TOWARDS MOLECULAR TARGETS FOR EPIDEMIOLOGIC STUDIES OF LEISHMANIASIS

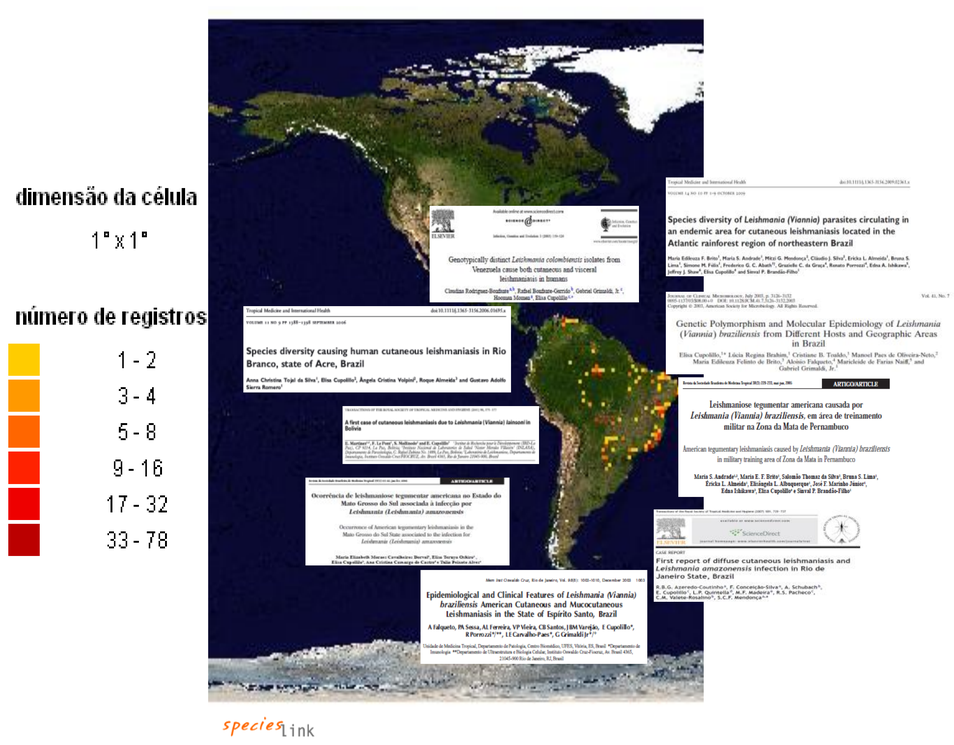
Elisa Cupolillo, Renato Porrozzi, Patricia Cuervo, Mariana Côrtes Boité, Gabriel Eduardo Melim Ferreira, Grazielle Cardoso da Graça, Barbara Neves dos Santos, Davi Coe Torres, Taíse Salgado de Oliveira

Laboratório de Pesquisa em Leishmaniose, Coleção de *Leishmania* do Instituto Oswaldo Cruz

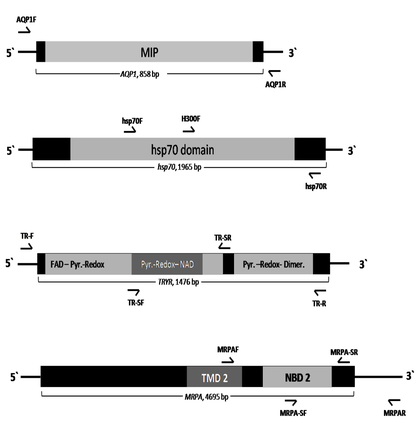
IOC/FIOCRUZ, Instituto Oswaldo Cruz/Fundação Oswaldo Cruz, Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ, Brasil

Leishmaniasis is a disease caused by a polymorphic group of protozoan parasite classified in the genus *Leishmania,* which are associated with several vertebrate and invertebrate hosts. Many species and their genetic variants are capable of producing a variety of clinical manifestations. Considering the public health importance of leishmaniasis in Brazil and the role of the genetic polymorphism of the parasites in the epidemiology of the disease we have developed/applied many molecular markers aiming to analyze the genetic variability of this parasite and to correlate this with eco-epidemiological features.

