

Investigation of the gene encoding chitinase from *Leishmania equatoriensis*, *Leishmania hertigi* and *Leishmania shawi*: implications in diagnosis and evolution

Gabriel L. Pereira¹, Felipe T. Jordão¹, Aline D. Cabral¹, Rodrigo B. Suzuki, Márcia A. Sperança¹

1. Center for Natural and Human Sciences, Universidade Federal do ABC, Campus São Bernardo do Campo, São Bernardo do Campo, São Paulo, Brazil.

Leishmaniasis is a complex of vector-borne zoonotic diseases, caused by parasitic protozoa belonging to *Leishmania* genus and Trypanosomatidae family, through the bite of an insect vector from genus *Lutzomyia*. Symptoms range from cutaneous to visceral forms. American Tegumentar Leishmaniasis (ATL) and the American Visceral Leishmaniasis (AVL) are expanding in large urban centers. More than one species of *Leishmania* can be endemic in the same region, for example in some regions from São Paulo State both, AVL and LTA species are endemic. Considering this situation, a specific diagnostic test is required for the control and prevention of human leishmaniasis. The recent reclassification of the species *L. hertigi* and *L. Equatoriensis* into the genus *Endotrypanum* was performed according to its biological cycle restricted to sloths found in Brazil, Colombia, Guyana and Central America, and also to molecular analysis using PCR-RFLP on SSU rDNA and cytochrome b gene sequences. The investigation of the chitinase encoded by a single copy gene highly conserved among *Leishmania* species, can generate unprecedented information for a better understanding of the species phylogenetic relationship. The samples used in the study were obtained from *Leishmania* collection of the Instituto Oswaldo Cruz of the National Reference Laboratory for *Leishmania*. *L. hertigi*, *L. equatoriensis*, and *L. shawi* reference species were maintained in M199 culture medium and exponentially growing cultures were centrifuged and used for DNA extraction. To obtain DNA, we used the DNeasy® Blood & Tissue Kit, according to supplier's instructions. Using oligonucleotides complementary to conserved regions of the *Leishmania* genus chitinase encoding gene, a PCR fragment was amplified and cloned into pGEM-T vector and submitted to sequencing by Sanger's method.

Key words: Leishmaniasis, Diagnostics, chitinase

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