading to the depletion of the nucleotide pool and thus prevents further DNA replication; Colchicine (Col), whose mechanism of action remains unknown outside of model eukaryotes, but it is known to block cytokinesis. Samples were read and analysed through flow cytometry and immunofluorescence. The protocols for chromatin enrichment were standardized. Samples of enriched chromatin will be submitted to LC-MS analysis. As results, flow cytometry data showed our cell cycle synchronization assays with HU and Col were successful: after HU treatment, on average, 55% of cells had a DNA content (C) heavily indicating they were synchronized in G1/S; after Col treatment, 53% had DNA content (2C) indicating they were synchronized in G2/M. In contrast, our unsynchronized controls had around 35% of cells in G1/S and 25% of cells in G2/M. We followed through with the chromatin enrichment protocol and SDS-PAGE analysis: bands around the expected molecular weight of histones, indicating chromatin proteins were enriched compared to cytoplasm and insoluble nuclei fractions. Currently, we are submitting our enriched chromatin samples for LC-MS analysis. Among all identified peptides, we expect those exclusively from Col-synchronized parasites, when compared to the controls, may provide new insights into how *T. cruzi* undergoes mitosis without condensing its chromatin. **Supported by:** CNPq **Keywords:** Trypanosoma; Chromatin; Histone.

PV – 025 - **Division of labor in trypanosome RNA processing and export through expanded Mex67 paralogs**

OBADO, S.[1]; CHUNG, C.[1]; JENSEN, B.[1]; ROUT, M.[2].
1. SEATTLE CHILDREN'S RESEARCH INSTITUTE, SEATTLE - UNITED STATES OF AMERICA; 2. ROCKEFELLER UNIVERSITY, NEW YORK - UNITED STATES OF AMERICA.

In animals and fungi, bulk mRNA export to the cytoplasm is mediated by the Mex67/Mtr2 (NXF1/NXT1) heterodimer and driven by an ATP-dependent remodeling machinery on the cytoplasmic side of nuclear pore complexes, the exclusive gateways of nucleocytoplasmic transport. Uniquely, we show that trypanosomes have three distinct Mex67 paralogs (TbMex67, TbMex67b and TbMex67L); each having a different non-redundant role in rRNA processing and mRNA export. TbMex67L is not involved in mRNA export but primarily associates with ribosome biogenesis factors. However, TbMex67 and TbMex67b retain canonical roles in mRNA export, *albeit* interacting with differing protein interactomes in the vertebrate host and insect vector forms of the parasite. Specifically, whilst TbMex67 and TbMex67b both associate with nuclear pore components, TbMex67b additionally associates with components of the TREX complex, long considered either absent or cryptic in these organisms except for Sub2 that is evolutionarily conserved. Moreover, TbMex67 and TbMex67b RNA export appears to be driven by the GTPase Ran export machinery, rather than ATP-dependent helicases, demonstrating substantial divergence in RNA export mechanisms in trypanosomes. Thus, in trypanosomes Mex67 paralogs have evolved distinct functions with implications for evolutionary origins and diversity in mechanisms that control gene expression and RNA metabolism. **Supported by:** NIH R01 AI192661-01A1 and R01 AI140429-01A1 **Keywords:** RNA export; Nuclear pore complex; Transcription.

PV – 026 - **Functional analysis of HAP2-coexpressed haptomonad surface proteins in Leishmania hybrid formation**

REINALDO, C.A.; SALVADOR, G.D.S.; MOSQUERA, K.D.; CATTA-PRETA, C.M.C.
LABORATÓRIO DE BIOLOGIA CELULAR EVOLUTIVA DE PROTISTAS, DEPARTAMENTO DE PARASITOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL.

Genetic exchange in *Leishmania* is well documented through whole-genome sequencing of natural hybrids and experimental crosses in sand fly vectors, which generate F1 recombinants with Mendelian inheritance. Yet the molecular mechanisms governing cell fusion remain unknown and committed sexual stages and gametes have not been identified. While HAP2-1 and HAP2-2, conserved gamete fusogenic proteins, are expressed in the sand fly midgut, membrane fusion requires more than a fusogenic protein; it demands accessory factors mediating cell-cell recognition. Importantly, these accessory surface proteins remain unknown. In gamma-irradiated fusion-competent cultured cells, haptomonad-specific markers (Catta-Preta et al., 2024, PNAS; p < 0.001) are co-expressed with *HAP2-1* and *HAP2-2*, linking haptomonad identity to mating competence. From 17 such markers, we selected four via in silico structural analysis. AlphaFold structural predictions identified two candidate protein families: Group 05 proteins possess compact globular ectodomains consistent with adhesive functions, whereas Group 31 proteins exhibit LRR-like solenoid architectures suggestive of cell-cell recognition. We will test whether these proteins are necessary for hybridization by generating CRISPR-Cas9 null mutants in L. tropica MA37 and L747, each expressing either eGFP-NEOr or mCherry-SATr. Next, we will evaluate fusion competence by crossing mutants in vitro after gamma-irradiation and quantifying hybrids via double-drug selection and flow cytometry. Fusion-deficient mutants will be prioritized for in vivo validation in *Lutzomyia longipalpis* infections. Determining whether these surface proteins are functionally required for hybridization will provide the first experimental evidence linking a *Leishmania* developmental stage to sexual reproduction in the sand fly vector. This will transform the cryptic sexual cycle from invisible into a tractable experimental system, enabling mechanistic studies of genetic exchange. **Supported by:** FAPESP **Keywords:** Leishmania tropica; Sexual Cycle; Membrane fusion.

PV – 027 - **Identification and functional characterization of the Vps26 component of the retromer complex in *Trypanosoma cruzi***

DE BRITO, M.S.; RODRIGUEZ, M.F.; DA SILVA, G.R.; HEISE, N.; KOELLER, C.M.
INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO - UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosoma cruzi is the protozoan parasite that causes Chagas disease, a neglected tropical disease that requires new targets for chemotherapy. 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. The retromer complex is involved in the retrograde transport of cargo between endosomes/lysosomes and the Golgi, as well as in receptor recycling and autophagy. This complex is composed of Vps5 and Vps17, involved in membrane deformation and vesicle formation, and Vps26, Vps29, and Vps35, responsible for cargo recognition and selection. Studies have shown that its components are essential for cell viability and the functioning of the secretory/endocytic pathways in protozoan parasites, such as *Trypanosoma brucei*. However, the composition and function of this complex remain unknown in *T. cruzi*. This study investigates the role of Vps26 in *T. cruzi* (TcVps26) biology. TcVps26 was identified in the Dm28cstrain genome through comparative genomics with orthologs from *S. cerevisiae*, *H. sapiens*, and *T. brucei.* RT-qPCR analyses indicated higher TcVps26 expression in epimastigotes in levels exceeding those of other cargo selection proteins. For functional analyses, knockout parasites were generated in epimastigotes using CRISPR/Cas9, employing guide RNAs and donor DNAs containing resistance markers to puromycin and/or blasticidin (BSD). Following selection, two BSD-resistant clones were obtained: one with a deletion in a single allele (SKO) and another with a deletion in both alleles (KO), confirmed by PCR. Both knockouts showed no changes in the proliferation of epimastigote forms; however, zymography gels revealed reduced cruzipain activity in the KO clone compared to the wild-type and SKO clones. Current analyses assess cruzipain processing by *Western blot* and the effects of Vps26 deletion on parasite differentiation and host cell infection. **Supported by:** CNPQ – FAPERJ **Keywords:** *Trypanosoma cruzi*; Retromer complex; Vps26.

PV – 028 - **The LmTRF Interaction Network: Proteomic Evidence for a Telomeric Hub in *Leishmania major***

BARBOSA, P.D.S.[1]; DE OLIVEIRA, I.P.[1]; PASSOS, A.D.O.[1]; ANDREASSA, E.D.C.[2]; DE SOUZA, T.D.A.C.B.[3]; CANO, M.I.N.[1].
1. UNESP, BOTUCATU - SP - BRAZIL; 2. FIOCRUZ, CURITIBA - PR - BRAZIL; 3. UNESP, CURITIBA - PR - BRAZIL.

Leishmaniasis is a neglected tropical disease affecting millions of people worldwide. Current therapeutic options remain limited by toxicity, high cost and the emergence of drug-resistant parasites. In this context, the molecular machinery responsible for telomere maintenance has emerged as a promising source of therapeutic targets. Despite the essential role of telomeres in chromosome stability, the composition and organization of the *Leishmania* telomeric chromatin remains underexplored. *LmTRF (L. major TTAGGG repeat binding factor) is one of the parasite's telomeric proteins that* plays a central role in telomere maintenance. In vertebrates, there are two TRFs, TRF1 and TRF2, but only one ortholog is found in trypanosomatids genome. Here, we characterized the LmTRF interaction network using pull-down assays coupled with shotgun mass spectrometry. Functional annotation revealed that LmTRF associates with proteins involved in chromatin organization, DNA repair, RNA metabolism and cell signaling. Among the most relevant candidates were proteins described as partners of the mammalian TRF2, including histones H3 and H2B, and putative orthologs of the Apollo nuclease and PNUTS, suggesting conservation despite the large evolutionary distance between these organisms. Additionally, proteins previously identified as *Leishmania* telomere-binding proteins, such as RPA1, RBP38 and CalA, were also pulled down with LmTRF. These findings suggest that LmTRF functions as a molecular hub integrating multiple pathways required for telomere homeostasis and provide evidence for a shelterin-like complex in *L. major*. Although additional validation is required to confirm these interactions, our results expand the current understanding of *Leishmania* telomere biology and support our previous findings about the composition of the parasite's telomeric chromatin. **Keywords:** Leishmania TRF; telomeric chromatin; interaction network.

PV – 029 - **Contribution to the taxonomy of *Apokeronopsis* (Ciliophora, Hypotricha) with characterization of a species from Guanabara Bay, Rio de Janeiro**

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

Among the Hypotricha Stein, 1859, the Kentrurostylida Paiva, 2020 is a clade of which some representatives produce bioactive compounds, including keronopsins, keronopsamides, and erythrolactones, synthesized by *Pseudokeronopsis rubra*, *P. ricci*, and *P. erythrina*, respectively. These molecules function as chemical defenses against predators and competitors, and are generally released via cortical granules or extrusomes—organelles still relatively understudied, but of remarkable morphological and functional diversity. Likewise, the cortical granules of *Apokeronopsis* represent promising targets for prospection of bioactive compounds. This study presents a brief revision of that genus and the morphology a species from Guanabara Bay, Brazil. *Apokeronopsis* currently comprises six species: *A. crassa* (type), *A. antarctica*, *A. bergeri*, *A. sinica*, *A. wrighti*, and *A. ovalis*, none of which has been studied in Brazil. The studied specimens were characterized *in vivo,* after protargol impregnation, and scanning electron microscopy. *Apokeronopsis* sp. measured ca. 100–130 µm *in vivo*; ca. 104 µm after protargol impregnation. Cells were flexible, slightly contractile, dorsoventrally flattened (3:1), elongated to fusiform in outline, with left-curved peristome. Cytoplasm densely granular, gray with yellowish tint near cortex, given the presence of two cortical granules types: (I), colorless, rounded in dorsal view, ca. <1 µm in diameter, arranged in longitudinal rows; (II), yellowish, optically hollow spheres, ca. 2–4 µm in diameter, distributed in the cytoplasm and beneath the pellicle. Adoral zone retroextended, occupying ca. 45% of the body length. Somatic ciliature consisted of an average of 39 cirral pairs forming a bicorona continuous with midventral complex; four buccal cirri, two frontoterminal, two pretransverse, 12 transverse, one left and one right marginal row with 31 and 39 cirri, respectively, and three dorsal kineties; caudal cirri absent. Nuclear apparatus distributed homogeneously throughout the cell, with numerous macronuclear nodules and relatively few micronuclei. With the exception of body shape and coloration, which resemble those of *A. bergeri*, all other studied morphological characteristics are consistent with *A. ovalis*. **Supported by:** CNPq **Keywords:** Protist; Systematic; Morphology.

PV – 030 - **The role of KAP6 and Topoisomerase II in topology and replication of *Trypanosoma cruzi* kDNA**

CAMELO, T.M.; MELO, N.S.; DE SOUZA, W.; MOTTA, M.C.M.

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Trypanosomatids presents a single branched mitochondrion that extends throughout the cell body and features a specialized region, the kinetoplast, which houses kDNA. Such mitochondrial DNA consists of concatenated maxicircles and minicircles forming an extensive network, whose arrangement varies according to the species and the trypanosomatid developmental stage. Kinetoplast Associated Proteins (KAPs) are involved in kDNA topology, replication, transcription, and repair. In addition to KAPs, mitochondrial topoisomerase II (TopoIImt) also participates in kDNA replication by releasing minicircles from the network to initiate this process. This study aims to investigate the roles of KAP6 and TopoIImt in the organization and replication of kDNA in *Trypanosoma cruzi* epimastigotes. To this purpose, KAP6 or TopoIImt mutant cells were generated using the CRISPR-Cas9 technique and subsequently gene expression for these proteins was evaluated, as well as protozoan proliferation, viability, cell cycle progression and ultrastructure. Cells with a deletion in only one of the alleles were obtained for both genes, indicating that they are equally essential. KAP6 and TopoIImt mutants showed approximately 80% reduction in gene expression compared to wild-type cells. Despite this, no significant changes in proliferation or cell viability were observed; however, delays in the cell cycle and kDNA replication were noted. Electron microscopy data revealed that both mutants exhibited morphological and ultrastructural alterations, including changes in kinetoplast shape and kDNA organization. We conclude that both proteins play important roles in the organization and replication of the kDNA network. **Supported by:** CAPES: 88887.139802/2025-00 **Keywords:** kinetoplast; kinetoplast associated proteins (KAPs); mitochondrial topoisomerase II.

PV – 031 - **Expression and localization of kinetoplast-associated proteins during *Trypanosoma cruzi* cell cycle**

LIMA, A.D.A.; MELO, N.S.; ZUMA, A.A.; GONCALVES, C.S.; DE SOUZA, W.; CAMELO, T.M.; MOTTA, M.C.M.

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

The kinetoplast is a specialized structure containing the mitochondrial DNA (kDNA) that is present in all trypanosomatids. The kDNA can exhibit distinct arrangements in different species and also during cellular differentiation, a process known as metacyclogenesis in *Trypanosoma cruzi*. Variations in kDNA topology are associated with Kinetoplast Associated Proteins (KAPs), which participate in the replication and repair of the mitochondrial genetic material and display distinctive localizations in the kinetoplast during *T. cruzi* metacyclogenesis. The main purpose of this study was to investigate whether the localization and expression of KAPs 4, 6, and 7 vary throughout the cell cycle of the *T. cruzi* epimastigote form. To this end, mutant cells were generated via CRISPRCas9 in order to express KAPs fused to mNeonGreen. To accurately assess the different cell cycle phases, cells were synchronized using hydroxyurea (HU) and subsequently, analyses using different light and electron microscopy methods, as well as flow cytometry and Western blotting, were performed. Fluorescence microscopy data revealed that KAPs 4, 6, and 7 exhibit distinct localizations within the kinetoplast: KAP4 is situated at the antipodal sites and within part of the kDNA network; KAP6 is oriented toward the posterior region of the kinetoplast, while KAP7 was observed exclusively at the antipodal sites. Western blot and flow cytometry analyses indicated a reduced expression levels of these proteins throughout the cell cycle. Preliminary results showed higher KAP4 expression during the G1/S phase, followed by KAP6, while KAP7 exhibited the lowest expression. During the G2/M phase, the expression of these three proteins decreased compared to the G1/S phase. Assays to evaluate the different stages of kDNA replication are underway, as well as electron microscopy analyses. With these results, we intend to better understand the role of KAPs in the organization and replication of *T. cruzi* kDNA. **Supported by:** CNPQ: 147514/2025-8 **Keywords:** kDNA; cell cycle; Kinetoplast Associated Proteins (KAPs).

PV – 032 - **Experimental evidence supporting TERRA-like transcripts in *Leishmania infantum* through integrated bioinformatic and molecular analyses**

RINALDI, J.E.D.S.; PASSOS, A.D.O.; DE OLIVEIRA, L.S.; CANO, M.I.N.
UNESP - UNIVERSIDADE ESTADUAL PAULISTA, BOTUCATU - SP - BRAZIL.

