Acta Biol. Venez., Vol. 26(2):35-47
Julio-Diciembre, 2006

TRENDS ON *TRYPANOSOMA (HERPETOSOMA) RANGELI* RESEARCH

TENDENCIAS EN INVESTIGACIONES DE *TRYPANOSOMA (HERPETOSOMA) RANGELI*

Ethel Bayer-Santos, Thaís Cristine Marques Sincero, Patrícia Hermes Stoco, Mário Steindel and Edmundo Carlos Grisard.

Laboratórios de Protozoologia e de Bioinformática, Departamento de Microbiologia e Parasitologia, Univer-sidade Federal de Santa Catarina, Florianópolis, SC, Brazil. Corresponding author: Edmundo C. Grisard - Universidade Federal de Santa Catarina (MIP/CCB), Florianópolis, SC, Brazil, 88040-900. E-mail: grisard@ccb.ufsc.br, fax: +55 (48) 33319258.

ABSTRACT

Trypanosoma rangeli is a hemoflagellate protozoan parasite presenting an overlapping distribution with *T. cruzi*, the etiological agent of Chagas disease, in a wide geographical area in Latin America. Despite considered as non-pathogenic for man, *T. rangeli* shares several characteristics with *T. cruzi* such as vertebrate and invertebrate reservoirs, vectors and approximately half of the soluble antigenic determinants. Despite the importance of specific detection, little is know about *T. rangeli* in comparison to *T. cruzi*; several questions lack proper answers, including the controversies concerning *T. rangeli´*s taxonomic position. In this context, this short review attempted to congregate current aspects on the research of this parasite, approaching several subjects as life cycle, vector suscepbility, specific genes studies and genomic data.

RESUMEN

Trypanosoma rangeli es un parásito hemoflagelado que comparte el área de distribución con *Trypanosoma cruzi*, agente etiológico de la enfermedad de Chagas, ampliamente distribuida en Latinoamérica. A pesar de no ser considerado como patogénico para los humanos, *T. rangeli* presenta características semejantes con *T. cruzi* tales como sus hospedadores vertebrados, vectores y aproximadamente la mitad de los antigenos solubles determinantes. A pesar de la importancia de que este tripanosomatídeo sea detectado en forma especifica, poco es el conocimiento adquirido cuando comparamos *T. rangeli* con *T. cruzi*, faltando respuestas adecuadas para innumerables cuestionamientos, uno de ellos las controversias en relación a su posición taxonómica. En este contexto, esta pequeña revisión intenta reunir algunos aspectos generales sobre la investigación de este parásito, abordando algunos aspectos del ciclo de vida, susceptibilidad del vector, estudios específicos de genes y datos genómicos.

Keywords: *Trypanosoma* (*Herpetosoma*) *rangeli,* biology, genetics.
Palabras clave: *Trypanosoma* (*Herpetosoma*) *rangeli,* biología, genética.

THE PARASITE

General features

The protozoan parasite *Trypanosoma rangeli* was originally described by the Venezuelan investigator Enrique Tejera in 1920 from flagellates found in the intestinal content of naturally infected *Rhodnius prolixus* collected in Venezuela (D´Alessandro 1976). Nowadays, *T. rangeli* be-longs to the subgenus *Herpetosoma* and infects triatomines and several wild and domestic mamma-lian species including humans in a wide geogra-phical area in Central and South American coun- tries (D´Alessandro-Bacigalupo and Saravia 1992).

The biology of *T. rangeli* in the invertebrate host presents some fascinating features. After colonization of the vector gut from which the parasite

reachs the hemocoel, epimastigotes multi-plicate in the hemolymph and invade the salivary glands where a large number of metatrypomastigotes (metacyclic trypomastigotes) are formed (D´Alessandro, 1976, Grisard *et al*. 1999a, Guhl and Vallejo, 2003). Transmission to the mammalian host occurs by bites of infected triatomines, espe-cially those from the genus *Rhodnius*, but some investigators had demonstrated that infection in mice by inoculation of infected triatomine feces is also possible. *T. rangeli* is completely harmless to the mammalian host where its biology is largely unknown. Intracellular multiplication in the vertebrate host remains controversial. Previous extensive histopathological studies on more than a hundred mice infected with *T. rangeli* did not show any evidence of intracellular parasite multiplication (Herbig-Sandreuter, 1955). On the contrary, Urdaneta-Morales and Tejero (1985) reported that lactating mice inoculated with *T. rangeli* presented parasitemia levels 7 times higher than the original inocula, suggesting parasite multiplication in the vertebrate host. In other studies, amastigote-like forms were found in cross-sections of heart, liver and spleen from young MMRI mice inoculated with the Venezuelan *T. rangeli* strain Perro-82 (De Scorza *et al.,* 1986, Urdaneta-Morales and Tejero, 1986). *In vitro* studies using the human promonocyte (U937) cell line infected with *T. rangeli* (San Agustin strain and Ub66-5b clone) demonstrated intracellular amastigote-like forms but without intracellular multiplication evidences (Osorio *et al.* 1995).

