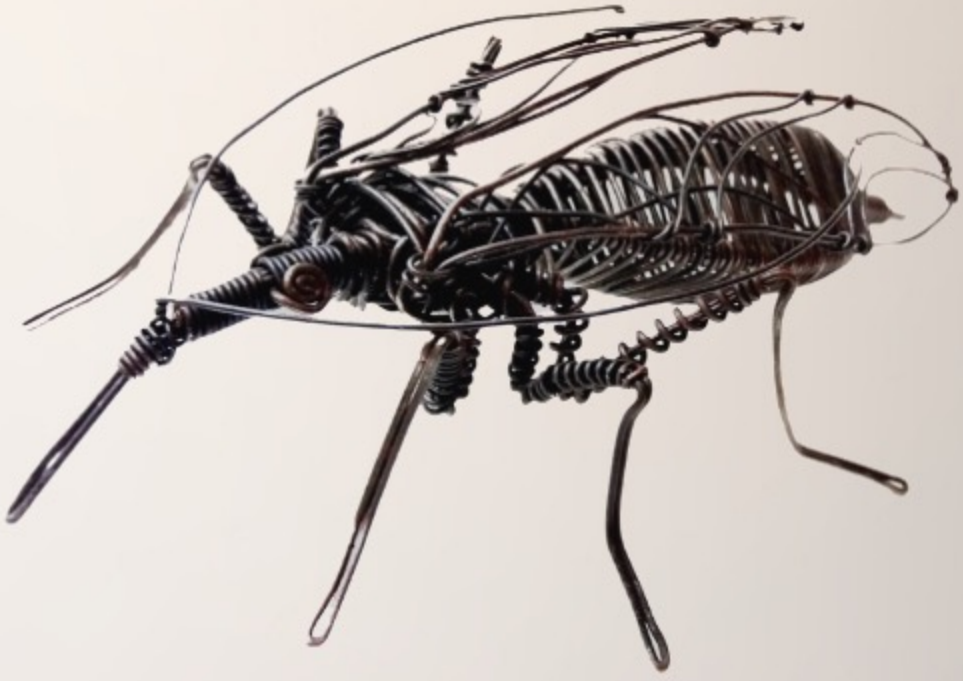
# XL Annual Meeting of the Brazilian Society of Protozoology

## LI Annual Meeting on Basic Research in Chagas' Disease



**Hotel Glória – Caxambu (MG), Brazil**

**November 10 – 12, 2025**

Abstract deadline August 5, 2025

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***PROCEEDINGS***
XL Meeting of the Brazilian Society of Protozoology
LI Annual Meeting on Basic Research in Chagas' Disease

*Hotel Glória, Caxambu, MG, BRASIL- C*axambu
10-12 November, 2025

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r.tonelli@unifesp.br

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TB – 020 -**Standardization of a LAMP-PCR Assay with Homemade Bst Enzyme for *Leishmania amazonensis* Detection**

DE CARVALHO, R.L.; GUERRA, G.T.; DE GODOY, S.A.M.; QUEIROZ, B.O.; VENTURA, B.M.L.; MARQUEZINI, L.M.B.; DE OLIVEIRA, L.L.; VASCONCELLOS, C.M.M.; FIETTO, J.L.R.; VASCONCELLOS, R.D.S..
UNIVERSIDADE FEDERAL DE VIÇOSA, VIÇOSA - MG - BRAZIL.

Leishmaniases are anthropozoonotic diseases affecting over 1 million people annually, transmitted by phlebotomine sand flies infected with more than 20 *Leishmania* species. In Brazil, the recent discontinuation of the Montenegro skin test reagent created a diagnostic gap, particularly for the tegumentary form, affecting smaller municipalities without access to advanced diagnostic methods and requiring patient referrals to reference centers. This problem is aggravated by limitations of current tests, which may present cross-reactivity with other parasites and often fail to identify the infecting species. This study aimed to validate the use of *L. amazonensis* primers in a LAMP-PCR assay with a laboratory-produced ("homemade") Bst enzyme, as well as to standardize the reaction, evaluate specificity, and determine the detection limit. Genomic DNA from several *Leishmania* species, *T. cruzi* strains, and *T. rangeli* was extracted and quantified. Reactions were optimized by comparing enzyme sources (commercial vs. homemade), incubation temperatures (60–68 °C), $MgSO_4$ concentrations (5–15 mM), and reduced primer concentrations. Detection limits for *L. amazonensis* PH8 DNA were determined, along with limiting dilutions of the enzyme. Specificity tests included all extracted parasite DNAs, and colorimetric detection with malachite green was applied. Amplification performance was similar between commercial and homemade enzymes. Optimal conditions were 64–65 °C and 8–12 mM $MgSO_4$. The homemade enzyme retained activity after tenfold dilution. The assay detected as little as 100 fg of *L. amazonensis* DNA. Primers showed 100% specificity. Both colorimetric and gel-based detection allowed rapid interpretation, highlighting the primers' high sensitivity and specificity and their potential for species-specific diagnostic kit development.
**Supported by:** Fapemig, CNPq, CAPES
**Keywords:** Lamp-PCR;Leishmania amazonensis;Molecular diagnosis.

TB – 021 - **Nucleosome Architecture and Chromatin Organization in *Trypanosoma cruzi***

CASTRO, B.S.[1]; ISSA, M.[2]; DA CUNHA, J.P.C.[3]; THIEMANN, O.H.[1].
1. IFSC - USP, SÃO CARLOS - SP - BRAZIL; 2. CSSB, HAMBURGO - GERMANY; 3. BUTANTAN, SÃO PAULO - SP - BRAZIL.

Chagas disease, caused by ***Trypanosoma cruzi***, affects millions of people worldwide, with no available vaccines or adequate treatments. The parasite's infectivity and the processes that enable its survival involve gene expression regulation, especially at a post-transcriptional level. Recent evidence suggests that epigenetics plays a crucial role in its pathogenicity, inciting the study of chromatin structure and dynamics. The most basic level of chromatin packaging is the nucleosome, composed of DNA interacting around an octameric protein complex that contains a pair of each histone - H2A, H2B, H3, and H4. The oligomeric structure of the octamer from homologue microorganisms is composed of two dimers of H2A/H2B and one tetramer of H3/H4. This study aims at the biochemical and biophysical characterization of ***Trypanosoma cruzi*** histones, in addition to the atomic structure of the histone octamer and the nucleosome. Conditions of the recombinant expression of histone coding genes of ***T. cruzi*** in ***Escherichia coli*** T7 Express strain were determined. The four histone protein purification protocols included refolding techniques, affinity chromatography, and size-exclusion chromatography. The ***Tc***H2A/***Tc***H2B and ***Tc***H3/***Tc***H4 oligomers were obtained as heterodimers, diverging from homologue histones in which H3 and H4 assemble as a tetramer. The characterization of the histones and the nucleosome assembly are currently under development. Moreover, crystallization screening and SEC-SAXS assays were performed in order to investigate the structural organization of these oligomers, aiming to explain the differences obtained. The current results support the need to continue the research to properly obtain the structure of the ***T. cruzi*** octamer through Cryo-Electron Microscopy (Cryo-EM), as well as the kinetic and thermodynamic properties of nucleosome assembly.
**Supported by:** CNPq # 130068/2025-0
**Keywords:** Trypanosoma cruzi;Nucleosome;Cryo-EM.