Telomeric repeat-containing RNA (TERRA) is a long non-coding RNA transcribed from subtelomeric regions toward chromosome ends and plays fundamental roles in telomere homeostasis, chromatin organization, and genome stability. Although TERRA has been described in several eukaryotes, its occurrence remains unexplored in *Leishmania infantum*, the causative agent of visceral leishmaniasis. This study investigated the presence of TERRA-like transcripts by integrating bioinformatic prediction with experimental validation. Public RNA-seq datasets from procyclic promastigote and amastigote stages (NCBI BioProject accession number: PRJNA1209106) were screened for reads containing telomeric repeats (TTAGGG)n, the conserved *Leishmania* subtelomeric CSB2/LCTAS motif, and the spliced leader sequence, followed by alignment against the *L. infantum* reference genome. This strategy identified recurrent subtelomeric TERRA-like transcript candidates in independent biological samples from both developmental stages. Northern blot analyses were performed using telomeric probes to experimental validate the predicted transcripts. Samples treated with DNase I and RNase A were used as controls. Specific hybridization signals were detected, demonstrating the presence of RNAs harboring telomeric repeat tracts, independently corroborating the in silico identification of recurrent subtelomeric transcript candidates. Collectively, these findings provide strong evidence supporting the occurrence of TERRA-like transcripts in *L. infantum*, establishing a foundation for future investigations into telomere-associated long non-coding RNAs and expanding current knowledge of telomere biology in trypanosomatids. **Supported by:** São Paulo Research Foundation (FAPESP), Process Number. 2026/02716-3. **Keywords:** Leishmania infantum; TERRA; Telomeres;

PV – 033 - **Tracking Evolutionary Patterns in the Transsulfuration and Cysteine Pathways in Kinetoplastids**

BUITRON, K.P.B.; SILBER, A.M.
ICB- UNIVERSIDADE DE SÃO PAULO | COMETA - CENTER OF METABOLISM, SÃO PAULO - SP - BRAZIL.

The sulfur amino acid pathway has undergone a complex evolutionary history shaped by horizontal gene transfer, vertical inheritance, and lineage-specific gene loss in Kinetoplastids. We previously showed that the methionine pathway evolved as a mosaic, with individual genes displaying distinct phylogenetic histories, presence/absence patterns, tree topologies, genomic localization, and synteny contexts. Selection analyses revealed episodic positive selection acting on genes of bacterial origin, while more conserved enzymes were predominantly shaped by mutational pressure. Building on this framework, we extended our comparative analysis to the full cysteine and transsulfuration pathways, characterizing gene distribution, microsynteny, and codon usage bias across Kinetoplastida to assess how these forces vary within a single metabolic module. Our results show that the transsulfuration and de novo cysteine synthesis pathways display progressively restricted distributions that correlate with parasitic lifestyle. The direct homocysteine-to-cysteine route (CBS, CGL) is broadly conserved across monoxenous and dixenous lineages, with sporadic losses in free-living euglenids and in Dutonella; notably, CGL, unlike CBS, persists in free-living organisms. The reverse route, mediated by CBL, shows a discontinuous distribution with multiple divergent, lineage-specific gene duplications rather than a single ancestral duplication event, as confirmed by OrthoFinder and phylogenetic analysis. The de novo synthesis enzymes SAT and CS were concordantly lost in Dutonella, Trypanozoon, and Phytomonadinae, suggesting complete and irreversible pathway elimination linked to host-derived cysteine acquisition in obligate parasites. Cysteine desulfurase duplicated into CD1 (broadly conserved since Euglena) and CD2 (restricted to Diplonema). Leishmaniinae uniquely retains the complete Met–Cys enzymatic module. A concatenated phylogeny of seven pathway enzymes recovers the canonical kinetoplastid topology with strong support, indicating that vertical inheritance predominates despite lineage-specific duplications and mixed evolutionary origins. These findings show that transsulfuration and de novo cysteine synthesis enzymes undergo recurrent, lineage-specific losses correlated with the transition to obligate parasitism, contributing to a broader understanding of how essential metabolic systems are preserved, restructured, or lost during the diversification of parasitic lifestyles. **Supported by:** 2023/07669-5 **Keywords:** Molecular evolution; Cysteine - transulfuration pathway; Kinetoplastids.

PV – 034 - **Effects of kinesore, a kinesin-1 modulator, on the ultrastructure of *Angomonas deanei* and the division of the symbiotic bacterium**

DE LIMA, L.S.G.; BARRINHA, A.; CAMELO, T.M.; DE SOUZA, W.; MOTTA, M.C.M.
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 kinetoplast. Suppression of tubulin expression by RNAi leads to the emergence of filamentous symbionts, indicating impaired bacterial division, as well as the appearance of protozoa with a *fat-cell* phenotype. 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 this work, we investigated the effect of kinesore, a kinesin-1 modulator, on the symbiotic relationship of *A. deanei*. This modulator prevents kinesin-1 from binding to cargo, while maintaining its motor activity, thereby promoting microtubule remodeling. Based on the literature, kinesore concentrations of 25, 50, and 100 µM were used in growth curves, viability tests, cell cycle assays and ultrastructural analyses by light and electron microscopy. The results showed that kinesore treatment did not significantly affect cell proliferation, viability, or cell cycle progression. However, alterations were observed in the organization of subpellicular microtubules, as well as in cell morphology and ultrastructure. Changes in the positioning of mitochondrial branches and an increase in membrane profiles in the cytosol and flagellar pocket were also observed. Flagellar biogenesis was affected, with a reduction or absence of the flagellum. In addition, kinesore treatment promoted the formation of filamentous endosymbionts unable to complete division, resulting in the emergence of aposymbiotic cells. Taken together, these results suggest the involvement of kinesin-1-related motor proteins in cellular organization and symbiont division in *A. deanei*. **Supported by:** CNPq e FAPERJ **Keywords:** Endosymbiosis in trypanosomatids; Microtubule cytoskeleton; Kinesore.

PV – 035 - **Investigation of the DNA damage response triggered by the presence of 5-ethynyl-2'-deoxyuridine (EdU) in the *Trypanosoma cruzi* genome**

DA MOTA, N.D.[1]; MENDES, I.C.[2]; MACHADO, C.R.[2]; ABUCHERY, B.E.[1]; DA SILVA, V.L.[1]; DA SILVA, M.S.[1].
1. CHEMISTRY INSTITUTE, UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL; 2. INSTITUTE OF BIOLOGICAL SCIENCES, FEDERAL UNIVERSITY OF MINAS GERAIS (UFMG), BELO HORIZONTE - MG - BRAZIL.

The thymidine analog EdU (5-ethinyl-2´-deoxyuridine) has been widely used to monitor DNA replication in several organisms. However, recent studies have shown that this compound is toxic to both mammalian cells and trypanosomatids. For *Trypanosoma cruzi* (the etiological agent of Chagas disease), a reduction in proliferation and viability was observed, along with an accumulation of cells in the S phase of the cell cycle, suggesting the presence of DNA damage. However, questions regarding the specific type of damage generated and whether DNA repair pathways are triggered in response to this damage remain open. Our main hypothesis is that the presence of EdU in the T. cruzi genome generates a conformational change in the DNA molecule that activates the NER repair pathway. To challenge and investigate this hypothesis, we assessed the behavior of WT and CSB-/+ T. cruzi strains in the presence of 50 µM and 100 µM EdU. CSB is a fundamental repair protein of the TC-NER pathway. Growth curve analysis revealed that 24 h after EdU removal from the medium (recovery), both strains showed reduced proliferation across all analyzed EdU concentrations. Interestingly, after this period, CSB-/+ parasites exposed to 50 µM EdU resumed their growth, diverging from the other experimental conditions, which maintained a reduced population and only slowly resumed growth after 48 h of recovery. Preliminary DNA content analyses by flow cytometry did not show significant differences among the strains analyzed. Currently, we are performing a kinetic assay following EdU removal to evaluate the mRNA levels of CSB, ATM, and ATR by RT-qPCR. The results of these approaches will allow us to infer whether TC-NER is indeed a repair pathway activated by EdU. If not, the involvement of the homologous recombination (HR) repair pathway will also be investigated. In this case, we will use Rad51-/+ and Rad51+/+/+ strains, since this recombinase is essential in the HR repair pathway. **Supported by:** FAPESP - 2025/26161- 8 **Keywords:** *Trypanosoma cruzi*; EdU; DNA damage response.

PV – 036 - **R-loop–associated ncRNAs' regulatory roles in *Leishmania major***

SEVERINO, A.C.; ISHIMOTO, A.Y.; CRUZ, A.K.
UNIVERSIDADE DE SÃO PAULO (USP), RIBEIRÃO PRETO - SP - BRAZIL.

Leishmaniasis is a group of neglected diseases caused by *Leishmania* protozoan parasites. In *Leishmania*, gene expression is predominantly regulated at the post-transcriptional level, with non-coding RNAs (ncRNAs) playing distinct and complementary roles. ncRNAs could act as regulatory RNAs when localized in the nucleus and/or cytoplasm as “free transcripts” and when associated with DNA in RNA-DNA hybrids, as R-Loops. In *Leishmania major*, *ncRNA.26.ODD3*, *ncRNA.27.c12*, *ncRNA.34.19*, and *ncRNA.35.25* colocalize with R-loop–enriched genomic regions. We aim to understand the roles of ncRNAs in gene expression regulation, either through association with R-loops or as independent regulatory ncRNAs. Overexpressing (OE) and/or knockout (KO) parasites for the four selected ncRNAs were generated using CRISPR/Cas9 and used to evaluate phenotypic alterations. We infected bone-marrow-derived macrophages (BMDMs) *in vitro* with KO, OE, and parental (PT) lineages at 4-72h. Mimicking metacyclogenesis conditions, we subjected the parasite to nutritional stress by culturing it in M199 without fetal bovine serum. Subcellular fractionation and RT-qPCR were used to evaluate *ncRNA.34.19* and *ncRNA.35.25* distribution. RT-qPCR showed no changes in ncRNAs’ levels across the parasite life cycle. *ncRNA.26.ODD3.*OE cells display reduced differentiation into infective forms and reduced amastigote survival in BMDMs. Modulation of *ncRNA.26.ODD3* affects stress response, probably related to an observed dysregulation of autophagy genes. *ncRNA.34.19* and *ncRNA.35.25* were detected both in the nucleus and cytoplasm, as were the other two, and smRNA-FISH analysis will proceed to confirm these data. Identification of *ncRNA.34.19* and *ncRNA.35.25*-bound proteins in each cell compartment is currently in progress, aiming to understand RNA-protein complex differences. Overall, ncRNAs in *Leishmania* have hidden regulatory mechanisms and may function in association with R-loops or as independent regulatory transcripts. **Supported by:** FAPESP - 2025/05210-0 / 2023/03015-0 **Keywords:** Leishmania major; Gene expression regulation; R-loops.

PV – 037 - **Characterization and functional analysis of Lupus La protein in *Leishmania braziliensis***

ANTUNIASSI, I.D.[1]; SANTOS, F.H.D.J.[1]; ESPADA, C.R.[2]; CASTILLO, R.E.H.[3]; CRUZ, A.K.[1].
1. FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL; 2. FACULDADE DE CIÊNCIAS FARMACÊUTICAS, UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 3. HOSPITAL ISRAELITA ALBERT EINSTEIN, SÃO PAULO - SP - BRAZIL.

Leishmaniasis is a group of diseases caused by the protozoan *Leishmania*, which, during its life cycle, passes through the gut of the sand fly vector and the phagolysosomes of phagocytic cells of the mammalian host. Thus, the organism must be agile to adapt and survive in these hostile environments. Its genetic organization, characterized by constitutive transcription, transfers the regulation of gene expression to the post-transcriptional level. In this context, RNA-binding proteins (RBPs) play an important role in the processing, stability and fate of messenger RNAs (mRNAs). Recent studies conducted by our laboratory identified the Lupus La protein as an RBP that binds to a long non-coding RNA (lncRNA) differentially expressed throughout the parasite's life cycle. Although this protein is conserved among eukaryotes and interacts with ncRNAs in trypanosomatids, its function in *Leishmania* remains poorly understood. Therefore, this project aims to investigate and functionally characterize Lupus La in *Leishmania braziliensis* using the CRISPR-Cas9 gene-editing system for gene tagging and knockout attempts. By fluorescence microscopy, we showed that the protein is localized in the nucleus. In addition, only heterozygous clones were obtained, suggesting that Lupus La may be essential for parasite survival, which prompted an alternative knockdown strategy using RNAi; the construct has been made and transfected into *L. braziliensis*. Phenotypic analyses of these transfectants revealed no significant alterations compared with parental cells. Furthermore, *in silico* analyses predicted the protein structure, domains, and potential protein interactions, while RNA-seq data analysis indicated an enrichment of ncRNAs associated with the protein. Thus, herein we present a preliminary characterization of Lupus La that indicates its functional relevance to understanding post-transcriptional gene regulation in *L. braziliensis*. **Supported by:** FAPESP (2024/20016-3 e 23/03015-0) **Keywords:** Leishmania; RNA binding protein; Lupus La.

PV – 038 - **The incorporation of the thymidine analogs BrdU and EdU causes DNA alterations and trypanosomatids react differently to them**

DA SILVA, M.S.[1]; DE OLIVEIRA, M.A.F.[1]; DA SILVA, V.L.[1]; TEIXEIRA, T.L.[1]; GEROLAMO, F.M.[1]; GAZIRI, T.[1]; SILVA, F.D.J.[1]; BLACK, J.A.[2]; CUCCOVIA, I.M.[3].
1. UNIVERSIDADE DE SÃO PAULO (USP), BELO HORIZONTE - SP - BRAZIL; 2. FMRP-USP, RIBEIRÃO PRETO - SP - BRAZIL; 3. UNIVERSIDADE DE SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL.

The thymidine analogs 5-Bromo-2′-deoxyuridine (BrdU), and 5-Ethynyl-2′-deoxyuridine (EdU) are widely used for monitoring DNA replication in eukaryotic cells. Although some studies have reported potential EdU toxicity in mammalian cells, to date there are no reports of cytotoxic effects associated with the use of any thymidine analog in trypanosomatids, which raises two possibilities: these nucleotides are non-toxic to these parasites; or no research group has been interested in reporting any observed toxicity. To discriminate between these two possibilities, we evaluated the toxicity of BrdU and EdU in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major.* Our findings demonstrate that EdU has a much higher cytotoxic and genotoxic potential than BrdU. However, the different trypanosomatid species analyzed respond markedly differently to the presence of these analogs, showing significantly different values for viability, DNA content, analog retention time, and DNA damage response. Additionally, our assays suggest that when incorporated into DNA, EdU increases the polarity of the ethynyl-containing region, thereby destabilizing the double helix, altering the number of hydrogen bonds between bases, and causing slight changes in the double helix's dihedral angles. The toxicity of these analogs, as reported here, may suggest biases in data from assays used to monitor DNA replication over extended periods. In other words, data involving the use of thymidine analogs in trypanosomatids (especially EdU) should be analyzed with caution. **Supported by:** FAPESP (processes 2019/10753-2 and 2023/07193-0) **Keywords:** DNA damage; DNA replication; Genotoxicity.

PV – 039 - **Evolutionary conservation of the 14-3-3 protein in Crithidia sp. LVH60a and other Leishmaniinae species: Implications for adaptation to the vertebrate host.**

LYRA, S.P.[1]; ROGERIO, L.A.[2]; MARUYAMA, S.R.C.[1].
1. SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO - FCFRP/USP, RIBEIRÃO PRETO - SP - BRAZIL; 2. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES - NIAID/NIH, ROCKVILLE - UNITED STATES OF AMERICA.

Visceral leishmaniasis is a neglected tropical disease caused by *Leishmania infantum* and transmitted in Brazil by the sandfly *Lutzomyia longipalpis*. Recently, *Crithidia sp.* LVH60a, a trypanosomatid phylogenetically related to *C. fasciculata*, was identified in coinfection with *L. infantum* in cases of visceral leishmaniasis, suggesting adaptation to the vertebrate host. Among the virulence-associated factors in Leishmania, the 14-3-3 protein stands out as a regulator of essential cellular processes. This study investigated the presence and characterization of the 14-3-3 isoforms in Crithidia sp. LVH60a. Orthologs were identified by BLASTn using *L. infantum* sequences as references. Coding sequences were translated using ORFfinder, compared by BLASTp, and their structures were predicted with AlphaFold and compared using PyMOL. Nucleotide and amino acid sequences were aligned with MUSCLE and used for phylogenetic reconstruction based on the Maximum Likelihood method. Phylogenetic analyses of nucleotide and protein sequences from *Crithidia sp*. LVH60a, *L. infantum, L. donovani, L. major, L. mexicana, L. amazonensis, L. braziliensis* and *Leptomonas pyrrhocoris* revealed two well-defined clades corresponding to the epsilon and omega isoforms, indicating a high evolutionary conservation across Leishmaniinae. Both isoforms were identified in *Crithidia sp.* LVH60a. BLASTp analyses showed high identity and coverage among proteins belonging to the same isoform and lower similarity between distinct isoforms. Predicted three-dimensional structures displayed a conserved architecture of nine α-helices with preserved amino acid organization in functional regions. Overall, results demonstrate that the 14-3-3 protein is highly conserved across Leishmaniinae and confirm the presence of both isoforms in *Crithidia sp.* LVH60a. This finding supports the hypothesis that this protein may contribute to the adaptation of this parasite to the vertebrate host, suggesting a role in infectivity. **Supported by:** FAPESP 2016/20258-0 **Keywords:** 14-3-3 protein; Crithidia sp; LVH60a; comparative genomics.