In another study Eger-Mangrich *et al.* (2001) infecting mice peritoneal macrophages, Vero cells and murine promocytes (J774) cell line, with culture trypomastigotes of *T. rangeli* (Choachi and SC-58 strains), showed the presence of intracellular non-dividing amastigote-like forms in both Vero and J774 cells until 144 hours. Parasites did not survive within macrophages. It is possible that *T. rangeli* reproduction in the mammalian host could depend on the characteristics of the parasite strain being used. However, the reproductive phase of *T. rangeli* in the vertebrate host is currently not known and the mentioned observations need to be confirmed using cloned strains of *T. rangeli* to eliminate any possibility of contamination with *T. cruzi* (D´Alessandro, 1976; Cuba, 1998; Grisard *et al.,* 1999b; Guhl and Vallejo, 2003)**.**

More than 2,600 cases of human infection by *T. rangeli* are related in the literature (D´Alessandro-Bacigalupo and Saravia, 1992). In several areas, *T. rangeli* has a known overlapping distribuition with *T. cruzi*, the causative agent of Chagas disease and, not rarely, mixed infections in mammals and triatomine bugs as well as in humans had been referred (D´Alessandro-Bacigalupo and Saravia, 1992; Grisard *et al.,* 1999b; Guhl and Vallejo, 2003)**.** Despite its harmless to the vertebrate host, *T. rangeli* infection in humans induces a humoral immune response resulting in antibodies that cross-react with *T. cruzi* in distinct serological assays. Afchain *et al.* (1979) demonstrated that culture epimastigotes of *T. cruzi* and *T. rangeli* share around 60% of their soluble antigens. This fact constitutes an additional problem for the diagnosis of Chagas disease since false-positive results can be expected in areas where these parasites are sym-patric (D´Alessandro-Bacigalupo and Saravia, 1992).

Detection of *T. rangeli* infection in humans is based on the same serological (indirect immunofluorescence, hemagglutination and ELISA) assays and parasitological techniques (hemoculture and xenodiagnosis) used for Chagas disease, allowing the occurrence of false-positive results in routine diagnosis (Añez *et al.,* 1985; Steindel *et al.,* 1991). Furthermore, *T. rangeli* single or mixed infections with *T. cruzi* have been reported in triatomines (Steindel *et al*., 1994; Gurgel-Gonçalves *et al.,* 2004), in wild mammals (Ramírez *et al.,* 2002) and in humans (Saldaña *et al.,* 2005).

Although *T. rangeli* can be distinguished from *T. cruzi* using several biological, immunological, biochemical and molecular methods, the characteristic biological behavior in the invertebrate host continues to be the best tool for distinguishing between these interesting parasites (Grisard *et al.,* 1999b).

Life Cycle

T. rangeli life cycle in the triatomine starts with the ingestion of the non-dividing blood trypomas-tigotes during a bloodmeal. After ingestion, trypomastigotes transform into large and short epimastigotes and spheromastigotes forms which multiply in the gut of the insect vector. Around

30 days after the infective bloodmeal epimastigotes and trypomastigotes start to be released with the vector´s urine or feces (D´Alessandro, 1976; Steindel *et al.,* 1993**).** It is noteworthy that trypomastigotes present in the triatomine feces are morphologically distinct from the metacyclic trypomastigotes derived from salivary glands.

Studies on the *T. rangeli* evasion from the intestine have proposed different patterns for this event. It has been recently suggested that the parasite crosses the cytoplasm of the midgut cells, causing cell damage reaching the hemocoel (De Oliveira and De Souza, 2001). However, it has been also proposed that *T. rangeli* crosses the intestinal barrier by an intracellular route without cell damage (Hecker *et al.,* 1990).

Although the concept of intracellular division of *T. rangeli* within hemocytes is commonly present in several manuscripts, no clear evidence of such division has been proved so far. For instance, it is not well established if the parasites are multiplying into the vector´s defense cells or if they were phagocyted and killed. De Oliveira *et al.* (2001), using transmission electron microscopy, showed that plasmatocytes were able to ingest epimastigote forms of the parasite forming a vacuole, but no evidence of dividing parasites were observed within such cells.

Recent studies using electron microscopy showed that epimastigotes coming from the insect hemocoel cross the basal lamina that surrounds the salivary glands and penetrate through the gland cell´s cytoplasm, and after reaching the gland lumen, epimastigotes forms remain adhered to the gland cell microvilli by their flagella, while metacyclic trypo-mastigotes are found swimming free in the saliva (Meirelles *et al.,* 2005). Since several mechanisms or process of *T. rangeli* - vector interaction are not well understood, the improvement or development of new tools to investigate the parasite life cycle, like green fluorescence protein (GFP) expressing parasites, are of great importance and can be used to determine cell-to-cell interactions, quantify parasite survival and penetration rates and address the little understood life cycle in the vertebrate hosts (Guevara *et al.,* 2005).