Using molecular markers, we have defined the *Leishmania* species that are circulating in many Brazilian endemic regions, associating species/strains with a particular transmission cycle

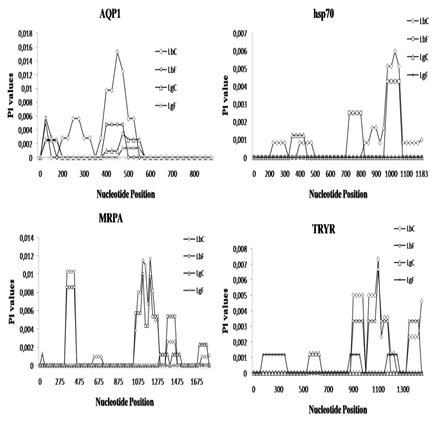


We observed DNA sequence polymorphisms in genes encoding proteins related to antimony resistance (*AQP1*, *hsp70*, *MRPA* and *TRYR*).

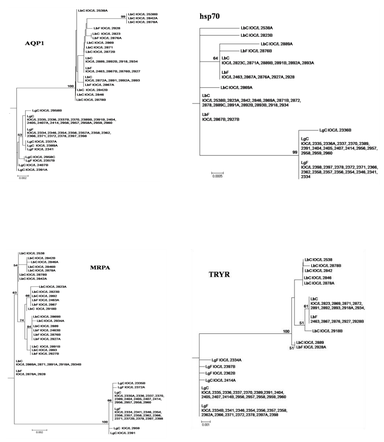


Schematic representation of the genes under study, showing the annealing regions of the oligonucleotides used for amplification and internal sequencing. The coding sequences are represented in rectangles and the black lines correspond to untranslated regions (UTR). Bold arrows show primer orientation. The different domains are represented in different gray scales. Domains represented in the genes: *AQP1* (MIP - major intrinsic protein domain), *hsp70* (hsp70 domain), *MRPA* (TMD 2 – second transmembrane domain; NBD 2 – second ATP-binding domain) and *TRYR* (FAD – Pyr.-Redox: FAD-dependent pyridine nucleotide-disulphide oxidoreductase; Pyr.-Redox–NAD: Pyridine nucleotide-disulphide oxidoreductase, NAD-binding region; Pyr.–Redox–Dimer.: Pyridine nucleotide-disulphide oxidoreductase, dimerisation).

It was observed a lower nucleotide diversity in the treatment failure isolates in comparison to those from cured patients. Based on a multiple logistic regression model, a significant therapeutic failure increase was observed among *L. braziliensis* isolates when there was a guanine instead of an adenine at position 1735 of the *hsp70* gene



Sliding window plot of the nucleotide diversity per site (π) comparing the level of genetic diversity through each gene sequenced from the parasites. Four groups are depicted: LbC, *L. braziliensis* parasites isolated from patients with therapeutic cure; LbF, *L. braziliensis* parasites isolated from patients with therapeutic failure; LgC, *L. guyanensis* parasites isolated from patients with therapeutic cure; and LgF, *L. guyanensis* parasites isolated from patients with therapeutic failure. The π values were calculated in DnaSP with a window length of 100 bp and step size of 25 bp.