PV – 040 - **Importance of the component TcVps5 of the retromer complex in *Trypanosoma cruzi* biology.**

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

The retromer complex is involved in the retrograde transport of cargo between endosomes/lysosomes and the Golgi complex, as well as in receptor recycling and autophagic processes. Studies have shown that its components are essential for cell viability and the functioning of the secretory/endocytic pathways in protozoan parasites, such as Trypanosoma brucei. However, the composition and function of this complex remain unknown in T. cruzi, which causes Chagas disease, a neglected tropical disease that requires new therapeutic approaches. In this study, we investigated the function of TcVps5 by assessing its role in intracellular protein trafficking and parasite biology. Initially, IFA analysis showed that TcVps5 localizes to the Golgi complex. Subsequently, TcVps5 knockout parasites (∆TcVps5) were generated using CRISPR/Cas9 and the absence of TcVps5 impaired epimastigotes proliferation. Also, ∆TcVps5 parasites showed reduced activity and processing of cruzipain and cathepsin B; alterations in cell surface composition, as evidenced by increased labeling with GS-IB 4 and tomato lectin, and reduced BSA endocytosis. Ultrastructural analyses also revealed Golgi complex disorganization in the mutants. Functionally, ∆TcVps5 parasites exhibited a reduced capacity to differentiate into metacyclic trypomastigotes and decreased amastigote proliferation, although no changes were observed in the release of infection-derived trypomastigotes. Taken together, the results demonstrate that TcVps5 plays a central role in maintaining T. cruzi secretory/endocytic pathways organization, it is essential for the proper processing of lysosomal proteases, maintenance of cell surface composition, endocytosis, Golgi complex integrity, and normal parasite life cycle progression. These findings represent the first functional characterization of a retromer complex component in T. cruzi and expand our understanding of the intracellular trafficking mechanisms involved in this parasite biology. **Supported by:** FAPERJ; CNPq **Keywords:** Vps5; retromer complex; *Trypanosoma cruzi*.

PV – 041 - **Aurora Kinase 3 (AUK3) Is Required for Normal Growth and Resistance to Hydroxyurea-induced Replication Stress in *Leishmania major***

DANTAS JUNIOR, D.M.; BLACK, J.A.; TOSI, L.R.O.
UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL.

Aurora kinases (AURKs) are a family of serine/theronine kinases that regulate cell cycle progression by coordinating the organization and segregation of chromosomes during mitosis and meiosis in eukaryotes. Typically, metazoans possess three AURKs (AURKA, AURKB and AURKC), each with distinct but partially overlapping functions. Dysregulation of AURKs leads to aneuploidy and defective cell division and has been implicated in different forms of human cancers. Among them, AURK3, the least characterized family member, is expressed predominantly in meiotic cell lines, where it plays roles in gametogenesis and chromosome segregation. *Leishmania major* genome encodes three kinases, in which Aurora kinase 3 (AUK3), most closely related to metazoan AURKC, still has an unknown biological function. As one of the earliest-diverging eukaryotic lineages, *Leishmania* exhibits several unique features of cell cycle regulation, by which AUK3 may play a role, making it an attractive model for investigating the evolution of Aurora kinase function. In this study, we aim to characterize the role of AUK3 during the regulation of cell cycle in *L. major.* Using CRISPR/Cas9 technology, we generated parasites with reduced AUK3 expression as well as parasites that expressed N-terminal *3xmyc-tagged* AUK3. Indirect immunofluorescence using anti-*myc* antibodies detected *3xmyc*::AUK3 signal in both the cytoplasm and the nucleus of L. major promastigotes. In AUK3-deficient cells, when compared to control cells, we observed a significant defect in cell proliferation, in addition to enhanced sensitivity to hydroxyurea-induced replication stress, suggesting that AUK3 may act during *L. major* cell cycle progression and, putatively, may play a role in the replicative stress response of this parasite. Ongoing investigation, including cell cycle analysis and proximity-labelling experiments using TurboID-tagged AUK3, will further reveal the molecular functions and interaction network of this kinase in *L. major.* **Supported by:** FAPESP 2025/25862-2 **Keywords:** Leishmania major; Aurora-Kinase 3; Cell Cycle.

PV – 042 - **Beyond target mutations: metabolic remodeling and drug resistance in *Trypanosoma cruzi***

EUFRASIO, A.G.; SFORCA, M.L.; PEREIRA, J.G.D.C.; ROJAS, A.E.L.; DE SOUSA, I.L.; CORDEIRO, A.T.
CENTRO NACIONAL DE PESQUISA EM ENERGIA E MATERIAIS - CNPEM, CAMPINAS - SP - BRAZIL.

Metabolic plasticity of *Trypanosoma cruzi* enables the parasite to adapt to environmental changes and may also facilitate its response to prolonged drug pressure. Here, we investigated adaptive mechanisms associated with resistance to two compounds targeting cytosolic NADPH-producing pathways: the quinazolinone QUI-34, associated with inhibition of glucose-6-phosphate dehydrogenase (G6PDH), and the sulfonamide AC-M109, associated with inhibition of cytosolic malic enzyme (MEc). *T. cruzi* epimastigotes were subjected to continuous drug pressure to generate resistant populations, which were characterized by susceptibility assays, target-gene sequencing, ^{1}H-NMR-based exometabolomics and transcriptomic analyses. Selection with QUI-34 generated a stable resistant population displaying an approximately 7-fold increase in EC50, whereas AC-M109 resistance resulted in a >10-fold EC50 shift. Both phenotypes remained stable after six months without drug pressure and were not associated with coding-sequence mutations in their respective target genes. Despite this common phenotype, the underlying adaptive responses differed. QUI-34-resistant parasites showed an attenuated metabolic response to compound exposure and transcriptional changes involving mitochondrial respiration, stress-response pathways and multidrug transporters. In contrast, AC-M109-resistant parasites remained metabolically responsive to the compound and displayed extensive remodeling of central carbon metabolism. Moreover, these parasites exhibited slight cross-resistance to G6PDH-targeting quinazolinones while remaining susceptible to benznidazole, supporting functional interplay between cytosolic NADPH-producing pathways. Together, these findings demonstrate that *T. cruzi* withstands prolonged drug pressure through distinct adaptive mechanisms, highlighting metabolic plasticity as a key contributor to drug resistance. **Supported by:** Fundação de Amparo à Pesquisa do Estado de SP (FAPESP), Grant number: 2019/23995-4; Financiadora de Estudos e Projetos (FINEP), Grant number: 01.22.0473.00 **Keywords:** *Trypanosoma cruzi*; resistance; omics.

PV – 043 - **Constrained in space, stochastic in time: single-cell analysis of DNA replication timing in *Leishmania*.**

DAMASCENO, J.D.[1]; NEGREIRA, G.H.[2]; DOMAGALSKA, M.[2]; MCCULLOCH, R.[3].
1. UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL; 2. INSTITUTE OF TROPICAL MEDICINE - ANTWERP, ANTWERP - BELGIUM; 3. UNIVERSITY OF GLASGOW, GLASGOW - SCOTLAND.

Leishmania exhibits extraordinary genomic plasticity through frequent gene copy-number variation and mosaic aneuploidy that fuel rapid adaptation, including drug resistance. Understanding genome duplication is therefore key to understanding how these parasites reconcile adaptive plasticity with faithful genome transmission. Using DNAscent to map initiation sites at single-molecule resolution and MFA-seq to profile bulk replication timing, we previously uncovered stochastic origin usage and strong timing compartmentalisation within and between chromosomes. Whether this organisation persists in individual cells, however, remained unknown.
Here, we combine single-cell whole-genome sequencing with marker-frequency analysis (scMFA-seq) to reconstruct genome-wide replication timing in *L. donovani* promastigotes throughout S phase. scMFA-seq reveals a programme that is spatially constrained yet temporally stochastic. The main early-replicating locus on each chromosome consistently overlaps the centromere and a heterochromatin domain, while intra- and inter-chromosomal timing compartments and the later replication of larger chromosomes persist in individual cells. Despite this stable spatial organisation, timing varies extensively: although genome duplication remains globally coordinated with S-phase progression, ~50% of locus-level replication events occur significantly earlier or later than predicted from population-average timing. Yet this variability is structured rather than arbitrary: earlier-than-predicted replication is associated with greater chromatin density, AT enrichment, higher transcription-initiation rates and chromosome size, but inversely associated with GC content and later population-level replication timing.
Our findings reveal an unexpected replication timing principle in *Leishmania*: a spatially constrained initiation landscape coexists with pervasive temporal variability. This single-cell view provides a framework for understanding how these parasites may combine faithful genome transmission with the flexibility that fuels genome evolution and adaptation. **Supported by:** FAPESP (25/10952-6) **Keywords:** Leishmania genome plasticity; Single-cell replication timing; DNA replication.

PV – 044 - **From autophagy to metacyclogenesis: TcZonda as a novel FKBP immunophilin associated with the endoplasmic reticulum in *Trypanosoma cruzi***

SCHOIJET, A.C.[1]; PARDO, C.[2]; ALONSO, G.D.[3].
1. INGEBI - CONICET - DEPARTAMENTO DE QUÍMICA BIOLÓGICA, FACULTAD DE CIENCIAS EXACTAS Y NATURALES, UNIVERSIDAD DE BUENOS AIRES, BUENOS AIRES - ARGENTINA; 2. INGEBI - CONICET, BUENOS AIRES - ARGENTINA; 3. INGEBI - CONICET - DEPARTAMENTO DE FISIOLOGÍA, BIOLOGÍA MOLECULAR Y CELULAR, FACULTAD DE CIENCIAS EXACTAS Y NATURALES, UNIVERSIDAD DE BUENOS AIRES, BUENOS AIRES - ARGENTINA.

Autophagy contributes to parasite adaptation and differentiation in *Trypanosoma cruzi*. TcVps34, a class III PI3K, and its regulatory partner TcVps15 are components of the autophagy pathway. Here, we found that overexpression of TcVps34 or TcVps15 enhanced autophagy and promoted metacyclogenesis, increasing the proportion of metacyclic trypomastigotes. These findings support a functional link between autophagy and parasite differentiation. To further investigate the molecular mechanisms underlying this connection, we searched for homologs of autophagy-associated proteins that could link these processes. In *Drosophila melanogaster*, Zonda (Zda), an immunophilin of the FKBP family, was identified as an early component of the autophagy pathway and shown to localize to ER-associated structures during nutrient starvation, where it nucleates at sites associated with omegasomes. However, autophagosome biogenesis and the contribution of ER-associated structures, including omegasome-like compartments, remain largely unexplored in trypanosomatids. Based on these findings, we searched for and identified candidate Zonda homologs in *T. cruzi*, leading to the identification of TcZonda. Bioinformatic analyses identified two candidate sequences with conserved FKBP domain organization, and RT-PCR established that the shorter sequence corresponds to the expressed TcZonda transcript. TcZonda-HA-overexpressing Dm28 parasites displayed a punctate intracellular distribution with partial overlap with the ER marker BiP under basal and nutrient starvation conditions. Strikingly, TcZonda overexpression increased the proportion of intermediate forms and metacyclic trypomastigotes during differentiation induced in TAU and TAU-3AAG media. Together, these findings point to a previously unrecognized connection between autophagy-related pathways, ER-associated processes and parasite differentiation in *T. cruzi*, identifying TcZonda as a candidate link between these processes. **Supported by:** GHIT H2025-101, Dr. Alonso **Keywords:** Autophagosome biogenesis; metacyclogenesis; *Trypanosoma cruzi*.

PV – 045 - **Comparative analysis of ionizing radiation resistance in trypanosomatids**

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

Parasitic infections are among the leading causes of morbidity and mortality worldwide. A substantial proportion of these human infections can be attributed to trypanosomatids, including *Trypanosoma cruzi* and *Trypanosoma brucei*. Studies have shown that trypanosomes exhibit remarkable resistance to gamma ionizing radiation (IR) compared with other eukaryotes. Growth and viability assays demonstrated that *T. cruzi* can resist up to 500 Gy, resuming proliferation after exposure to lower doses (100 Gy of γ-ray) while maintaining relatively stable viability. In contrast, *T. brucei* undergoes complete cell death within 24 h after 100 Gy IR. Flow cytometric DNA content analysis revealed viable *T. cruzi* cells distributed across all phases of the cell cycle, along with a marked cell cycle arrest at G0 state/G1 phase, consistent with the reduced proliferation observed during the initial post-IR period. Immunofluorescence assays using α-γH2A confirmed that IR induced DNA damage in both trypanosomatids species. However, the damage was almost completely resolved in *T. cruzi* after 10 days, particularly in the groups exposed to lower radiation doses (up to 100 Gy). In *T. cruzi*, RT-qPCR analyses showed a slight increase in the expression of DNA repair genes, including ATM, ATR, PARP, and RAD51, between 1 and 24 h after IR, especially in the groups exposed to 300 and 500 Gy, followed by a significant decrease in the expression of all analyzed DNA repair genes at 192 h. These findings are consistent with a possible reduction in cellular metabolism associated with a quiescent (dormant) state, as suggested by the reduced proliferation, G1 accumulation, and sustained viability throughout the experimental period. Experiments conducted to date demonstrate that *T. brucei* possesses significantly lower resistance to IR than *T. cruzi*, and our next steps aim to investigate the reason for this behavior. **Supported by:** FAPESP 2024/21950-1 **Keywords:** DNA damage response; Chagas disease; sleeping sick.

PV – 046 - **What does Polo-like kinase do in *Leishmania major*?**

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

Polo-like kinases (PLKs) are a family of evolutionarily conserved serine/threonine kinases that perform important roles in eukaryotic cell cycle regulation, specifically during mitosis. Our understanding of this family of kinases in divergent eukaryotic pathogens such as *Leishmania* remains incomplete. Unusually, *Leishamania* possess one PLK (LmPLK), whilst related trypanosomatids, like *Trypanosoma brucei* and *Trypanosoma cruzi*, have two PLK isoforms. Why *Leishmania* relies on a single PLK is unclear. Previous work localized PLK to the basal body of *Leishmania*, a specialized structure located at the base of the flagellum. To investigate *Leishmania major* PLK in greater detail we first confirmed the subcellular localization using CRISPR-Cas9 editing to endogenously tag both alleles of the LmPLK gene with as N-terminal 3×Myc epitope (^{3xMyc}LmPLK). Immunofluorescence showed that ^{3xMyc}LmPLK localizes near the base of the flagellum, consistent with previous findings. Using ^{3xMyc}LmPLK cells, we will examine LmPLK localisation in greater detail across the *L. major* cell cycle. In parallel, we will use CRISPR-Cas9 to generate LmPLK deletion mutants and characterize their phenotype, to better understand PLK function in *L. major*. We hypothesize that studying PLK in *Leishmania* we will advance understanding of this parasite's cell cycle and, more widely, contribute to knowledge of PLK function across the Eukarya. **Supported by:** FAEPA - HCFMRP – USP **Keywords:** Leishmania; Polo-like kinase; Basal body.

PV – 047 - **EXPLORING THE ROLE OF CRK1 IN THE MORPHOLOGY AND METACYCLOGENESIS OF *Trypanosoma cruzi***

CORRÊA, A.D.P.; CALDERANO, S.G.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Trypanosoma cruzi exhibits a complex life cycle characterized by transitions between cellular forms with distinct morphological, metabolic, and functional features. Cell cycle progression and maintenance of cellular architecture are essential for parasite development and may be directly related to its ability to respond to different environmental stimuli. Metacyclogenesis, for instance, requires coordinated cellular reorganization for the formation of metacyclic trypomastigotes. In this context, CDK-related kinases may perform functions beyond the regulation of cell proliferation. CRK1 is a poorly characterized kinase in *T. cruzi*, and its contribution to cellular organization and differentiation remains unclear. Therefore, we investigated the impact of CRK1 depletion on epimastigote morphology and metacyclogenesis efficiency. Recombinant *T. cruzi* lines were generated using CRISPR/Cas9 in the Dm28c-Cas9 strain and validated by PCR screening, followed by growth curve analysis. For phenotypic characterization, the Nucleus-Kinetoplast-Flagellum (NKF) distribution pattern was analyzed, along with parasite morphometry, considering body length, flagellum length, and total length, with image analysis performed using CellSens software. Differentiation capacity was evaluated through metacyclogenesis assays, with quantification of epimastigote, intermediate, and metacyclic trypomastigote forms. Partial deletion of CRK1 was confirmed by PCR and did not impair epimastigote proliferation or the NKF distribution profile. However, the CRK1 +/- Blast lineage exhibited significant morphological alterations, with increased body length and reduced flagellum length ($p < 0.05$), resulting in a lower flagellum-to-body ratio ($p = 0.0227$). In addition, CRK1 hemizygosity impaired metacyclogenesis, with an approximately 50% reduction in the formation of metacyclic trypomastigotes (16% vs. 33%; $p < 0.01$) and an increase in intermediate forms (31% vs. 14%). Partial CRK1 depletion does not affect proliferation but significantly alters epimastigote morphology and impairs metacyclogenesis progression, highlighting its role in *T. cruzi* differentiation. **Supported by:** FAPESP 2024/23849-6 **Keywords:** Kinases; Cellular Plasticity; Differentiation.

PV – 048 - **Ultrastructural and organizational changes in *Trypanosoma cruzi* induced by novel drug candidates for the treatment of Chagas disease**

LEITE, J.M.R.M.[1]; MARCHESE, L.[1]; FARIA, J.M.D.L.[2]; BORGES, B.S.[3]; CORDEIRO, A.T.[1].
1. CNPEM/LNBIO, CAMPINAS - SP - BRAZIL; 2. CNPEM/LNNANO, CAMPINAS - SP - BRAZIL; 3. IFSC/USP, SÃO CARLOS - SP - BRAZIL.