Vector susceptibility

The pleomorphism of *T. rangeli* prevents differentiation of epimastigote forms of the parasite from other trypanosomatids by morphology. Thus, the presence of flagellates similar to *T. rangeli* (*T. rangeli*-like) in triatomine intestines does not imply that such species have vectorial capacity. The definitive evidence consists of demonstrating the presence of metacyclic trypomastigotes in the saliva or in the salivary glands and their infectivity to a vertebrate (Guhl and Vallejo, 2003).

In this way, the *Rhodnius* genus is particularly susceptible to infection by *T. rangeli* and transmi-ssion by salivary inoculation has been demonstrated for 12 out of 15 species of this genus using naturally or experimentally infected insects (Guhl and Vallejo, 2003). Although with low infection rates in salivary glands, the susceptibility of *Panstrongylus megistus*, *Triatoma infestans*, *T. sordida*, *T. braziliensis* and *T. vitticeps* to *T. rangeli* infection was recently reported (De Stefani Marquez *et al.,* 2006).

While *T. cruzi* infection reveals no pathological effects to triatomines, infection by *T. rangeli* determines well described pathologies (Cuba, 1998). Such pathological characteristic may be reflecting differences on the co-evolution of these parasites with their invertebrate hosts (D´Alessandro-Bacigalupo and Saravia, 1992; Eichler and Schaub, 2002).

T. rangeli is also a useful model to study immune modulation in *Rhodnius* species as demonstrated by some studies involving the role of substances in hemocyte micro-aggregation, nitric oxide production and proPO system (Gomes *et al.* 2003, Machado *et al.,* 2006), and inferences on the interaction of the parasite with the vector *R. prolixus* were recently reviewed (Whitten *et al.,* 2001; Azambuja and Garcia, 2005; Azambuja *et al.,* 2005).

***T. rangeli* specific features**

Studies involving antigenic, genetic and biochemical characteristics of the parasite have been performed with three main objectives: I) To enhan-

ce the knowledge about the parasite biology itself; II) To establish differential diagnostics between *T. cruzi* and *T. rangeli*; III) To address phylogenetic and evolutionary issues.

Characterization of *T. rangeli* has been extensively reviewed by Grisard *et al.* (1999) and Guhl and Vallejo (2003). However, recent approaches by analysis of the parasite transcriptome have been started (Snoeijer *et al.,* 2004; Rodrigues, 2005).

Comparative genomic, transcriptomic and/or pro-teomic studies of distinct *T. cruzi* and *T. rangeli* populations will allow the identification, purification and characterization of differentially expressed pro-teins that are involved in the induction of host immune response, directly contributing to prevent false positive results and incrementing the sen-sitivity and specificity of Chagas disease diagnosis as well as to reinforce the taxonomic position of *T. rangeli* (Stevens *et al.,* 1999).

In this context, comparisons of the total protein profiles from *T. rangeli* and *T. cruzi,* according to their morphological and physiological stages (epimastigotes and trypomastigotes) showed a differential gene expression during the life cycle, inclu- ding some species-specific proteins (Mejia *et al.,* 2004). Among the differently expressed genes detected by a substrative approach used in this study for the isolation and characterization of species-specific expressed genes, a mucin-like gene described by Abate *et al.* (2005) was clearly expressed only by *T. cruzi*.

Another example of differential protein expression are GPI-anchored proteins from the plasmatic membrane of *T. rangeli* and *T. cruzi* epimas-tigotes, which were isolated and characterized by Añez-Rojas *et al.* (2006). This study revealed different patterns of GPI-anchored proteins be-tween *T. rangeli* (90, 85 and 56 kDa) and *T. cruzi* (30, 70 and 100 kDa). These differences in size and the specific antigenicity observed, lead the authors to consider these proteins as a biochemical marker to identify and differentiate these parasites.

Genetic Characterization

Little is known about genetic structures and gene expression in *T. rangeli*. Comparing the current number of nucleotide sequences, proteins, structures and citations on PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db= PubMed) of *T. rangeli* with other Trypanosomatid species entries in the GenBank, a small number of *T. rangeli* nucleotide sequences or genes (150) and proteins (73) have been characterized and the overall picture of the genome is still absent (Table 1).

Despite the reduced number of nucleotide sequences also observed for *T. vivax* and *T. congolense*, the Sanger Institute (http://www. sanger.ac.uk/) is carrying out a partial genome project for both species, which will increment their databases in the near future.