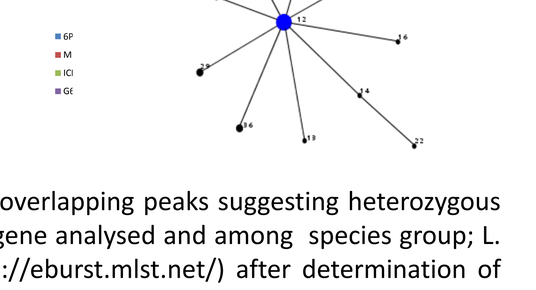


Neighbor-joining original trees depicting the relationships between different *L. braziliensis* and *L. guyanensis* isolates related to treatment outcomes using four independent gene data sets (*AQP1*, *hsp70*, *MRPA* and *TRYR*). Each isolate is described by species (Lb: *L. braziliensis* and Lg: *L. guyanensis*), therapeutic response (C, cure, and F, failure), IOC/L number and a final letter that may or may not be present, which corresponds to the clone sequenced. The distance matrices were prepared using the evolutionary models HKY+I (*AQP1* and *TRYR*), HKY (*hsp70*) and TIM (*MRPA*). Numbers below or above the branch indicate bootstrap values based on 1,000 replications. The scale bar represents the genetic distance.

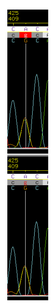
Using microsatellite analysis, we detected a lower observed heterozygosity (mean Ho=0.015) than expected (mean He=0,180) in *L. infantum*, indicating high prevalence of inbreeding, that is supported by mean F_{IS}. Clustering method and F_{ST} estimates indicated strains grouping in two populations. Structure in Brazilian *L. infantum* populations does not seem to be geographically related

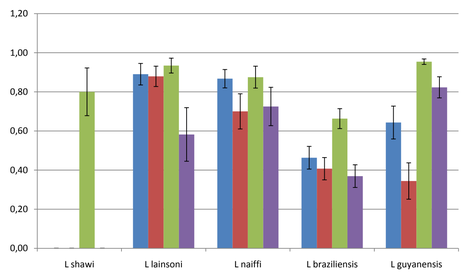
Estimated population structure and substructure of *L. infantum* from Brazil as inferred by the STRUCTURE program

Results are based on MLMT of 11 microsatellite markers obtained for 103 *L. infantum* strains studied. In the barplots each strain is represented by a single vertical line divided into *K* colors, where *K* is the number of populations assumed. According to Δ*K* the most probable number of populations in the data set is two, corresponding to green and red bars.



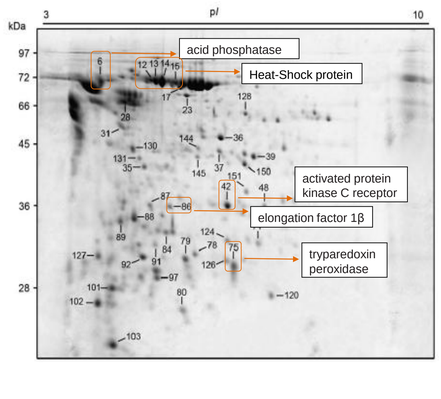
Multi-locus sequence analysis using housekeeping genes are also being conducted by our group. We have analyzed the *L.(Viannia)* subgenus by sequencing the enzyme-coding genes MPI, ICD, G6P and 6PG. Polymorphisms were detected as well as species specific alleles. Heterozygotic samples were identified. These studies generate a new outlook on *Leishmania* and provide a solid basis for new generation genome based molecular epidemiology studies





Figures presented in the following order: Example of overlapping peaks suggesting heterozygous site in a chromatogram; Haplotype diversity for each gene analysed and among species group; L. braziliensis clonal complex obtained by e-burst (http://eburst.mlst.net/) after determination of sequence type for the four loci studied.

Proteomic approach was carried out and indicates that *Leishmania* promastigotes secrete proteins involved in immunomodulation, signal transduction, and intracellular survival, such as HSP70, acid phosphatase, activated protein kinase C receptor, elongation factor 1β, and tryparedoxin peroxidase



Proteins detected in the secreted fraction collected from promastigotes. Protein profile obtained after separation of secreted fraction by 2DE (first dimension: IEF pH range 3–10 non-linear, second dimension: 12% SDS-PAGE) and staining by colloidal Coomassie Brilliant Blue G-250. Protein spots were identified by MALDI-TOF/TOF mass spectrometry. The gel is a reliable replicate of five independent gels. Numbers on the right side indicate themolecular mass standards expressed in kDa.

Supported by

FIOCRUZ
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
FAPERJ
FINEP