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains a neglected tropical disease with limited therapeutic options, highlighting the need for new drug candidates. This study investigated the ultrastructural effects of three compounds targeting essential T. cruzi metabolic pathways: quinazolinone derivatives targeting TcG6PDH, TCMDC-143160 targeting TcTDH, and TCMDC-143108 targeting TcMEc. Epimastigotes of the Dm28c strain (DTU-TcI) were cultured in LIT medium supplemented with 10% FBS at 28 °C and treated with the compounds at 3× EC_{50} for 24 h. Ultrastructural analyses were performed by transmission electron microscopy (TEM) after high-pressure freezing and freeze substitution, and by cryo-electron tomography (Cryo-ET). TEM analysis of TCMDC-143160-treated parasites revealed mitochondrial swelling, blebbing near the kinetoplast, altered chromatin organization, nuclear envelope disorganization, and pronounced plasma membrane undulation. Cryo-ET further enabled three-dimensional visualization of intracellular structures under near-native conditions. Segmentation and 3D reconstruction of a TCMDC-143160-treated parasite allowed the mitochondrion and nucleus to be individually delineated, revealing the extended morphology of the mitochondrion, its relationship with the kinetoplast region, and nuclear organization and connections. These structural features may be associated with the effects of TCMDC-143160; however, Cryo-ET data from untreated wild-type parasites are still required to determine whether the observed features are specifically induced by the compound. Overall, these preliminary results demonstrate the feasibility of combining Cryo-ET and 3D segmentation to characterize ultrastructural alterations associated with experimental drug treatments in T. cruzi. **Supported by:** CAPES - 88887.252523/2026-00 **Keywords:** *Trypanosoma cruzi*; Cryo-electron tomography; Drug-induced ultrastructural alterations.

PV – 049 - **DNA polymerase theta promotes genome stability and resistance to genotoxic stress in *Leishmania major***

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

Leishmania parasites cause leishmaniasis, a neglected tropical disease affecting millions of people worldwide. *Leishmania* exhibits remarkable genomic plasticity, which contributes to parasite adaptation and survival under environmental and host-associated stresses, with DNA repair mechanisms playing a central role. Although the major DNA repair pathways have been characterized in *Leishmania*, microhomology-mediated end joining (MMEJ), an alternative pathway for the repair of DNA double-strand breaks, remains poorly understood. DNA polymerase theta (Polθ), a key factor in MMEJ, may therefore contribute to genome maintenance and genomic plasticity in this parasite. Here, we investigated the *PolQ* homolog LmjF.24.0890 in *Leishmania major*. In silico analyses identified it as the ortholog of human *POLQ*. Comparative sequence analyses revealed conservation of Polθ among *Leishmania* species and other trypanosomatids, with high sequence similarity between their polymerase domains and the polymerase region of human Polθ. AlphaFold structural predictions further showed that the secondary structure of the *L. major* Polθ polymerase domain is largely conserved relative to its human homolog. Using CRISPR-Cas9, we generated parasites expressing Polθ with 3xMyc tag at the N-terminal domain. Immunofluorescence analysis showed that Polθ localizes predominantly to the nucleus. Additionally, a heterozygous *PolQ* knockout line exhibited reduced growth and increased sensitivity to zeocin, etoposide, cisplatin, and camptothecin, alongside increased resistance to methyl methanesulfonate (MMS). Western blot analysis revealed elevated γH2A levels in the *PolQ* knockout line following treatment with MMS, camptothecin, and cisplatin, indicating increased DNA damage. Cell-cycle analysis following MMS synchronization showed delayed cell-cycle progression in knockout parasites. Together, our findings identify *Leishmania* Polθ as a conserved nuclear polymerase that contributes to genome maintenance and the response to genotoxic stress, supporting a role for Polθ in alternative end-joining DNA repair in this parasite. **Supported by:** FAPESP 2025/09797-6; FAEPA HCFMRP-USP **Keywords:** Leishmania; DNA Polymerase Theta; Microhomology-mediated end joining.

PV – 050 - **Study of the effects of treatment with a mitochondrial pyruvate carrier inhibitor on *Leishmania (V.) braziliensis***

DOS SANTOS, V.X.; VILETE, J.V.C.; MENNA-BARRETO, R.F.S.; BOMBAÇA, A.C.S.; PEREIRA, L.D.O.R.
FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Leishmaniasis, a group of neglected tropical diseases that includes cutaneous and visceral forms, is caused by parasites of the genus *Leishmania*. *Leishmania (Viannia) braziliensis* is a causative agent of american tegumentary leishmaniasis (ATL) and alternates between promastigote and amastigote forms in distinct environments: the phlebotomines gut and the parasitophorous vacuoles of phagocytic cells in the vertebrate host. Pyruvate plays a central role in energy metabolism and must be transported from the cytoplasm into the mitochondrial matrix by the mitochondrial pyruvate carrier (MPC), whose function in *L. braziliensis* remains poorly characterized. Evidence from other trypanosomatids suggests that MPC inhibition promotes metabolic reprogramming, affecting parasite growth and infectivity. Orthologs of MPC1 and MPC2 were identified in the *L. braziliensis* genome, suggesting the presence of a functional MPC complex. This study evaluated the effects of the MPC inhibitor UK-5099 on promastigotes cultured in Schneider's medium in the presence or absence of fetal bovine serum (FBS). Parasite viability, mitochondrial membrane potential, reactive oxygen species (ROS) production, and membrane integrity were assessed. In the presence of FBS, UK-5099 treatment promoted increased parasite proliferation, whereas under serum-free conditions it induced a dose-dependent reduction in viability (IC_{50}= 53.4 μM). Mitochondrial membrane potential was maintained in FBS-supplemented cultures but significantly reduced in the absence of serum, with no detectable changes in ROS levels or membrane integrity. These findings suggest that MPC inhibition could induce metabolic plasticity and energetic adaptation in *L. braziliensis*, reinforcing the importance of pyruvate transport in mitochondrial bioenergetics. Future studies will investigate the metabolic remodeling associated with MPC inhibition, contributing to a better understanding of *L. braziliensis* metabolism and the biology of ATL. **Supported by:** PROEP-Fiocruz, FAPERJ, CNPq **Keywords:** Leishmania (Viannia) braziliensis; trypanosomatid biochemistry; mitochondrial pyruvate carrier.

PV – 051 - **Toxic effects of the drug ibuprofen on ciliates (phylum Ciliophora)**

ALTHAUS, G.L. PUCRS, PORTO ALEGRE - RS - BRAZIL.

Ibuprofen is a widely consumed anti-inflammatory drug and an emerging contaminant of concern in aquatic ecosystems. This study aims to evaluate the ecotoxicological and physiological effects of ibuprofen exposure in ciliates. Different concentrations were evaluated after 24, 48, and 72 h using the MTT assay as an indicator of cellular metabolic activity. NOEC and LOEC values were determined, and oxidative stress will also be assessed. Data were analyzed using Shapiro-Wilk, One-Way ANOVA, and Dunnett's test. After 24 h, ANOVA indicated significant differences among treatments ($p = 0.023$), with 200 mg/L differing from the control ($p = 0.0335$) and showing increased metabolic activity. The NOEC was 100 mg/L and the LOEC was 200 mg/L. After 48 h, ANOVA was also significant ($p = 0.00258$), with 200 mg/L differing from the control ($p = 0.0248$) and again showing increased metabolic activity. The NOEC was 100 mg/L and the LOEC was 200 mg/L. After 72 h, no significant differences were observed ($p = 0.233$), resulting in a NOEC of 200 mg/L and a LOEC > 200 mg/L. Overall, ibuprofen did not reduce the metabolic activity of Tetrahymena thermophila under the conditions tested. Instead, 200 mg/L induced a transient stimulatory response after 24 and 48 h, which was no longer observed after 72 h. Oxidative stress assessment will help determine whether physiological effects occur despite the absence of metabolic inhibition. **Supported by:** CNPQ **Keywords:** Ciliates; Ecotoxicology; Ibuprofen.

PV – 052 - **BENZNIDAZOLE-INDUCED NON-MITOCHONDRIAL OXIDATIVE STRESS IN *Trypanosoma cruzi* EPIMASTIGOTES *IN VITRO***

CAVALCANTE, A.L.B.G.; CAMINHA, M.A.; BOMBAÇA, A.C.S.; VALENTE, R.H.; MENNA-BARRETO, R.F.S.
INSTITUTO OSWALDO CRUZ, FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Benznidazole (Bz) is the frontline therapy for Chagas disease, a neglected tropical disease caused by *Trypanosoma cruzi*. However, its clinical use is limited by variable efficacy and severe side effects. The emergence of Bz-resistant *T. cruzi* strains may explain these treatment limitations. Decreased drug bioactivation by type I and II nitroreductases (NTRs) has been proposed as a key mechanism of resistance. NTR I promotes damage to parasite DNA, whereas NTR II produces superoxide. Despite ongoing debate regarding the role of reactive oxygen species (ROS) in the drug's mechanism of action, Bz is thought to deplete antioxidant defenses by forming thiol adducts. To better understand the mechanisms underlying Bz resistance in *T. cruzi*, we evaluated the contribution of mitochondria, the primary source of ROS, and oxidative stress to the trypanocidal effect of Bz. First, a Bz-resistant strain (Res) was generated by exposing epimastigotes of the wild-type (WT) Dm28c strain to selective pressure. This resistant phenotype was confirmed, as treatment with Bz at the WT IC_{50}/24h (25.6 ± 3.4 µM) did not cause lysis in the Res strain. Increased ROS levels were observed exclusively in Bz-treated WT, with only the antioxidant N-acetylcysteine (NAC) protecting the parasites from the trypanocidal effect. Ultrastructural analysis revealed that Bz induces a mitochondrion swelling phenotype in WT, which was prevented by NAC and absent in the Res strain. Mitochondrial function was also evaluated, showing decreased mitochondrial oxygen consumption in WT and reduced mitochondrial membrane potential, both of which were reversed by NAC, whereas the Res strain remained unaffected. In a preliminary analysis, proteomic data revealed that both the Res and Bz-treated WT strains exhibit lower abundance of mitochondrial complexes and increased NTR I levels, along with differential modulation of antioxidant enzymes by Bz. Surprisingly, MitoTEMPO failed to block ROS production or mitochondrial damage in the WT strain, suggesting that Bz-induced oxidative damage is not primarily mitochondrial, but rather due to a depletion of thiol levels. Our data support the notion that although other mechanisms may act in concert to mediate trypanocidal activity, the *T. cruzi* mitochondrion is a key target of Bz and may contribute to parasite resistance.
Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001 **Keywords:** *Trypanosoma cruzi*; Benznidazole; Resistance.

PV – 053 - **Characterization of candidate components of the neddylation pathway in *Leishmania infantum***

MARUYAMA, F.[1]; GOMES, M.J.M.[1]; DE CORREIA, C.R.S.T.B.[2]; TORRES, C.[2]; TEIXEIRA, F.R.[1].
1. UFSCAR, SÃO CARLOS - SP - BRAZIL; 2. USP, RIBEIRÃO PRETO - SP - BRAZIL.

Leishmania infantum, the etiological agent of visceral leishmaniasis, is a trypanosomatid protozoan with a life cycle alternating between invertebrate and vertebrate hosts, whose adaptation depends on cellular regulatory mechanisms. Among these, the ubiquitin-proteasome system plays a fundamental role in trypanosomatids, regulating processes such as cell-cycle progression, differentiation between parasite forms, and infectivity. In this context, neddylation, a post-translational modification characterized by the conjugation of the ubiquitin-like protein NEDD8 to target proteins, regulates the activity of cullin-RING ubiquitin ligases (CRLs) and consequently modulates the ubiquitination of different substrates. Like ubiquitination, neddylation occurs through an enzymatic cascade composed of an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ligase. Despite its importance, neddylation has not yet been characterized in *L. infantum*. Therefore, this study aimed to contribute to the functional characterization of candidate proteins involved in this pathway in the parasite. Genes encoding orthologous or structurally similar proteins to those of the *H. sapiens* neddylation system were cloned into mammalian expression vectors to validate their interactions. LinfUBA3 and LinfNAE1, which form the neddylation E1 heterodimer, and two potential proteins involved in neddylation regulation, LinfCAND1 and LinfE3 NEDD8, were initially investigated. Co-immunoprecipitation assays in HEK293T cells detected an interaction between LinfUBA3 and LinfNAE1 and their association with LinfNEDD8, demonstrating that these proteins are components of the parasite neddylation system. Interactions among the other selected components of the neddylation pathway will be evaluated, as well as the interaction between CAND1 and Cullin-1 directly in *L. infantum*. These results may contribute to the characterization of neddylation in *Leishmania*.
Supported by: FAPESP: 2025/27394-6 **Keywords:** Neddylation; Leishmania infantum; Ubiquitin-proteasome system.

PV – 054 - **Gene Silencing in *Trypanosoma cruzi*: Establishing the CRISPR-Cas13d System – Construction of Expression Vectors**

DUDZEVICIUS, E.; CALDERANO, S.G.; GONÇALVES, É.M.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

The functional study of genes in *Trypanosoma cruzi* is hampered by the lack of efficient genetic tools for gene knockdown, such as RNAi. The CRISPR-Cas13 system, a programmable RNA-guided RNase, emerges as an innovative tool for specific RNA silencing. Unlike the well-established Cas9, which cleaves DNA, Cas13 is directed to a target mRNA through a guide RNA (crRNA) and degrades it, preserving genome integrity. However, Cas13 activation can trigger off-target effects, where nonspecific RNAs may be degraded, leading to cellular toxicity. To overcome this limitation, we chose the HfCas13d variant from *Ruminococcus flavefaciens*, which carries mutations that minimize collateral degradation of nonspecific RNAs due to its compact size and high fidelity. The strategy of this project is to reduce the potential toxicity of Cas13 in the parasite by generating two recombinant strains: one with constitutive expression (pLew13 vector) and another with tetracycline-inducible expression (pTcIndex vector). This dual approach will allow us to select the most stable and least toxic form for knockdown assays. In this initial stage, the focus was on constructing the expression vectors. The HfCas13d sequence was amplified by PCR, digested, and cloned into the pLew13 and pTcIndex vectors. The constructs will be confirmed by restriction digestion and colony PCR. Preliminary results aim to confirm the successful generation of the pLew13-HfCas13d and pTcIndex-HfCas13d plasmids, as the production of these vectors constitutes the fundamental basis of the project, enabling the subsequent steps of transfection and selection of recombinant *T. cruzi* epimastigote strain. The implementation of this protocol will open new perspectives for the functional genetics of the parasite, accelerating the validation of pharmacological targets and the study of essential genes. **Supported by:** FAPESP 2024/20554-5 **Keywords:** CRISPR Cas13d; *Trypanosoma cruzi*; RNA Knockdown.

PV – 055 - **Genomic characterization of the clinical isolate LVH66 of *Crithidia sp*. LVH60A associated with visceral leishmaniasis: insights from long-read sequencing and genome annotation**

OLIVEIRA, V.D.S.[1]; GARCIA, A.M.L.[2]; TORRES, C.[1]; ROGERIO, L.A.[3]; DE ALMEIDA, R.P.[4]; MARUYAMA, S.R.C.[2].
1. RIBEIRÃO PRETO MEDICAL SCHOOL, UNIVERSITY OF SÃO PAULO (FMRP-USP), RIBEIRÃO PRETO - SP - BRAZIL; 2. SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO (FCFRP-USP), RIBEIRÃO PRETO - SP - BRAZIL; 3. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NATIONAL INSTITUTES OF HEALTH (NIAID/NIH), ROCKVILLE - UNITED STATES OF AMERICA; 4. FEDERAL UNIVERSITY OF SERGIPE (UFS), ARACAJU - SE - BRAZIL.

Monoxenous trypanosomatid infections in humans are atypical but have been reported in Brazil, including Crithidia spp. isolated from patients with visceral leishmaniasis (VL). These infections remain poorly explored. The clinical isolate LVH66, obtained from a patient with VL in Sergipe, was sequenced using Oxford Nanopore Technology and Illumina to assess its relationship with the previously characterized Crithidia sp. LVH60A and generate an improved genome assembly. Long reads underwent quality control and assembly with Flye, Canu, or NextDeNovo, followed by hybrid polishing with short and long reads. The Flye and NextDeNovo assemblies showed the best performance, with genome sizes of 34.1 to 34.7 Mb and N50 values of 1.19 to 1.34 Mb, respectively. Whole genome alignments (WGA) using NUCmer (MUMmer) showed that all contigs from the Flye and NextDeNovo assemblies aligned to all 38 pseudochromosomes of the Crithidia sp. LVH60A reference genome (GCA_030078075.1). NextDeNovo showed greater structural continuity and was selected for downstream analyses. QUAST analysis estimated 96.78% coverage of the Crithidia sp. LVH60A reference genome, while PyANI+ estimated an average nucleotide identity of 98.73%. BUSCO analysis using the Euglenozoa dataset indicated 98.9% completeness, with 98.5% of orthologs present as single copies. The NextDeNovo assembly resulted in 31 chromosome-level sequences and was annotated with Companion using C. fasciculata as reference. A total of 10,524 gene models were identified, including 10,272 protein-coding genes, 289 pseudogenes, and 252 noncoding genes; functional annotation was assigned to 6,112 genes. Overall, these results support the genomic assignment of LVH66 to the Crithidia sp. LVH60A lineage, provide a high-quality genomic resource for this putative novel species, and establish a robust basis for future functional and comparative analyses within Trypanosomatidae. **Supported by:** CNPq 132058/2026-0 **Keywords:** Crithidia sp; LVH60A; comparative genomics; gene annotation.