Kinetoplast and mini-exon gene studies

Important contributions were done studying sequences conserved in all Kinetoplastida: the kinetoplast DNA (kDNA), a peculiar form of mitochondrial DNA (Sturm *et al.,* 1989, Vallejo *et al.,* 1994), and the nuclear mini-exon (spliced-leader or SL) gene, which generates a 39 nucleotide (nt) long sequence added to the 5' end of all transcribed mRNA (Grisard *et al.,* 1999a).

Table 1: Number of sequences, proteins, structures and citations on PubMed from distinct *Trypanosoma* species available at the GenBank*.

Data type	*T. vivax*	*T. cruzi*	*T. brucei*	*T. congolense*	*T. evansi*	*T. rangeli*
Nucleotide	68	169.329	111.179	111	158	150
Protein	165	45.656	27,077	96	114	73
Structure	9	69	77	0	0	10
Citations on PubMed	476	8.265	5.881	209	496	320

*Data extracted from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) on December 2006.

The kDNA network consists of several thousand mini-circles and a few dozen maxi-circles that are concatenated. The mini-circles are known to differ in nucleotide sequence among distinct spe-cies, but are homogeneous in size and can commonly cross-hybridize (Vallejo *et al.,* 1994). Interestingly, at least one copy of a conserved ~100–200 nt long region containing an almost invariant universal 12 nt sequence, has been found on the kDNA mini-circles of all trypanosomatid species studied so far (Sturm *et al.,* 1989; Vallejo *et al.,* 1994).

There are four copies of a conserved region of 120bp in *T. cruzi* mini-circles organized at 90° to each other. On the other hand, *T. rangeli* contains three distinct classes of kDNA mini-circles which differ in size and molecular organization, as follows: KP1 mini-circles contain a single conserved region, KP2 contains two conserved regions located at 180° to each other, and KP3 contains four conserved regions located at 90°, as observed for *T. cruzi* (Vallejo *et al.,* 1994; Guhl *et al.,* 2002).

In this outstanding study, Vallejo *et al.* (1994) described that conserved regions of the mini-circles of both species have high similarity, allowing the design of primers for specific and sensitive detection of these trypanosomes by PCR. Furthermore, Vallejo *et al.* (2002) developed a duplex PCR assay allowing the amplification of all types of mini-circles, which is of major importance for strain characterization and to address the parasite´s epidemiology.

Using that approach in a study carried out in many countries of Latin America, the authors have determined that *T. rangeli* strains isolated from *R. prolixus* presented all three amplification products, while the strains isolated from other *Rhodnius* species presented amplification products derived only from KP2 and KP3 mini-circles, but not from KP1. *T. rangeli* strains presenting KP1 mini-circles (KP1+) or not (KP1-) were found in the intestine of several specimens of *R. colombiensis*, but KP1(+) strains were not able to escape to the haemolymph and invade the salivary glands. The same authors observed a similar situation in *R. prolixus*, where it was possible to find both *T. rangeli* KP1(-) and KP1(+) in the intestine, but only KP1(+) strains in the salivary glands (Vallejo *et al.,* 2002; Urrea *et al.,* 2005).

These data suggests that some *Rhodnius* species are preferably susceptible to *T. rangeli* KP1(+) strains while others are susceptible to KP1(-). Thus, each *Rhodnius* species would select in nature the parasite subpopulations that are able to reach the salivary glands and to be transmitted by the saliva while probing the vertebrate host (Guhl and Vallejo, 2003). Such observation was formerly pointed out in a previous study that revealed this vector-parasite relationship when studying the susceptibility of distinct *Rhodnius* species to some *T. rangeli* strains isolated from different geographical areas (Machado *et al.,* 2001) and confirmed by a recent study by De Stefani Marquez *et al.,* (2006).

Studies on *T. rangeli* mini-exon gene also allowed the division of *T. rangeli* strains in two distinct lineages, according to sequence differences. This gene is a sequence of 39 nt found in tandem repeats on the parasite nuclear DNA. Such repeats are 582 nt long in *T. cruzi* and 858 nt in *T. rangeli,* due the insertion of the small subunit of the ribosomal (5S rDNA) gene between the *T. rangeli* mini-exon gene repeats, which is absent in *T. cruzi* (Hernandez-Rivas *et al.,* 1992). Therefore, these two organisms may be distinguished primarily by the electrophoretic mobilities of their respective amplification products (Murthy *et al.,* 1992). Also, inter as well as intra-specific variability of different strains of *T. rangeli* can be assessed by the SL gene (Grisard *et al.,* 1999a; Guhl and Vallejo, 2003; Urrea *et al.,* 2005). However, due the low copy number of this gene (~200-300/cell) the SL gene is not a good target for direct differential diagnosis by PCR (Grisard *et al.,* 1999a).

Independent mitochondrial (Vallejo *et al.,* 2003) and nuclear (Grisard *et al.,* 1999a) molecular markers initially showed a clear division of *T. rangeli* into two major phylogenetic groups associated with specific vectors in Latin America countries.