PV – 056 - **DNA metabarcoding reveals seasonal dynamics of microeukaryotic communities along a pollution gradient in Guaíba Lake, Brazil**

PEREIRA, S.D.M.[1]; UTZ, L.[2].
1. PUCRS, PELOTAS - RS - BRAZIL; 2. PUCRS, PORTO ALEGRE - RS - BRAZIL.

Several factors affect the functioning of freshwater ecosystems and influence their trophic state. In this context, environmental changes directly impact biodiversity. Unicellular eukaryotes (i.e., protists) play a fundamental role in ecosystem functioning, contributing to nutrient cycling and structuring aquatic food webs. The composition of these communities is considered a valuable indicator of biological quality, used to monitor aquatic ecosystems and assess their ecological status and trophic condition. In this study, we investigated the effects of water quality and seasonal variation on unicellular eukaryotic communities in Guaíba lake, southern Brazil, using DNA metabarcoding. Both water quality and seasonality significantly influenced community composition at the sampled sites. Autotrophic and heterotrophic organisms exhibited distinct seasonal patterns, with some showing greater abundance in specific water quality and seasons. These patterns suggest their potential use as bioindicators of water quality. Overall, our study highlights the significant effects of water quality and seasonal variation on the structure of unicellular eukaryotic communities in a large subtropical lake. **Supported by:** CAPES E CNPQ **Keywords:** biomonitoring; environmental DNA; freshwater.

PV – 057 - **Molecular observations of Plasmodium in mosquitoes, birds blood and feces samples at Cruzeiro do Sul, Acre: an exploratory study.**

DAMASCENO, V.L.S.[1]; DA CUNHA, T.P.[2]; ABREU, W.U.[1]; BRILHANTE, A.F.[3]; DOS SANTOS, T.C.[1]; COMPAROTI, G.L.M.[1]; FERREIRA, L.J.[1]; BISPO, T.R.[4]; RODART, I.F.[4]; CABRAL, A.D.[4]; LAPORTA, G.Z.[5]; SPERANCA, M.A.[1].
1. UFABC - UNIVERSIDADE FEDERAL DO ABC, SÃO BERNARDO DO CAMPO - SP - BRAZIL; 2. FMABC - FACULDADE DE MEDICINA DO ABC, SANTO ANDRÉ - SP - BRAZIL; 3. UFAC - UNIVERSIDADE FEDERAL DO ACRE, RIO BRANCO - AC - BRAZIL; 4. FACULDADE DE CIÊNCIAS MÉDICAS DA SANTA CASA DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 5. FMABC - FACULDADE DE MEDICINA ABC, SANTO ANDRÉ - SP - BRAZIL.

The hemoparasite of the *Plasmodium* genus are widely distributed, being transmitted by Diptera from the *Culicidae* family. Even though the molecular framework has evolved, there is still a gap in information about *Plasmodium* distribution, especially in the Amazon region. This study report presents molecular evidence and presence of *Plasmodium* in preliminary results in mosquitoes, blood and feces samples in domestic birds gathered in Projeto de Assentamento Dirigido (PAD) Santa Luzia, Cruzeiro do Sul, Acre. The PAD was divided into sectors to better identify samples. 721 mosquitoes were collected via Shannon trap in 2024. 14 blood samples of domestic ducks (*Anas platyrhynchos domesticus*) were collected in 2025. For fecal matter, 17 samples were collected from domestic chickens (*Gallus gallus domesticus*). Samples were submitted to DNA extraction by TRIzol-chloroform method; PCR-CytB (*Haemoproteus, Plasmodium, and Leucocytozoon*); (5'CATATATTAAGAGAAITATGGAG), (3' ATAGAAAGATAAGAAATACCATTC), generating 500pb amplicon; PCR-Nested (*Haemoproteus and Plasmodium*); (5' ATGGTGCTTTCGATATATGCATG), (3'GCATTATCTGGATGTGATAATGGT) generating a 479pb amplicon and molecular confirmation via Sanger sequencing. Thus, Plasmodium sp. was detected in some samples. In a Culex (Cux.) sp. mosquito, Sanger sequencing of the CytB amplicon confirmed Plasmodium sp., also detected in domestic ducks, with 3 positive samples for both molecular markers. One domestic chicken fecal sample was also positive, suggesting infected vector ingestion and possible spread of avian *Haemosporidian* or *Plasmodium* in the regional ecosystem. The positive samples were found in areas interconnected by access roads, suggesting spreading in the regional ecosystem. The detection in fecal matrix, although requiring validation via sequencing, emerges as a new perspective for tracking based on feeding habits. **Supported by:** FAPESP 2022/10392- 2 **Keywords:** Plasmodium; Molecular epidemiology; Nested-PCR.

PV – 058 - **Exploring the Evolutionary Dynamics of Ribosome through Amoebozoa Genomic Architecture and Deep Eukaryotic Phylogenomics**

BIM, M.A.[1]; BARBOSA, J.P.A.[1]; KATZ, L.A.[2]; BROWN, M.[3]; LAHR, D.J.G.[1].
1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. SMITH COLLEGE, NORTHAMPTON - UNITED STATES OF AMERICA; 3. MISSISSIPPI STATE UNIVERSITY, STARKVILLE - UNITED STATES OF AMERICA.

Ribosomes are universally conserved, but their evolutionary dynamics remain poorly explored in non-model eukaryotes. Here, we address ribosome evolution through a dual-scale approach: investigating rDNA architecture in testate amoebae (Amoebozoa: Arcellinida) and tracing ribosomal protein (RP) evolution throughout the eukaryotic tree of life. We sequenced and assembled long-read genomic data from two testate amoeba species, Arcella uspiensis and Cryptodifflugia operculata. Both encode their rDNA within putative circular extrachromosomal elements (rECEs) (~16.2 kb and ~10.1 kb) that contain canonical rDNA operons alongside non-coding short tandem repeat (STR) regions. Experimental long-range PCR revealed structural size variations within the STRs in A. uspiensis. This represents the first report of rDNA-encoding extrachromosomal elements in Arcellinida, the most diverse order within Amoebozoa. Concurrently, we reconstructed preliminary maximum-likelihood trees for 10 RPs with a three-domain, taxon-rich dataset using the EukPhylo pipeline. None of the trees show deep paralogy. Most eukaryotic groups lack ingroup paralogs, including lineages with generally high paralogy levels, such as Amoebozoa. This result supports the hypothesis that the fixation of duplicated genes encoding proteins for large macromolecular complexes is strongly constrained because of the need for equimolar concentrations of their components (gene dosage balance hypothesis). However, specific lineages show ingroup expansions: land plants have paralogs resulting from whole-genome duplications, while dinoflagellates maintain multiple expressed paralogs, suggesting potential differential expression in this group. Moreover, the trees highlight clear endosymbiotic gene transfer events in photosynthetic eukaryotes. By combining long-read genomics and deep phylogenomics, this work illuminates rDNA architecture in Arcellinida and provides the first taxon-rich evolutionary framework for eukaryotic RPs. **Supported by:** CAPES 88887.986366/2024-00 **Keywords:** ribosomal proteins; extrachromosomal rDNA; Arcellinida.

PV – 059 - **Characterization of the antiparasitic activity of the L-type calcium channel modulator FPL64176 in *Trypanosoma cruzi* for target deconvolution and mechanism-of-action studies**

DE FREITAS, F.R.N.[1]; SANTOS, M.M.D.J.[1]; FREITAS JUNIOR, L.[2]; MORAES, C.B.[1].
1. FACULDADE DE CIÊNCIAS FARMACÊUTICAS - USP, SÃO PAULO - SP - BRAZIL; 2. INSTITUTO DE CIÊNCIAS BIOMÉDICAS - USP, SÃO PAULO - SP - BRAZIL.

Chagas disease remains a public health concern. The limited efficacy and high toxicity of available treatments underscore the need for new therapeutic targets. In this context, FPL64176, an L-type calcium channel modulator, exhibits antiparasitic activity against *Trypanosoma cruzi* in phenotypic screens. However, its mechanism of action remains unclear. Thus, the goal of this study is to characterize the activity of FPL64176 and investigate its mechanism of action and deconvolution in *T. cruzi*. We evaluated antiparasitic activity in intracellular amastigotes from clones of different DTUs using a strain panel and time-kill assays to characterize kinetics over time. In the strain panel, FPL64176 exhibited consistent antiparasitic activity (EC_{50} = 5.9–7.0 µM) across T. cruzi clones. However, cytotoxicity (CC_{50} = 4.6–41.5 µM) was more variable. Time-kill assays revealed an increase in antiparasitic potency with exposure time. In Y cl H10-S, the EC_{50} decreased from 0.04 µM after 72 hours to 0.01 µM after 120–144 hours (maximum activity: 85%). In Sylvio cl X10/1, the EC_{50} was 6.2 µM at 72–96 hours, decreasing to 2.0 µM at 144 hours (maximum activity: 100%). The time-kill data refer to the first replicate, with extra replicates currently being analysed to check if the results can be reproduced. The process of selecting for resistance in *T. cruzi* YH10S epimastigotes began with an initial concentration of 0.28 µM (the EC_{50}), followed by a gradual increase in the concentration of FPL64176 over the course of the passages. The current selective pressure is 12 µM. The goal is to reach 50 X the initial EC_{50}, which is 14 µM. Genomic sequencing and RNA-seq using resistant populations will enable the identification of genetic and transcriptional alterations associated with resistance. Candidate alterations will then be validated through gene editing approaches. This integrated strategy will advance the deconvolution of targets. It will also elucidate the mechanism of action of FPL64176 in *T. cruzi*. **Supported by:** 2025/05894-7 **Keywords:** *Trypanosoma cruzi*; Target deconvolution; FPL64176.

PV – 060 - **Analysis of regulated transcripts and the cellular localization of the TRMT6/TRMT61A heterodimer in Leishmania mexicana.**

DE MELO, L.C.R.P.[1]; BEZERRA, A.M.[1]; REZENDE, A.M.[2]; REIS, C.R.D.S.[1]; MOURA, D.M.N.[1].
1. INSTITUTO AGGEU MAGALHÃES - FIOCRUZ PE, RECIE - PE - BRAZIL; 2. INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BELO HORIZONTE - MG - BRAZIL.

Trypanosomatids rely extensively on post-transcriptional mechanisms to regulate gene expression. RNA modifications have emerged as important components of this regulatory landscape, with the potential to influence transcript stability, processing, and translation. Among these modifications, N1-methyladenosine (m1A) has been detected in trypanosomatid RNA, although its biological functions in these parasites remain poorly understood. In other eukaryotes, m1A is deposited by the TRMT6/TRMT61A methyltransferase complex. The presence of conserved components of this machinery in trypanosomatids suggests that m1A methylation may contribute to post-transcriptional gene regulation in these organisms. Based on a transcriptome analysis following CRISPR-mediated knockout of the TRMT61A gene in *L. mexicana*, we identified specific genes exhibiting differences in expression. To further characterize these TRMT6 and TRMT61A orthologs, the present study aimed to validate the differential expression of selected genes impacted after knockout and determine the subcellular localization of the heterodimer in *L. mexicana*. Using RT-qPCR, the expression of selected transcripts in the TRMT61A knockout strain was analysed and compared with previously obtained RNA-seq data. In parallel, the cellular localization of TRMT6 and TRMT61A tagged to a HA epitope was analyzed by immunofluorescence. Therefore, the RT-qPCR assays corroborated the differential expression pattern for upregulated genes (eif3h and hsp60) and downregulated genes (L1a and lysophospholipase), suggesting a potential regulatory function of this protein. Furthermore, the subcellular localization results displayed a predominant nuclear pattern for both proteins, which is consistent with a potential function as an RNA methyltransferase. Therefore, the results showed here contributed to the functional characterization of TRMT6 and TRMT61A proteins in *Leishmania mexicana.* **Supported by:** FACEPE/ BIC-1703-2.02/26
Keywords: m1A; RNA methylation; gene expression.

PV – 061 - **Promising drug candidates targeting the *Leishmania* telomerase**

FONTES, V.S.[1]; SERAFIM, M.S.M.[2]; DE OLIVEIRA, E.G.[2]; SALVADOR, G.H.M.[1]; AGOSTINO, V.D.S.[3]; GERES, L.F.[3]; DE OLIVEIRA, R.B.[2]; FONTES, M.R.D.M.[1]; COELHO, A.C.[3]; FERNANDES, C.A.H.[1]; MALTAROLLO, V.G.[2]; ALVES, R.J.[2]; CANO, M.I.N.[1].
1. UNIVERSIDADE ESTADUAL PAULISTA, BOTUCATU - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL; 3. UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS - SP - BRAZIL.

Leishmaniasis is caused by parasites of the genus *Leishmania,* and the treatment is still a challenge due to the toxicity and limited efficacy of available drugs. This study aims to investigate telomerase, particularly its catalytic subunit, telomerase reverse transcriptase (TERT), as a feasible therapeutic target in *Leishmania* by integrating computational screening, biological assays, and mechanistic investigation. Initially, our target-based screening using molecular docking performed with GOLD 5.1 was employed with the Brazilian Compound Library (BraCoLi, *n* = 1,176). Seventeen compounds with favorable predicted interactions with the *L. major* TERT were prioritized, of which twelve were available for *in vitro* evaluation. Two compounds termed CAPCL and CAMNF, derivatives of an aryloxypropylamine scaffold, showed the most promising antileishmanial activity against promastigotes of *L. major* and *L. infantum*, with EC_{50} values of approximately 30 µM for CAPCL and 5 µM for CAMNF. These two compounds were previously reported with antileishmanial activity against *L. amazonensis* promastigotes, with EC_{50} values of 10 µM for CAMNF and 3 µM for CAPCL. We also performed additional assays using TERT-knockout lines previously generated by our group, whose parasites lack a functional telomerase. These transgenic lines showed reduced activity for both compounds, supporting the possible involvement of telomerase as a potential target. The most promising compound, CAMNF, showed moderate cytotoxicity on bone-marrow murine macrophages *in vitro* (CC_{50} ~20 µM). Therefore, CAPCL and CAMNF are promising scaffolds for further optimization and synthesis of more selective analogs. Overall, these findings support the potential of the parasite telomerase as a drug target and provide the basis for the development of new antileishmanial drug candidates. **Supported by:** FAPESP 2025/13050-3, CNPQ, CAPES
Keywords: Leishmaniasis; drug discovery; TERT.

PV – 062 - **Does RAD1 contribute to replication stress response in *Leishmania major*?**

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

Leishmania parasites display remarkable genome plasticity, yet must preserve sufficient genome integrity to survive replication-associated stress. The RAD9–RAD1–HUS1 (9-1-1) complex is a conserved DNA damage checkpoint clamp, but the specific role of RAD1 in *Leishmania major* remains poorly understood. Here, we investigated RAD1 through evolutionary, structural and functional approaches. Sequence and phylogenetic analyses showed strong conservation among *Leishmania* orthologues, while AlphaFold2 modelling identified a conserved PCNA-like core interrupted by kinetoplastid-enriched, low-confidence regions. CRISPR–Cas9-mediated editing generated a partially disrupted RAD1+/Δ::NEO line and N-terminally 3×Myc-tagged parasites. Partial RAD1 disruption did not cause a sustained growth defect under standard culture conditions, but significantly increased sensitivity to hydroxyurea (HU) genotoxic replication stress, as shown by reduced survival across intermediate HU concentrations and a lower area under the survival curve ($P = 0.0247$). Biochemical cellular fractionation showed preferential recovery of RAD1 in the nuclear-enriched fraction, whereas total RAD1 abundance remained broadly unchanged during recovery from HU exposure. Preliminary γH2A imaging further suggested that reduced RAD1 function may alter replication-stress-associated chromatin signalling and flow-cytometric analysis after acute and chronic treatment that RAD1 depletions may be involved in late fork collapse. These findings support RAD1 as a structurally conserved but kinetoplastid-divergent component of the 9-1-1 machinery and indicate that RAD1 contributes to replication-stress tolerance in *L. major*. Further genetic complementation and direct measurements of DNA synthesis will be required to define the molecular mechanisms underlying this phenotype. **Supported by:** FAPESP 2024/07364-2 **Keywords:** Replication checkpoint; Rad1; DNA damage.

PV – 063 - **Functional characterization and cellular localization profile of the kinase CRK3 during the cell cycle of *Trypanosoma cruzi***

PANTOJA, A.B.; CORRÊA, A.D.P.; GONÇALVES, É.M.; CALDERANO, S.G.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

The cell cycle in eukaryotes is strictly regulated by cyclin-dependent kinases (CDKs). In *Trypanosoma cruzi*, homologous kinases include CRK1, acting in the G1/S phase, and CRK3, associated with the G2/M phase. Both possess conserved phosphorylation sites for Wee1, a CDK-inhibitory kinase involved in response to DNA damage. This study aimed to characterize the functional role and establish the cellular localization profile of the CRK3 protein in T. cruzi, investigating its essentiality and dynamics throughout the cell cycle. For this purpose, recombinant lines were generated using CRISPR/Cas9-mediated genome editing in the Dm28c strain, targeting the knockout of the CRK3 gene and the endogenous insertion of a Myc epitope at the 3' end of the coding region. Genomic edits were confirmed by PCR molecular validation. Phenotypic characterization was evaluated by monitoring the growth curve over 10 consecutive days. The cellular localization profile was mapped by immunofluorescence (IF) in the CRK3-Myc line. Additionally, the phosphorylation profile at Y15 was investigated by anti-Y15 Western blotting (WB). CRISPR/Cas9 editing resulted exclusively in the generation of the single-knockout line (CRK3+/-), which displayed a growth and proliferation profile comparable to the control line. IF analyses revealed that CRK3 exhibits nuclear localization and a signal associated with kDNA, with its nuclear presence being consistent with the site of action of Wee1 kinase. Additionally, WB assays demonstrated that the Y15 residue is exclusively phosphorylated in CRK3 when compared to CRK1. In conclusion, CRK3 exhibits dynamic nuclear localization and phosphorylation regulation via Wee1. The inability to obtain a homozygous knockout confirms the essentiality of the CRK3 gene in T. cruzi, consolidating this kinase as a promising target against Chagas disease. **Supported by:** FAPESP 2024/20554-5 **Keywords:** *Trypanosoma cruzi*; CRK3; cell cycle.

PV – 064 - **Is Wee1 the Only One? Conserved functions and new insights into inhibitory CRK regulation in *Trypanosoma cruzi***

GONÇALVES, É.M.; PANTOJA, A.B.; CALDERANO, S.G.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Although Wee1 is a conserved kinase that regulates cell cycle progression and genome integrity through the inhibition of Cyclin-dependent kinases (CDKs) in eukaryotes, its role in trypanosomatids remains poorly understood. In *Trypanosoma cruzi*, orthology analyses identified Wee1 as the only CDK-inhibitory protein, with no other canonical inhibitors detected. Here, we characterized Wee1 expression and localization and investigated the effects of its loss on cell cycle, DNA damage, and response to benznidazole (BZN). CRISPR/Cas9 was used to insert a c-Myc tag at the endogenous Wee1 locus and generate double-knockout (Wee1-/-) parasites. Wee1 expression peaked during S phase, exhibited predominantly nuclear localization, and was absent from the nucleus during mitosis. Wee1 was also undetectable in metacyclic forms. Preliminary observations suggest that Wee1 loss may affect parasite proliferation, DNA damage accumulation, zoid formation, and may increase sensitivity to BZN. Antibodies recognizing CDK Y15 and T14 phosphorylation in mammals also detected corresponding phosphorylated proteins in T. cruzi, supporting conservation of these regulatory sites. Notably, Y15 phosphorylation decreased in Wee1-/- parasites, whereas T14 phosphorylation remained unchanged, suggesting a conserved role for Wee1 in Y15 phosphorylation and possible regulation of T14 by a distinct kinase, such as a MYT1 analog. Notably, Y15 phosphorylation was detected in Cyclin-related kinases (CRKs), specifically CRK3 but not CRK1, consistent with CRK3 nuclear localization and suggesting that CRK3 may be regulated by Wee1. Overall, these findings indicate conserved features of T. cruzi Wee1, including cell cycle-dependent expression, mitotic nuclear depletion, and absence in non-proliferative cells. Ultimately, Wee1 appears to contribute to the maintenance of DNA integrity and cell proliferation, highlighting its potential as a target for combinatorial therapies with genotoxic agents. **Supported by:** FAPESP (2024/23848-0 and 2024/20554-5) **Keywords:** Trypanosomatids; Cell cycle regulation; CRK phosphorylation.

PV – 065 - **Transcriptomic analysis of *Trypanosoma cruzi* epimastigotes expressing a dominant-positive Ras family protein (TcRlp-Q61K) reveals alterations in vesicular trafficking, glycocalyx remodeling, and metabolic adjustments**

DE FARIAS, K.L.; NEPOMUCENO-SILVA, J.L.; LEAL, M.D.S.
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, MACAE - RJ - BRAZIL.

Ras family GTPases act as essential molecular switches controlling cell proliferation, growth, and differentiation by alternating between active (GTP-bound) and inactive (GDP-bound) states. The parasite *Trypanosoma cruzi* possesses a single Ras-family gene encoding the protein TcRlp (*T. cruzi* Ras-like protein), whose exact physiological role remains uncharacterized. Previously, our group demonstrated that expression of the GTP-locked dominant-positive mutant TcRlp-Q61K reduces epimastigote proliferation, increases *in vitro* metacyclogenesis, and causes ultrastructural alterations in the mitochondria adjacent to the kinetoplast. To understand the functional impact of this constitutive activation, this work aimed to characterize the comparative transcriptomic profile of epimastigotes expressing TcRlp-Q61K versus control lineages during the logarithmic growth phase, identifying differentially expressed genes (DEGs) to map affected signaling pathways and potential Ras effectors. Total RNA from parasites expressing TcRlp-Q61K and control lineages was extracted and submitted to mRNA sequencing (Illumina NovaSeq X). Bioinformatic analysis integrated quality control, trimming, alignment to the *T. cruzi* Dm28c reference genome (Berna et al., 2018), quantification, PCA, Volcano Plot, Heatmap, and functional annotation by Gene Ontology (GO). We identified 34 significant DEGs (21 upregulated and 13 downregulated). Gene Ontology (GO) analysis revealed an integrated cellular response, highlighting the 60S ribosomal protein L11 (C4B63_38g161) and Group V trans-sialidase (C4B63_134g54) as key functional hubs associated with translational machinery and cell surface/secretory pathway remodeling, respectively. These hubs were accompanied by coordinated adjustments in lipid homeostasis (sphingolipid metabolism, acyltransferases, and UDP-dependent glycosylation), amino acid and peptide turnover (arginine/glutamine pathways and glutamyl carboxypeptidases), and chromatin/transcriptional regulation (Histone H3 and RNA polymerase II subunits). These findings indicate that constitutive activation of TcRlp triggers a focused compensatory response impacting translational tuning, lipid membrane composition, and glycocalyx dynamics rather than generalized metabolic stress. Currently, individual characterization of DEGs, variant screening, and KEGG pathway analyses are in progress to fully integrate TcRlp into the *T. cruzi* functional signaling network. **Supported by:** CAPES **Keywords:** Ras GTPase; Transcriptomics; Chagas disease.

PV – 066 - **From *in silico* screening to drug repurposing: identification of Encorafenib and Dabrafenib as candidate TgCDPK1 inhibitors against *Toxoplasma gondii***

CARVALHO, J.P.B.; DE SANTANA, G.A.; TELACIO, P.J.; MEZZICH, G.; ANTUNES, D.; RAMOS, A.C.G.; ADESSE, D.
FIOCRUZ, RIO DE JANEIRO - RJ - BRAZIL.

Toxoplasma gondii is an obligate intracellular parasite and the etiological agent of toxoplasmosis, a major cause of infectious retinochoroiditis worldwide, with particularly severe disease cases reported in Brazil. Current therapy, primarily based on sulfadiazine and pyrimethamine, is effective against the proliferative stages of *T. gondii* but has limited efficacy against persistent tissue cysts, and may cause severe adverse effects, which highlights the need for alternative therapeutic strategies. Calcium-dependent protein kinase 1 of *T. gondii* (TgCDPK1), which participates in essential processes of parasite invasion and replication, represents an attractive target for antiparasitic drug development. Here, we investigated whether structure-based screening of existing pharmacological compounds could identify candidates for repurposing against TgCDPK1. Virtual screening using Boltz-2 identified the BRAF inhibitors encorafenib and dabrafenib as the first- and second-ranked candidates, respectively. Subsequent molecular-dynamics analysis supported a stable predicted interaction of dabrafenib with TgCDPK1, with protein root mean square deviation (RMSD) remaining below 3 Å and ligand RMSD below 4 Å, while the compound remained within the predicted binding site. To determine whether these candidates displayed acceptable host-cell tolerability, cytotoxicity was evaluated over a concentration range of 1.56–200 µM for up to 48 h. CellTiter-Glo assays in Vero cells and MTT assays in ARPE-19, HFF and NHDF cells showed substantial preservation of host-cell viability across a broad concentration range, although concentration- and exposure-dependent reductions were observed at the highest concentrations. Together, these findings identify encorafenib and dabrafenib as repurposing candidates emerging from a TgCDPK1-directed *in silico* screening strategy and establish a concentration range for subsequent evaluation of their antiparasitic activity against *T. gondii.* **Supported by:** CNPq, CAPES, FAPERJ, FIOCRUZ. **Keywords:** Toxoplasmosis; drug repurposing; in silico, chemotherapy.

PV – 067 - **Molecular characterization of the *Trypanosoma rangeli* inhibitor of serine peptidase 2 (TrgISP2) and comparison with TcISP2 aimed at differential diagnostic methods for Chagas disease.**

FERREIRA, L.J.; SANTANA, C.M.; JORDÃO, F.T.; COMPAROTI, G.L.M.; DAMASCENO, V.L.S.; CABRAL, A.D.; SPERANCA, M.A.
UNIVERSIDADE FEDERAL DO ABC, SÃO BERNARDO DO CAMPO - SP – BRAZIL

.

The hemoflagellate protozoan *Trypanosoma rangeli* shares hosts and vectors with *Trypanosoma cruzi*, the causative agent of Chagas disease. Although non-pathogenic to humans, the marked antigenic similarity between both species poses challenges to the accuracy of differential diagnosis. In *T. cruzi*, inhibitor of serine peptidase 2 (ISP2) has been described as an immune evasion factor and a promising molecular marker; however, little is known regarding the role of its homolog in *T. rangeli* (TrgISP2) in parasite-host interactions. Therefore, this study aims to molecularly characterize the gene encoding TrgISP2 and its corresponding recombinant protein via PCR, gene cloning, protein expression, and enzymatic activity assessment. The proposal was prompted by the prior identification of *T. rangeli* in samples processed by the research team, enabling preliminary amplification steps of the target gene fragment. However, sequencing data analysis revealed non-specific products, indicating the need to adjust stringency conditions and design specific primers. To address this limitation and ensure assay accuracy, reference isolates of *T. rangeli* are being incorporated as positive controls for reaction standardization and the validation of subsequent expression steps. Elucidating the structural and functional differences between TrgISP2 and TcISP2 may reveal specific markers for the differential diagnosis of Chagas disease, enhancing accuracy in epidemiological studies and the development of biotechnological tools. Expected outcomes include confirming the presence and expression of the *TrgISP2* gene, as well as establishing preliminary parameters for protein functionality, consolidating a solid foundation for future public health research. **Supported by:** FAPESP 2026/00251-3 **Keywords:** Trypanosoma rangeli; ISP2; Molecular diagnosis.

PV – 068 - **Characterization of the tRNAs Isopentenylation Pathway in *Trypanosoma cruzi***

DE AMORIM, J.E.C.[1]; BRAGA, M.S.[2]; SILBER, A.M.[2]; NASCIMENTO, J.D.F.[1].
1. LABORATORY OF TRYPANOSOMATIDS TRNA BIOCHEMISTRY, CHEMISTRY INSTITUTE, UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL; 2. LABORATORY OF BIOCHEMISTRY OF TRYPS - LABTRYPS, DEPARTMENT OF PARASITOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES (ICB II), UNIVERSITY OF SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL.

Chagas disease is caused by *Trypanosoma cruzi*, which has the ability to transition between invertebrate and vertebrate hosts and employs several mechanisms that enable it to adapt to different environments. Many of these regulatory mechanisms depend on the rapid regulation of gene expression, which occurs predominantly at the post-transcriptional level. In this context, the modulation of translation through post-transcriptional modifications (PTMs) in tRNA nucleotides stands out. One such modification is N6-(isopentenyl) adenosine (i^6A), which is found at adenosine 37 in the anticodon loop of cytosolic and mitochondrial tRNAs that recognize codons initiated by uridine (U). In other eukaryotes, this modification is catalyzed by the isopentenyltransferase enzyme MOD5, using dimethylallyl pyrophosphate derived from the isoprenoid and sterol biosynthetic pathways as a substrate. Because this PTM is located near the codon: anticodon pairing region, the presence of i^6A may influence translation efficiency and fidelity. Although *T. cruzi's* genome codes for a putative ortholog of MOD5, little is known about the biological relevance of the tRNA isopentenylation pathway in this organism. Therefore, the present project aims to functionally unveil the role of the enzyme MOD5 of *T. cruzi.* To this end, genetically modified strains were generated using CRISPR-Cas9 technology, resulting on one hand in Mod5 knockout parasites and on the other hand in Mod5 endogenously fused to the Shark2 and an auxin-inducible degron, which enable knockdown of the mRNA and the protein of interest, respectively. These lineages were subsequently subjected to phenotypic analyses, which showed that diminished MOD5 expression does not alter the proliferation of the parasite's epimastigote form. Thus, elucidating the impacts of this post-transcriptional modification may broaden the understanding of the parasite biology, as well as contribute to the improvement of genetic manipulation and gene silencing strategies in *T. cruzi.* **Supported by:** PIBIC Dow CRIE (2025-3220)
Keywords: tRNAs; isopentenylation; post-transcriptional modifications.

PV – 069 - **Copper coordination compound LR3024 has anti-*Trypanosoma cruzi* activity at nanomolar concentration and Golgi complex alteration**

DO CARMO, M.A.S.[1]; MOREIRA, F.F.[1]; RODRIGUES, Y.M.[1]; ZOCATELI, P.I.A.[1]; JÚNIOR, A.H.[2]; FERNANDES, C.[2]; DAMATTA, R.A.[1]; SEABRA, S.H.[1].
1. UENF, CAMPOS DOS GOYTACAZES - RJ - BRAZIL; 2. UFSC, FLORIANÓPOLIS - SC - BRAZIL.

Chagas disease, caused by *Trypanosoma cruzi*, is a neglected illness with current therapies limited by high toxicity and low efficacy. This study evaluated the antiparasitic activity of three metal coordination compounds against *T. cruzi* epimastigotes (Dm28c strain) *in vitro*. Comparative screening incubating parasites with zinc (JC2025), copper (LR3024), and cobalt (LR1225) compounds at 5 µM (24–96 h) using direct counting with a Neubauer chamber showed that LR3024 and LR1225 reduced parasitic load by >50% at 24 h, whereas JC2025 reached this reduction at 48 h. Notably, LR3024 eliminated 100% of parasites at 72 h. The IC_{50} for LR3024 was ~300 nM. Cytotoxicity assessed by MTT assay in LLC-MK2 cells (72 and 96 h) revealed selectivity indices of 11.3 (72 h) and 10.8 (96 h). Transmission electron microscopy of parasites treated with 300 and 1000 nM of LR3024 for 72 and 96 h demonstrated ultrastructural alterations in the Golgi complex. LR3024 exhibited potent nanomolar trypanocidal activity and induced organelle damage in *T. cruzi*, highlighting its potential as a promising candidate for novel antichagasic drug development. **Supported by:** **Keywords:** Trypanossoma cruzi; Coordination compound; Nanomolar activity.

PV – 070 - **Phylogenetic study of Caenomorphidae (Ciliophora, Intramacronucleata) with new data from Brazilian strains of *Caenomorpha* sp. and *Sulfonecta* sp.**

YOSHINO, J.M.D.O.[1]; BORGES, B.D.N.[1]; PAIVA, T.D.S.[2].
1. UFPA, BELÉM - PA - BRAZIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRAZIL.

The Caenomorphidae comprise a ciliate clade of the subphylum Intramacronucleata, restricted to anoxic or microaerophilic aquatic environments, which harbor methanogenic prokaryote symbionts. Knowledge of their diversity—still poorly explored in Brazil—as well as their natural history, is essential for understanding the evolution of anaerobiosis in ciliates. In the present study, we analyze the phylogenetic affinities of caenomorphids based on 18S rDNA sequences present in the NCBI/GenBank, with addition of newly obtained ones from *Caenomorpha* sp. and *Sulfonecta* sp., collected from a eutrophic pond in Rio de Janeiro, Brazil. Based on our results and revision of previous phylogenetic studies, the systematics of Caenomorphidae is discussed. Phylogenetic analysis (maximum likelihood) supported the separation of the genera *Caenomorpha* and *Sulfonecta*, and did not reject the monophyly of Caenomorphidae relative to other intramacronucleate clades. However, their phylogenetic position is unstable due to high sensitivity to nucleotide alignment parameters variation. Such instability is consistent with previous studies. The Caenomorphidae are normally classified within the class Armophorea, and observations on their ontogeny and overall morphology, coupled with accumulated phylogenetic evidence, suggest they either represent a derived clade diverging at the base of Armophorea or should be raised to class level. **Supported by:** FAPESPA **Keywords:** Anaerobic; Ciliates; Evolution.