Along with the use of the SL gene and the kDNA mini-circles, several other markers (5S rDNA gene, internal transcribed spacers – ITS and RAPD) have been used for inter and intra-specific

differentiation (Aksoy *et al.,* 1992; Steindel *et al.,* 1994; Grisard *et al.,* 1999a; Barnabe *et al.,* 2003; Da Silva *et al.,* 2004; Beltrame-Botelho *et al.,* 2005).

These studies allowed phylogenetic and taxonomic studies of *T. rangeli* isolates from man, triatomines and wild mammals, allowing the recent indication of the occurrence of four distinct *T. rangeli* lineages (Maia Da Silva *et al.,* 2004).

Despite the divergence on the number of *T. rangeli* lineages, such studies support either clonal evolution or speciation in *T. rangeli* populations, probably derived as a secondary adaptation to their parasitic condition in triatomine vectors (Guhl and Vallejo, 2003; Gurgel-Gonçalves *et al.,* 2004; Urrea *et al.,* 2005).

Since different degrees of polymorphism among *T. rangeli* strains isolated from different geographical regions, hosts and vectors were detected, we reinforce our former suggestion to use as many well characterized strains and biochemical, immunological and molecular markers for intra and interspecific characterization as possible (Grisard *et al.,* 1999a).

Study of some other specific genes

Currently an increasing number of studies have been carried out addressing *T. rangeli* specific genes or using *T. rangeli* genes for comparative biochemical and/or molecular biology studies.

The discussion concerning the antigenic particularities of *T. cruzi* and *T. rangeli* is accen-tuated when the sialidases and transsialidases are in question. Some authors have described that *T. rangeli* sialidases lack the trans-sialidase activity (Buschiazzo *et al.,* 1993; Pontes-De-Carvalho *et al.,* 1993; Buschiazzo *et al.,* 1997; Sousa *et al.,* 2005), or with similar activity in distinct pH. While *T. rangeli* sialidase is more efficient in acid pH (5.0), the optimal activity of the *T. cruzi* enzyme occurs in neutral pH. Nevertheless, the function of *T. rangeli* sialidase still remains unknown, but some authors have suggested the enzyme´s invol-vement in the parasite development inside the insect vector (Medina-Acosta *et al.,* 1994).

The high resolution structures of free and inhibitor-bound *T. rangeli* sialidase and its comparison with *T. cruzi* trans-sialidase, revealed a highly conserved catalytic center, where subtle structural differences account for strikingly different enzymatic activities and inhibition properties (Amaya *et al.,* 2003). Other recent work characterized an ORF encoding a protein with high identity with members of *T. cruzi* gp85/trans-sialidase family, which function also remains uncertain, but the expression was confirmed and many copies of this gene were located next to the telomeres, as well as several surface proteins of the trypanosomatids (Añez-Rojas *et al.* 2005).

Another *T. rangeli* gene already studied is the tyrosine-aminotransferase (Bontempi *et al.* 2000). This study revealed that this gene is organized in tandem multicopy arrays, encoding a protein of 420 aminoacids (aa) with a predicted molecular mass of 46.4 kDa, having confirmed the enzymatic activity.

The kinetoplastid membrane protein´s 11 gene (KMP-11 antigen), which seems to play a key role in the induction of humoral and celular immune response, was also characterized in *T. rangeli* (Uruena *et al.,* 2004). The locus encoding this protein is formed by four gene units organized in tandem with 97% identity to the deduced aa sequence of *T. cruzi,* and are located in different chromosomes of *T. rangeli* KP1(+) and KP1(-) strains.

Cuervo *et al.* (2006) studied the hystone H2A gene as a differential marker for KP1(+) strains by LSSP-PCR. The locus encoding H2A protein in KP1(+) strains is formed by at least 11 gene units of 799bp, organized in tandem and located in two distinct chromosomes of approximately 1.9 and 1.1Mb in size, whereas in KP1(-) strains these genes are on pairs of chromosomes of about 1.7 and 1.9Mb (Puerta *et al.,* 2000; Cuervo *et al.,* 2006).

Vargas *et al.* (2000) identified a *T. rangeli*-specific repetitive element of 542 nt (named P542) in the *T. rangeli* genomic library by Southern and Slot Blot. The element has approximately 10^3 copies per parasite genome and is distributed in several chromosomal bands. Based on the nucleotide sequence of this element, a PCR-based assay allowed the detection of 1/10 of the DNA content of a single parasite and no amplification

products were observed for other trypanosomatids. This approach is very sensitive and allows specific diagnosis of *T. rangeli´*s presence even in mixed infections (Vargas *et al.,* 2000).

Already reported for several trypanosomatids, a recent work described and characterized the D-glucose transport activity in both epimastigote and trypomastigote forms of *T. rangeli*, revealing a high affinity transport in both forms and a larger identity of the predicted aa sequence with the homologous *T. cruzi* fragment (Miletti *et al.,* 2006).