PV – 071 - **1,4-NAPHTHOQUINONE EXHIBITS POTENT TRYPANOCIDAL ACTIVITY AGAINST *TRYPANOSOMA CRUZI* THROUGH MITOCHONDRIAL IMPAIRMENT AND REDOX DISRUPTION**

BOMBAÇA, A.C.S.[1]; BERNAL, Y.E.[2]; RAMOS, V.F.D.S.[1]; GUIMARAES, A.C.R.[3]; BARRAGAN, M.L.[2]; JÚNIOR, E.N.D.S.[4]; MENNA-BARRETO, R.F.S.[1].
1. LABORATÓRIO DE BIOLOGIA CELULAR – INSTITUTO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL; 2. FACULTAD DE CIENCIAS, UNIVERSIDAD ANTONIO NARIÑO, BOGOTÁ - COLOMBIA; 3. LABORATÓRIO DE GENÔMICA APLICADA E BIOINOVAÇÕES – INSTITUTO OSWALDO CRUZ, FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL; 4. DEPARTAMENTO DE QUÍMICA – INSTITUTO DE CIÊNCIAS EXATAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRAZIL.

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects millions of people worldwide, particularly in the Americas. Current treatments are based on benznidazole and nifurtimox, but their use is hampered by limited and variable efficacy in established chronic disease and adverse effects that may lead to treatment discontinuation. These limitations highlight the need for more effective drugs. Our group has focused on the synthesis and identification of novel naphthoquinoidal compounds with potent antiparasitic activity. Among these molecules, β-lapachone, a compound with pro-oxidant activity, has been extensively investigated for its effects on reactive oxygen species (ROS) production and mitochondrial function in *T. cruzi*. Here, we evaluated the trypanocidal activity of 5-hydroxy-6-iodo-2-methyl-1,4-naphthoquinone (NQ) and investigated its mechanism of action. This compound was active against all three parasite forms, displaying low IC_{50}/24 h values. In infective bloodstream trypomastigotes, the IC_{50}/24 h value was 658.0 ± 80.0 nM, whereas for intracellular amastigotes it was 326.0 ± 27.0 nM. Cytotoxicity assays in peritoneal macrophages revealed a selectivity index of 44.6. The IC_{50}/24 h value in epimastigotes was 7.8 ± 0.9 µM, and pre-incubation with the antioxidants N-acetylcysteine (NAC), glutathione (GSH), and trypanothione [$T(SH)_2$] protected parasites from the trypanocidal effect. Ultrastructural analyses revealed marked mitochondrial swelling, loss of cristae, and a washed-out matrix. These alterations may occur early during NQ treatment, as evidenced by reductions of 45% in mitochondrial membrane potential ($\Delta\Psi m$) and 40% in O_2 consumption after 2 h. Interestingly, trypanothione reductase (TR) activity was reduced by 42%, and GSH pre-treatment protected enzyme activity. These findings suggest that NQ interferes with the cellular GSH/GSSG pool and that depletion of the reduced form compromises the antioxidant system and parasite viability. **Supported by:** Fiocruz, CAPES, CNPq and FAPERJ **Keywords:** *Trypanosoma cruzi*; chemotherapy; mitochondria.

PV – 072 - **Functional characterization of dihydroorotate dehydrogenase in *Trypanosoma cruzi***

NUNES, T.A.D.L.[1]; MORAES, G.L.P.[1]; VAIDERGORN, M.D.M.[2]; NONATO, C.[2]; SCHENKMAN, S.[1].
1. UNIVERSIDADE FEDERAL DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. UNIVERSIDADE DE SÃO PAULO - RIBEIRÃO PRETO, RIBEIRÃO PRETO - SP - BRAZIL.

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains an important public health problem, particularly in the Americas. Current therapeutic options are limited, especially during the chronic phase of the disease, reinforcing the need to identify and validate new molecular targets. *T. cruzi* dihydroorotate dehydrogenase (TcDHODH, EC 1.3.98.1) is a flavoenzyme involved in de novo pyrimidine biosynthesis and potentially associated with redox homeostasis. *Tc*DHODH catalyzes the oxidation of dihydroorotate to orotate, coupled to the reduction of flavin mononucleotide (FMN), and subsequently uses fumarate as an electron acceptor, producing succinate. Despite its central metabolic function and potential as a drug target, the biological relevance of *Tc*DHODH throughout the parasite life cycle remains incompletely understood. Here, we investigated the functional role of *Tc*DHODH in *T. cruzi,* particularly its contribution to parasite proliferation, pyrimidine metabolism, redox homeostasis, and adaptation to different environmental conditions. To address these questions, *T. cruzi* epimastigotes overexpressing TcDHODH were generated and characterized using molecular and cellular approaches. Western blot analysis using an anti-*Tc*DHODH antibody confirmed the recognition of recombinant *Tc*DHODH and detected a protein band compatible with *Tc*DHODH in parasite extracts. Immunofluorescence assays further supported *Tc*DHODH expression in epimastigotes and revealed increased fluorescence in the overexpressing population compared with wild-type parasites, consistent with increased protein expression. Finally, the epimastigotes overexpressing the enzyme were able to proliferate for more generations in synthetic medium lacking pyrimidines in contrast with the wild type, which differentiates into metacyclic trypomastigotes. Together, these results indicate that pyrimidines can be used by epimastigotes and, in their absence, perform de novo biosynthesis. Further analysis using the overexpressors can additionally help to understand the pyrimidine role in metabolic adaptation in *T. cruzi* and provide additional tools for evaluating this enzyme as a potential therapeutic target for Chagas disease. **Supported by:** FAPESP 2025/15013-8 **Keywords:** *Trypanosoma cruzi*; Pyrimidine Biosynthesis; TcDHODH.

PV – 073 - **The mitogenome of *Cryptodifflugia operculata* and mitochondrial RNA editing in Arcellinida (Amoebozoa)**

BARBOSA, J.P.A.[1]; BANSON, I.B.[2]; PÁNEK, T.[3]; PORFIRIO-SOUSA, A.[4]; FIGUEIREDO, T.[1]; KLEITZ-SINGLETON, F.[2]; BIM, M.A.[1]; BROWN, M.[2]; LAHR, D.J.G.[1].
1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. MISSISSIPI STATE UNIVERSITY, STARKVILLE - UNITED STATES OF AMERICA; 3. CHARLES UNIVERSITY, PRAGUE - CZECH REPUBLIC; 4. TEXAS TECH UNIVERSITY, TEXAS - UNITED STATES OF AMERICA.

Tubulinea is one of three major lineages of Amoebozoa and the only one with no evidence for mitochondrial RNA (mtRNA) editing. The most diverse order of Tubulinea is Arcellinida, a monophyletic group of shelled amoebae with more than 68 described genera. *Cryptodifflugia operculata* is a shelled amoeba with global distribution. We investigated insertions, deletions and substitutions in *C. operculata* mtRNA post-transcriptional processing. We successfully sequenced the mitochondrial DNA, assembled the mitogenome and compared it to transcriptomic data of the same monoclonal eukaryotic culture. We found evidence for insertional edition comparing RNA data with DNA data. Applying a frequency-based confidence threshold to candidate editing sites we recovered more mitochondrial genes (62) than either no editing (54) or unfiltered application of every candidate site (57), indicating that low-confidence candidate sites are enriched for noise rather than true editing events. Thus, filtering low-frequency sites is critical for accurate gene annotation in this system. Using barcode data, we found evidence for mtRNA editing in other Arcellinida lineages. Editing occurs in tRNA, electron transport chain genes and rRNA. Its pattern is unique within Amoebozoa, but convergent to another eukaryotic lineage (Heterolobosea). In Arcellinida, editing is essential for functional recovery, but its machinery and evolutionary origin remain unknown. **Supported by:** FAPESP 2025/23098-3 **Keywords:** RNA editing; Mitochondria; Amoebozoa.

PV – 074 - ***Toxoplasma gondii*** **impairs neuronal CX3CL1 shedding and alters microglial responses during chronic infection**

DE MORAES, J.A.
INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL.

In previous work, we screened conditioned media from Toxoplasma gondii cyst-bearing neurons to identify altered neuronal signals that could mediate neurovascular injury and serve as targets for restoring CNS homeostasis. This analysis highlighted CX3CL1/fractalkine, a membrane-bound neuronal chemokine released by proteolytic shedding to signal through CX3CR1 on microglia. We therefore investigated how chronic neuronal infection disrupts this regulatory axis. Primary mouse cortical neurons were infected with ME49 tachyzoites and analyzed at 7 days post-infection, when tissue cysts were detected. Neuronal conditioned media (nCM) were analyzed by proteome array and ELISA; CX3CL1 distribution and shedding enzymes by immunofluorescence, RT-qPCR, and immunoblotting. The pathway was evaluated in mouse cerebral cortices at 10 and 40 days post-infection and functionally tested by treating BV-2 microglia with nCM with or without recombinant CX3CL1 (rCX3CL1). Soluble CX3CL1 decreased from 4.29 ng/mL in control nCM to undetectable levels following infection, despite unchanged Cx3cl1 expression. Conversely, CX3CL1 immunoreactivity increased in cyst-bearing neurons, indicating impaired release of neuronal CX3CL1. In vivo, the 85-kDa soluble CX3CL1 fraction decreased by approximately 40% at both time points, whereas Cx3cr1 and Ctss expression and ADAM17 protein increased at 40 days. In microglia exposed to infected nCM, rCX3CL1 reduced nitrite production and altered polarization-associated genes, increasing Nos2 and decreasing Arg1 expression. These findings indicate that T. gondii impairs neuronal CX3CL1 shedding and disrupts neuron-microglia communication during chronic infection, potentially sustaining neuroinflammation. Ongoing studies are investigating the mechanisms underlying CX3CL1 dysfunction through longitudinal analysis of neuronal CX3CL1, membrane-cytosolic fractionation, and CX3CL1/CX3CR1 immunostaining in chronically infected mouse brains. **Supported by:** FAPERJ, CNPQ, CAPES, Fiocruz **Keywords:** Toxoplasma gondii; fractalkine; neuroinflammation.

PV – 075 - **Obtaining a System for Constitutive Expression of the Calcium Sensor Yellow Cameleon Nano-50 in Leishmania for Screening of Antileishmanial Compounds**

SILVA, L.A.[1]; YAGI, A.C.L.[1]; MAIA, M.A.C.[2]; DA SILVA, V.L.[3]; COELHO, N.C.S.C.[1]; DE LIMA, J.S.[1]; DELLAGOSTIN, O.A.[2]; DA SILVA, M.S.[4]; GRAMINHA, M.A.S.[1].
1. UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" (UNESP), ARARAQUARA - SP - BRAZIL; 2. UNIVERSIDADE FEDERAL DE PELOTAS (UFPEL), PELOTAS - RS - BRAZIL; 3. UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" (UNESP), BOTUCATU - SP - BRAZIL; 4. UNIVERSIDADE DE SÃO PAULO (USP), SÃO PAULO - SP - BRAZIL.

Leishmaniasis are parasitic diseases caused by protozoan species of the genus Leishmania that represent an important public health problem in the Americas. Currently, the drugs available for the treatment of these diseases have limitations related to administration, dosing, and toxicity, highlighting the need for new therapeutic strategies. In this context, Ca2+ signaling mechanisms represent promising drug targets, as its homeostasis is involved in important metabolic processes and parasite infectivity. In this context, the use of genetically modified organisms for the screening of compounds with therapeutic potential represents a promising approach. Thus, this study aims to construct a transgenic strain of Leishmania amazonensis constitutively expressing the calcium indicator Yellow Cameleon-nano50 (YC-nano50) for future application as a screening tool for antileishmanial compounds. For this purpose, the YC-nano50 gene was successfully cloned into the pLEXSYneo-2.1 plasmid, which contains homology arms for the 18S SSU locus of Leishmania. The sequence was linearized with SwaI, purified, and a total of 2 ug of linear DNA was transfected into L. amazonensis promastigotes at 1×10^7 cells/mL by electroporation. The clones were selected by comparison with an untransfected control under 10 µg/mL geneticin selective pressure. However, it was not possible to select antibiotic-resistant parasites, indicating that the transfection was unsuccessful, which may be explained by the insufficient amount of DNA used compared with that recommended for the LEXSY expression system (10 µg). Therefore, a higher concentration of the purified fragment is required for future assays. Thus, although the methodology adopted enabled the construction of the system for constitutive expression of YC-nano50 in Leishmania, transgenic strains have not yet been obtained. Therefore, new transfections are being performed using a higher amount of linear DNA. **Supported by:** FAPESP (processo: 2024/23195-6) **Keywords:** Leishmania; Ca2+ signaling; constitutive expression.

PV – 076 - **Functional and molecular characterization of a potentially essential long non-coding RNA in *Leishmania braziliensis***

SANTOS, F.H.D.J.; CRUZ, A.K.
UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO - SP - BRAZIL.

Background: *Leishmania* parasites have an unusual genome organization, with gene expression regulated predominantly at the post-transcriptional level. Previous transcriptomic surveys by our group identified approximately 11,000 putative ncRNAs in *L. braziliensis*, nearly 3,000 of which are differentially expressed across developmental stages. Five lncRNAs preferentially expressed in metacyclic promastigotes or amastigotes were selected for functional investigation. **Results:** *lncRNA.12.36* is located within the 3′ UTR of the gene *LBRM2903_120010*. Complete removal of *lncRNA.12.36* from the genome was only possible when a third plasmidial copy was provided, suggesting potential essentiality. In contrast, *LBRM2903_120010* and another lncRNA in the same 3′ UTR (*lncRNA.12.37*) were successfully knocked out. *LBRM2903_120010* KO clones exhibited increased proliferation, greater capacity to generate axenic amastigotes, and higher metabolic activity under nutrient-deprivation conditions. Add-back parasites re-expressing *LBRM2903_120010* have also been obtained for phenotypic complementation and to investigate its subcellular localization, mNeonGreen was fused to the N-terminus of the protein, while BirA-tagged versions were generated with the tag fused to either the N- or C-terminus. Because conventional knockout of *lncRNA.12.36* was unsuccessful, an RNA interference (RNAi) strategy was employed. The *lncRNA.12.36* RNAi line exhibits reduced proliferation compared with the parental line, and RT-qPCR is being performed to confirm *lncRNA.12.36* reduction. To investigate its molecular function, we performed an *in vitro* S1m affinity pull-down assay and identified six proteins as probable *lncRNA.12.36*-binding partners. We are now implementing the RAPID/BoxB assay to identify, *in vivo*, proteins that interact with *lncRNA.12.36*. **Conclusion:** These results support a biologically relevant and potentially essential role for *lncRNA.12.36* in *L. braziliensis*. Its functional characterization may reveal post-transcriptional regulatory mechanisms involved in parasite proliferation and differentiation. **Supported by:** FAPESP - 2024/09219-0 **Keywords:** Leishmania; non-coding RNA; Knockout.

PV – 077 - **D-Serine as a functional carbon source in *Trypanosoma cruzi*: fuelling oxidative metabolism, one-carbon network, nutritional stress resistance, and developmental differentiation**

ALENCAR, M.B.; ALENCAR, V.J.B.; AGRIPINO, J.D.M.; BALLARI, M.S.; TEVERE, E.; DURAN, J.J.R.; SILBER, A.M.
INSTITUTO DE CIÊNCIAS BIOMÉDICAS II - USP, SÃO PAULO - SP - BRAZIL.

Trypanosoma cruzi, the causative agent of Chagas disease, undergoes dramatic metabolic shifts throughout its life cycle, relying on alternative carbon sources when glucose is scarce. D-amino acids represent a potentially relevant (in mammalian and invertebrate hosts) but unexplored nutrient source for the parasite. Here, we investigated the metabolic fate of D-serine (D-Ser) in *T. cruzi* epimastigotes and its role in key biological processes. Using ^{1}H-NMR and $^{14}CO_2$ trapping assays, we show that D-Ser is catabolized by epimastigotes, yielding CO_2, acetate, and low levels of alanine, consistent with partial racemization to D-Ser. D-Ser also promoted glycine accumulation (4.7-fold above PBS) in the supernatant, comparable to L-Ser, suggesting both stereoisomers feed the one-carbon metabolic network via serine hydroxymethyltransferase (SHMT). Inhibition of the mitochondrial pyruvate carrier (by UK-5099) reduced $^{14}CO_2$ production by 59.2% at 1h and 33.0% at 3h, indicating dual carbon routing: oxidative catabolism via pyruvate dehydrogenase (PDH) and sphingolipid biosynthesis via serine palmitoyltransferase (SPT). High-resolution respirometry revealed that D-Ser significantly increases routine respiration (1.57 ± 0.09-fold) and ET-capacity (2.04 ± 0.23-fold) above carbon-starved controls, with a concomitant 2.21 ± 0.12-fold rise in intracellular ATP levels. Under prolonged nutritional stress (72–120h), D-Ser preserved parasite proliferative capacity equivalently to L-Ser and L-His. Surprisingly, D-Ser stimulated metacyclogenesis as a sole carbon source, reaching ~12% metacyclic forms at peak (day 5). Taken together, these results reveal a remarkable metabolic flexibility in *T. cruzi* epimastigotes, with D-Ser simultaneously feeding oxidative energy metabolism via PDH, sphingolipid biosynthesis via SPT, and the one-carbon network via SHMT, while sustaining viability under stress and promoting metacyclogenesis. **Supported by:** FAPESP: 2022/16078-8 and 2021/12938-0 **Keywords:** Bioenergetics; Nutritional stress; D-aminoacids.

PV – 078 - **Partial knockout of potential Pre-Replication Complex interactors suggests a role in *Trypanosoma cruzi* replication**

DE OLIVEIRA, M.M.[1]; AOKI, J.I.[2]; SABBAGA, M.C.Q.B.E.[2].
1. UNIVERSIDADE DE SÃO PAULO, SÃO PAULO - SP - BRAZIL; 2. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

DNA replication in *Trypanosoma cruzi*, the etiological agent of Chagas disease, is a poorly understood process. In model eukaryotes, replication begins with the recruitment of an ORC1-6 hexamer which, together with other proteins (Cdc6, Cdt1, and MCM2-7) forms the pre-replication complex. In *T. cruzi*, only a single protein, Orc1Cdc6, has been described as a replication initiator. In contrast, Orc1Cdc6 of *T. brucei* was described interacting with Orc2, Orc4, Orc5 and Orc1b, forming part of the pre-RC. Based on this, our objective was to characterize the presence and functional role of these interactors in *T. cruzi* by investigating the effects of deleting the genes coding these proteins using CRISPR/Cas9-mediated gene knockout, as well as by generating tagged lines for localization and expression. The knockout of Orc2 (Tb09.10.3120), Orc4 (Tb927.10.13380), and Orc5 (Tb927.10.7980) was performed in *T. cruzi* (Dm28c Cas9) and for "Orc4" (Tc_C4B63_4g186) and "Orc2" (Tc_C4B63_63g42), after selection, we observed that only one allele was replaced by the drug resistance marker (blasticidin), and viable clones were generated, all of which were partial knockouts. The transfection strategy (two rounds of electroporation) did not favor the generation of a full knockout, but it appeared to favor clone selection. The growth curves of “Orc4” C4B63_4g186 showed that some clones reach stationary phase at higher cell density compared to the Cas9 control. This suggests that the partial deletion of this gene affects the epimastigote cell cycle, may be modulating DNA replication. The complete phenotypic characterization, as well as the generation of full knockouts for this and the other mentioned orthologous proteins, is currently underway and may indicate the involvement of these interactors in the machinery of pre-replication in the parasite. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and by São Paulo Research Foundation (FAPESP) [Grant number: 2020/00694-6]. **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001; Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Processo 2020/00694-6. **Keywords:** *Trypanosoma cruzi*; DNA Replication; Pre-replication complex.

PV – 079 - **The RNAP III Transcription Machinery in *Trypanosoma cruzi*: The dynamics of RPC4 and Tfc1 in the cell cycle and metacyclogenesis**

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

Transfer RNAs (tRNAs) are transcribed by RNA polymerase III (RNAP III) and play a central role in protein synthesis. In *Trypanosoma cruzi*, the etiologic agent of Chagas disease, knowledge regarding the machinery responsible for transcribing these loci, as well as the mechanisms regulating tRNA gene expression, remains limited. Previous work from our group demonstrated that tDNAs exhibit a three-dimensional cluster organization and that the degree of chromatin compaction at these loci changes across life stages and correlates with tRNA abundance. The tRNA transcription machinery consists of RNAP III—containing 17 subunits—and the multiprotein transcription factors TFIIIB and TFIIIC. This study aimed to characterize two components of the tRNA transcription complex, the RPC4 subunit of RNAP III and Tfc1 of the transcription factor TFIIIC, as well as to understand how the regulation and functional organization of this machinery occur in the nucleus across different life stages and cell cycle phases of *T. cruzi*. We investigated the subcellular localization, expression levels, genomic distribution, proteomic effects of partial depletion, and role in cellular differentiation of both the RPC4 subunit of RNAP III and the Tfc1 subunit of TFIIIC, which were recently identified by our group through *in silico* analyses. Experimental approaches such as CRISPR/Cas9 genome editing, immunofluorescence assay (IFA), and mass spectrometry were employed. We successfully generated genetically modified cell lines, including: (i) tagged cell lines for RPC4 (Ty1-RPC4) and Tfc1 (Ty1-Tfc1), and (ii) partial knockout cell lines for RPC4 ($RPC4^{+/-}$) and Tfc1 ($Tfc1^{+/-}$), whose edits were validated by Western blot and/or PCR. The partial knockout cell lines exhibited slow growth; however, only the $Tfc1^{+/-}$ line showed a reduced differentiation rate from epimastigotes to metacyclic forms compared to parental cells. Proteomic analysis revealed shared sets of differentially expressed proteins between the partial knockout cell lines, reinforcing the functional association between these subunits. Furthermore, IFA analysis of the Ty1-RPC4 cell line indicated cell cycle-dependent modulation of protein expression. Taken together, our findings provide new insights into the role of the RNAP III transcription machinery in *T. cruzi*. **Supported by:** 2018/15553-9 **Keywords:** RNAP III machinery; tDNA; Transcription.

PV – 080 - **Construction of a Dual-Reporter Vector for the Functional Analysis of Candidate Cis-Regulatory Elements in *Trypanosoma cruzi***

LOPES, L.D.S.[1]; DE LIMA, P.L.C.[2]; PIRES, D.[3]; KELLY, J.M.[4]; TAYLOR, M.[4]; DA CUNHA, J.P.C.[3].
1. USP/ICB, SÃO PAULO - SP - BRAZIL; 2. USP/INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL; 3. INSTITUTO BUTANTAN, SAO PAULO - SP - BRAZIL; 4. LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON - UNITED KINGDOM.

The etiological agent of Chagas disease, *Trypanosoma cruzi*, has a genome organised into linear compartments: the core compartment, containing housekeeping genes, and the disruptive compartment, enriched in virulence genes. Its genes are transcribed as polycistronic transcription units (PTUs), which exhibit distinct patterns of gene expression. Our research group has investigated the chromatin-level mechanisms regulating *T. cruzi* gene expression. We have shown that core regions exhibit fewer DNA–DNA interactions and higher chromatin accessibility, consistent with increased transcriptional activity, whereas disruptive regions display more chromatin loops, lower accessibility, and reduced transcriptional activity. We also found that divergent strand-switch regions (dSSRs) are associated with H2B.V-enriched domains and display distinct epigenetic signatures and cis-regulatory elements depending on the genomic context. In addition, we mapped numerous transcription start sites (TSSs) at and outside dSSRs using small triphosphate RNA sequencing (3pRNA-seq) in epimastigotes. Based on these findings, we aimed to functionally evaluate selected 3pRNA-enriched sequences and determine their ability to activate transcription in vivo. To this end, we constructed the pLuc reporter vector, containing a multiple cloning site for insertion of candidate cis-regulatory elements followed by two reporter genes, *luciferase* and *mNeonGreen*. We generated pLuc constructs carrying eight selected cis-regulatory elements from distinct genomic contexts, including core, disruptive, and mixed dSSRs, as well as core and disruptive PTUs. Following integration into the *T. cruzi* genome, these constructs will enable us to assess the contribution of each element to transcription initiation. This study establishes a reporter-based platform for the functional characterisation of cis-regulatory elements and provides new insights into the mechanisms underlying gene expression regulation in *T. cruzi*. **Supported by:** FAPESP: 2018/15553-9, 2025/20165-1 e 2022/15610-8 **Keywords:** Trypanosoma; Cis-elements; Regulation.

PV – 081 - **Integration of computational approaches and quantitative analyses to decipher the 3D genome organization of *Trypanosoma cruzi* in infective and non-infective forms.**

DE LIMA, P.L.C.; BELLINI, N.K.; MENEZES, A.P.D.J.; DE SOUZA, V.C.; DA CUNHA, J.P.C.
INSTITUTO BUTANTAN, SÃO PAULO - SP - BRAZIL.

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains a public health challenge for which no fully effective treatment is available, partly due to the complexity of gene expression control in the parasite. Unlike most eukaryotes, *T. cruzi* lacks conventional promoters for individual genes, and gene expression is regulated predominantly at the post-transcriptional level; the three-dimensional organization of the genome has been proposed as an additional regulatory layer, but its relationship with the linear organization of the genome and with transitions between the parasite life forms has remained poorly explored. Here, we show that chromatin contacts are not broadly redistributed as the parasite transitions between the replicative epimastigote and the infective metacyclic form. Instead, the changes are concentrated at specific genomic regions, particularly centromeres, tRNA loci, and dSSRs. Contacts associated with the Core compartment in epimastigotes become more strongly associated with the Disruptive and GpDr compartments in the infective form, independently of the genomic size of each compartment. This targeted reorganization is especially notable because it involves the compartment that contains much of the parasite's virulence machinery precisely when the parasite becomes infective. Together, these findings suggest that three-dimensional genome organization may provide an additional layer of regulation, even in genomic regions that lack classical promoters. **Supported by:** 2018/15553-9 **Keywords:** *Trypanosoma cruzi*; 3D genome organization; Hi-C.

PV – 082 - **Understanding the 3D genomic architecture of *Trypanosoma cruzi* Dm28c epimastigote and metacyclic trypomastigote stages via Hi-C analysis**

VERGARA, P.O.T.; DE LIMA, P.L.C.; DE SOUZA, V.C.; BELLINI, N.K.; DA CUNHA, J.P.C.
LABORATORY OF CELL CYCLE, BUTANTAN INSTITUTE, SAO PAULO - SP - BRAZIL.

Trypanosoma cruzi, the causative agent of the chagas disease in Latin America, has long lacked a high-quality, complete genome assembly. Recently, a telomere-to-telomere level chromosome assembly of the Dm28c strain was completed, revealing conserved structural organization across the 32 *T. cruzi* chromosomes: core and disruptive compartments. High-throughput chromosome conformation capture (Hi-C) has demonstrated that topologically associated domain (TADs)-like within core compartments are shorter and more compact than those in disruptive. The parasite's complex four-stage life cycle remains incompletely characterized at the chromatin level; while the epimastigote stage has been extensively characterized by Hi-C, critical gaps remain regarding other development stages. We aimed to characterize and compare the three-dimensional (3D) chromatin architecture between the non-infective epimastigote and infective metacyclic trypomastigote stages of *T. cruzi* Dm28c using Hi-C data. We generated Hi-C libraries from two biological replicates, each of epimastigote and metacyclic trypomastigotes. Given the repetitive nature of the *T. cruzi* genome, we employed the mHiC pipeline to capture multimapping reads and generate accurate Hi-C matrices. Downstream analysis was performed using Hicexplorer and FanC tools. Metacyclic trypomastigote exhibited significantly higher frequencies of inter-chromosomal (trans) contacts compared to epimastigote. Subtelomeric regions exhibited distinct chromatin architectural differences between the two stages. Furthermore, loop-like structures were identified in Hi-C contact matrices of metacyclic trypomastigote, particularly in regions containing dSRR and cSRR. The preliminary findings reveal substantial stage-specific differences in higher order chromatin architecture, indicating that each development stage employs distinct 3D genome organization. The epimastigote stage displays more compact chromosome conformations, while metacyclic trypomastigote exhibits relatively relaxed chromatin interactions. These results provide the first evidence that development stages transitions in *T. cruzi* are accompanied by remodelling of 3D chromatin architecture, potentially reflecting adaptations for host interactions. **Supported by:** FAPESP:2018/15553-9; Fundação Butantan 61.189.445/0001-56 **Keywords:** Hi-C; Epimastigote; Metacyclic trypomastigote.

PV – 083 - **From syntrophy to bioenergetic integration: an evolutionary marriage between *Angomonas deanei* and its bacterial endosymbiont**

BARRINHA, A.[1]; DA SILVA, B.N.M.[1]; DE SOUZA, W.[2]; GALINA FILHO, A.[3]; MOTTA, M.C.M.[2].
1. IBCCF - UFRJ, BELO HORIZONTE - RJ - BRAZIL; 2. CPMP, CENTRO NACIONAL DE BIOLOGIA ESTRUTURAL E BIOIMAGEM, CENTRO DE CIÊNCIAS DA SAÚDE, UFRJ, RIO DE JANEIRO - RJ - BRAZIL; 3. LABORATÓRIO DE BIOENERGÉTICA E FISIOLOGIA MITOCONDRIAL, INSTITUTO DE BIOQUÍMICA MÉDICA LEOPOLDO DE MEIS, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Angomonas deanei is an excellent model for investigating organelle origin and cellular evolution, as this trypanosomatid co-evolves with an endosymbiont belonging to the β-Proteobacteria class. This mutualistic relationship is characterized by extensive metabolic exchange, in which the endosymbiont complements several biosynthetic pathways of the host, including the biosynthesis of heme, essential amino acids, and vitamins. In turn, the bacterium benefits from a supply of host-derived phospholipids. The wild-type strain (AdWt) exhibits predominantly oxidative metabolism, whereas the aposymbiotic strain (endosymbiont-free; AdApo) displays a more fermentative phenotype. In this study, we combined proteomics and high-resolution respirometry to characterize the respiratory chain of A. deanei and its endosymbiont, as well as to investigate the influence of the symbiotic bacterium on the host bioenergetics. To this end, the AdApo strain served as a comparative model together with treatments with dichloroacetate (DCA), a bioenergetic modulator that promotes the metabolic redirection of pyruvate toward mitochondrial oxidation, and bedaquiline, a specific inhibitor of bacterial ATP synthase activity. Our results showed that the AdWt mitochondrion is more dependent on pyruvate and exhibit a 76.8% increase in ATP-linked to O 2 consumption, when compared to AdApo cells. Furthermore, electron transfer capacity, maximal respiratory capacity, and respiratory reserve were increased by 40.6%, 39.5%, and 50.2%, respectively, in the AdWt strain. In addition, proteomic analysis revealed that the endosymbiont expresses a partial repertoire of enzymes involved in glycolysis, the Krebs cycle and the respiratory chain. Taken together, these data indicate that the endosymbiont modulates metabolic plasticity, contributing to the bioenergetic integration of the host protozoan. **Supported by:** CNPq (305299/2022-0) and FAPERJ (E-26/201.011/2021). **Keywords:** bioenergetic integration; endosymbiosis in trypanosomatids; metabolic coevolution.

PV – 084 - **Ecdysone signaling coordinates immunity, microbiota, and Leishmania development in *Phlebotomus perniciosus***

VIEIRA, C.S.[1]; MARCO, S.[2]; TELLERIA, E.L.[3]; VOLF, P.[3].

1. DEPARTMENT OF PARASITOLOGY, FACULTY OF SCIENCE, CHARLES UNIVERSITY/DEPARTMENT OF GENERAL BIOLOGY, INSTITUTE OF BIOLOGY, FLUMINENSE FEDERAL UNIVERSITY, RIO DE JANEIRO - RJ - BRAZIL; 2. DEPARTMENT OF BIOLOGY, UNIVERSITY OF NAPLES FEDERICO II, NAPLES - ITALY; 3. DEPARTMENT OF PARASITOLOGY, FACULTY OF SCIENCE, CHARLES UNIVERSITY, PRAGUE - CZECH REPUBLIC.

Introduction: *Phlebotomus perniciosus* is the natural vector of *Leishmania infantum*, the causative agent of visceral leishmaniasis in the Mediterranean basin. Ecdysone signaling has been implicated in the regulation of insect immunity and gut microbiota homeostasis, potentially influencing parasite development. Here, we investigated how *L. infantum* infection modulates ecdysone signaling and how disruption of this pathway affects immunity, microbiota, parasite load, and developmental forms. **Methods:** Double-stranded RNA targeting the ecdysone receptor (dsEcR) or a control sequence (dsLacZ) was microinjected into infected females. Gene expression of ecdysone-related genes, immune pathway components, and antimicrobial peptides was assessed by RT-qPCR; parasite and bacterial loads were estimated using *Leishmania* actin and bacterial 16S rRNA, respectively. Parasite morphology was analyzed at 5 days post-infection, distinguishing procyclic promastigotes, elongated nectomonads, leptomonads, and metacyclic promastigotes. **Results:** *L. infantu*m infection induced ecdysone- and immunity-related genes and altered gut bacterial abundance. EcR silencing disrupted hormonal and immune signaling, altered microbiota dynamics, and significantly reduced parasite load at 5 days post-infection. Importantly, EcR knockdown also shifted parasite development, increasing the proportion of procyclic promastigotes, reducing leptomonads, and eliminating detectable metacyclic forms. **Discussion:** Our results suggest that *L. infantum* activates ecdysone signaling and shape vector immunity, suppressing gut microbiota which favors parasite establishment. Disruption of this pathway leads to microbiota proliferation and impaired parasite survival. These findings highlight ecdysone signaling as a key regulator of sand fly immunity and *Leishmania* development, supporting hormonal pathways as potential targets for vector control. **Supported by:** Marie Skłodowska-Curie Actions (MSCA) – European Commission and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro). **Keywords:** Phlebotomus perniciosus, ecdysone receptor;innate immunity, microbiota;Leishmania infantum, RNA interference.