Recently, the ability to hydrolyze extracellular ATP was described for *T. rangeli* epimastigote forms (Fonseca *et al.,* 2006), such activity was stimulated by carbohydrates involved in the attachment / invasion of salivary glands of *R. prolixus* and by lipoprotein circulating in the haemolymph.

Other than this work, the physiological role of ecto-ATPases is still poorly understood in many orga-nisms. Some authors have suggested a role in cell protection against extracelular ATP citolytic effects (Filippini *et al.,* 1990), regulation of ecto-quinase concentration (Plesner, 1995), envolve-ment in sinal transduction (Margolis *et al.,* 1990), and cellular adhesion mechanisms (Kirley, 1997).

Another interesting report describes an ecto-phosphatase activity in *T. rangeli* (Gomes *et al.,* 2006). Distinct ecto-phosphatase activities were detected in both short and long epimastigote forms of *T. rangeli* and cytochemical analysis demons-trated the localization of these enzymes on the parasite surface (cell body, flagellum and intrace-llular vacuoles) showing that the main ecto-phosphatase activity was phosphotyrosine phosphatase (PTP) (Gomes *et al.,* 2006). The function of these enzymes in trypanosomatids is not well established, but experimental evidences show that they participate in process like celular differentiation (Bakalara *et al.* 1995; Szoor *et al.,* 2006) increase of infectivity (Furuya *et al.,* 1998) and cellular nutrition (Gottlieb and Dwyer, 1981; Remaley *et al.,* 1985a; Remaley *et al.,* 1985b).

However, the corresponding gene(s) for both ecto-ATPase and tyrosine phosphatase activities have not been identified so far.

Toaldo *et al.* (2001) studied the molecular karyotype and chromosomal localization of genes encoding beta-tubulin, cysteine proteinase, a heat shock protein (HSP 70) and actin in *T. rangeli* strains from distinct geographical regions. The beta-tubulin karyotype profile allowed the separation of *T. rangeli* in two distinct groups, while cysteine proteinase, HSP 70 and actin genes were useful to differentiate *T. cruzi* and *T. rangeli*. These data reinforced the high degree of polymorphism among *T. rangeli* strains isolated in Latin America, remarkably among strains from Southern Brazil and strains iso-lated from Central and Northern of South America.

Genomic data

Considering the recent effort to sequence the genomes of the TriTryps, three highly important trypanosomatids from the public health point of view (*T. cruzi*, *T. brucei* and *Leishmania major*), and aiming at obtaining a general overview of the *T. rangeli* genome, our group has been working during the last years to generate an overview of the parasite transcriptome (GOLD, http://www. genomesonline.org/). The study is based on the generation of *Expressed Sequence Tags* (EST) (Adams, 1994) and *Open Reading Frame EST* (ORESTES) (Dias Neto *et al.,* 2000) from both epimastigote and trypomastigote forms from two distinct *T. rangeli* strains.

Among the different approaches to evaluate the gene expression, the generation of the EST and ORESTES represents a useful tool for the physical mapping of genomes, with the advantage of generating cDNA libraries that can represent different developmental stages or specie-specific genes (Verdun *et al.,* 1998; Dias Neto *et al.,* 2000).

Initially, normalized and non-directional cDNA libraries were constructed using epimastigote forms of the Choachi strain, resulting in a total of 656 valid EST, among which, 386 showed similarity with Trypanosomatids sequences and over 37% of the valid EST returned no hits and may represent specific genes, unknown genes, or even 5' or 3' end untranslated regions-UTR (Snoeijer *et al.,* 2004).

Using the *in vitro* metacyclogenesis method (Koerich *et al.,* 2002) to obtain trypomastigote

forms, further cDNA libraries of both parasite forms were constructed and sequenced in order to perform both intra and inter-specific comparative studies.

Following the generation of ORESTES profiles from both epimastigotes and trypomastigotes from *T. rangeli* SC-58 and Choachi strains, the project is now in the annotation process of about 10.000 sequences (EST and ORESTES). The annotation process in based on a system specially designed to analyze genomic data. Composed of selected bioinformatics software packages and an intuitive web-based interface named GARSA (*Genomic Analyses Resources for Sequence Annotation*) (Davila *et al.,* 2005), the system will offer a comprehensive and user-friendly *T. rangeli* data-base allowing easy search for genes and their annotations.

With the release of the *T. cruzi* (El-Sayed *et al.,* 2005), *T. brucei* (Berriman *et al.,* 2005) and *L. major* (Ivens *et al.,* 2005) genome databases, the existence of well annotated sequences from distinct *T. rangeli* forms will allow comparative biochemical, taxonomic and phylogenetic studies.

Upon publication, the *T. rangeli* transcriptome will be publicly available through the BiowebDB Consortia website (http://www.biowebdb.org), were curated and annotated sequences can be assessed, as well as through the GenBank.

REFERENCES

ABATE, T., M. RINCON, Z. DIAZ-BELLO, L. SPENCER.& A. RODRIGUEZ-ACOSTA
2005. A mucin-like gene different from the previously reported members of the mucin like gene families is transcribed in *Trypanosoma cruz*i but not in *Trypanosoma rangeli*. *Mem Inst Oswaldo Cruz,* 100:(4)391-395.

ADAMS, M. D.
1994. Expressed sequence tags as tools for physiology and genomics (71-76). In: Adams, M. D., Fields, C., Venter, J. C., editors. *Automated DNA sequencing and analysis.* London: Academic Press.

AFCHAIN, D., D. LE RAY, J. FRUIT & A. CAPRON
1979. Antigenic make-up of *Trypanosoma cruzi* culture forms: identification of a specific component. *J. Parasitol.,* 65:(4)507-514.

AKSOY, S., G. L. SHAY, M. S. VILLANUEVA, C. BEARD & F. RICHARDS
1992. Spliced leader RNA sequences of *Trypanosoma rangeli* are organized within the 5S rRNA-encoding genes. *Gene,* 113(2):239-243.

AMAYA, M., A. BUSCHIAZZO, T. NGUYEN & P. M. ALZARI
2003. The high resolution structures of free and inhibitor-bound *Trypanosoma rangeli* sialidase and its comparison with *T. cruzi* trans-sialidase. *J. Mol. Biol.,* 325(4):773-784.

AÑEZ-ROJAS, N., P. GARCIA-LUGO, G. CRISANTE, A. ROJAS & N. AÑEZ
2006. Isolation, purification and characterization of GPI-anchored membrane proteins from *Trypanosoma rangeli* and *Trypanosoma cruzi. Acta Trop.,* 97(2):140-145.

AÑEZ-ROJAS, N., A. PERALTA, G. CRISANTE, A. ROJAS, N. AÑEZ, J. RAMIREZ & M. A. CHIURILLO, M. A.
2005. *Trypanosoma rangeli* expresses a gene of the group II trans-sialidase superfamily. *Mol. Biochem. Parasitol.,* 142(1):133-136.

AÑEZ, N., J. VELANDIA & A. M. RODRIGUEZ
1985. *Trypanosoma rangeli* Tejera, 1920. VIII. Response to reinfections in 2 mammals. *Mem. Inst. Oswaldo Cruz,* 80(2):149-153.

ANTHONY, R. L., T. S. CODY & N. T. CONSTANTINE
1981. Antigenic differentiation of *Trypanosoma cruzi and Trypanosoma rangeli* by means of monoclonal-hybridoma antibodies. *Am. J. Trop. Med. Hyg*., 30(6):1192-1197.

ANTHONY, R. L., C. JOHNSON & O. E. SOUSA
1979. Use of micro-ELISA for quantitating antibody to *Trypanosoma cruzi and Trypanosoma rangeli*. *Am. J. Trop. Med. Hyg*., 28(6):969-973.

AZAMBUJA, P.& E. S. GARCIA
2005. *Trypanosoma rangeli* interactions within the vector *Rhodnius prolixus*: a mini review. *Mem. Inst. Oswaldo Cruz*, 100(5):567-72.

AZAMBUJA, P., N. A. RATCLIFFE & E. S. GARCIA
2005. Towards an understanding of the interactions of *Trypanosoma cruzi and Trypanosoma rangeli* within the reduviid insect host *Rhodnius prolixus*. *An. Acad. Bras. Cienc*., 77(3):397-404.

BAKALARA, N., A. SEYFANG, T. BALTZ & C. DAVIS
1995. *Trypanosoma brucei* and *Trypanosoma cruzi*: life cycle-regulated protein tyrosine phosphatase activity. *Exp. Parasitol*., 81(3):302-312.

BARNABE, C., S. BRISSE & M. TIBAYRENC
2003. Phylogenetic diversity of bat trypanosomes of subgenus *Schizotrypanum* based on multilocus enzyme electrophoresis, random amplified polymorphic DNA, and cytochrome b nucleotide sequence analyses. *Infect. Genet. Evol*., 2(3):201-208.

BASSO, B., L. CERVETTA, E. MORETTI, Y. CARLIER & C. TRUYENS
2004. Acute *Trypanosoma cruzi* infection: IL-12, IL-18, TNF, sTNFR and NO in *T. rangel*i-vaccinated mice. *Vaccine,* 22(15-16):1868-1872.

BELTRAME-BOTELHO, I. T., D. GASPAR-SILVA, M. STEINDEL, A. M. DAVILA & E. C. GRISARD
2005. Internal transcribed spacers (ITS) of *Trypanosoma rangeli* ribosomal DNA (rDNA): a useful marker for inter-specific differentiation. *Infect. Genet. Evol*., 5(1):17-28.

BERRIMAN, M., E. GHEDIN, C. HERTZ-FOWLER, G. BLANDIN, H. RENAULD, D. BARTHOLOMEU, N. LENNARD, E. CALER, N. HAMLIN, B. HAAS, U. BOHME, L. HANNICK, M. ASLETT, J. SHALLOM, L. MARCELLO, L. HOU, B. WICKSTEAD, U. ALSMARK, C. ARROWSMITH, R. ATKIN, A. BARRON, F. BRINGAUD, K. BROOKS, M. CARRINGTON, I. CHEREVACH, T. CHILLINGWORTH, C. CHURCHER, L. CLARK, C. CORTON, A. CRONIN, R. DAVIES, J. DOGGETT, A. DJIKENG, T. FELDBLYUM, M. FIELD, A. FRASER, I. GOODHEAD, Z. HANCE, D. HARPER, B. HARRIS, H. HAUSER, J. HOSTETLER, A. IVENS, K. JAGELS, D. JOHNSON, J. JOHNSON, K. JONES, A. KERHORNOU, H. KOO, N. LARKE, S. LANDFEAR, C. LARKIN, V. LEECH, A. LINE, A. LORD, A. MACLEOD, P. MOONEY, S. MOULE, D. MARTIN, G. MORGAN, K. MUNGALL, H. NORBERTCZAK, D. ORMOND, G. PAI, C. PEACOCK, J. PETERSON, M. QUAIL, E. RABBINOWITSCH, M. RAJANDREAM, C. REITTER, S. SALZBERG, M. SANDERS, S. SCHOBEL, S. SHARP, M. SIMMONDS, A. SIMPSON, L. TALLON, C. TURNER, A. TAIT, A. TIVEY, S. VAN AKEN, D. WALKER, D. WANLESS, S. WANG, B. WHITE, O. WHITE, S. WHITEHEAD, J. WOODWARD, J. WORTMAN, M. ADAMS, T. EMBLEY, K. GULL, E. ULLU, J. BARRY, A. FAIRLAMB, F. OPPERDOES, B. BARRELL, J. DONELSON, N. HALL, C. FRASER, S. MELVILLE, & N. EL-SAYED
2005. The genome of the African trypanosome *Trypanosoma brucei*. *Science,* 309:(5733)416-422.

BONTEMPI, E. J., G. A. GARCIA, A. BUSCHIAZZO, J. HENRIKSSON, C. PRAVIA, A. RUIZ, U. PETTERSSON & V. PSZENNY
2000. The tyrosine aminotransferase from *Trypanosoma rangeli*: sequence and genomic characterization. *FEMS Microbiol. Lett.,* 189(2):253-257.

BRENIERE, S. F., M. BOSSENO, C. BARNABE, S. URDANETA-MORALES & M. TIBAYRENC
1993. Population genetics of *Trypanosoma cruzi* and *Trypanosoma rangeli*: taxonomical and epide-miological purpose. *Biol. Res*., 26(1-2):27-33.

BUSCHIAZZO, A.,O. CAMPETELLA & A. FRASCH
1997. *Trypanosoma rangeli* sialidase: cloning, expression and similarity to *T. cruzi* trans-sialidase. *Glycobiology,* 7(8):1167-1173.

BUSCHIAZZO, A., M. CREMONA, O. CAMPETELLA, A. FRASCH & D. SANCHEZ
1993. Sequence of a *Trypanosoma rangeli* gene closely related to *Trypanosoma cruzi* trans-sialidase. *Mol. Biochem. Parasitol.*, 62(1):115-116.

CUBA CUBA, A.
1998. Review of the biologic and diagnostic aspects of *Trypanosoma* (*Herpetosoma*) *rangeli***.** *Rev. Soc. Bras. Med. Trop*., 31(2):207-220.

CUERVO, C., M. LOPEZ & C. PUERTA
2006. The *Trypanosoma rangeli* histone H2A gene sequence serves as a differential marker for KP1 strains. *Infect. Genet. Evol*., 6(5):401-409.

D´ALESSANDRO-BACIGALUPO, A. & N. G. SARAVIA
1992. *Trypanosoma rangeli*. In: Kreier, J. P., Baker, E. J. R., editors. *Parasitic Protozoa.* (1-54) 2ed. London: Academic Press.

D´ALESSANDRO, A.
1976. Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. In: Lumsden, W. H. R., Evans, D. A., editors. *Biology of Kinetoplastida*. (328-403) London: Academic Press.

D´ALESSANDRO, A. & N. SARAVIA
1999. *Trypanosoma rangel*i. In: Gille, H. M., editor. *Protozoal Diseases*. (398-412) Arnold.

DA SILVA, F. M., H. NOYES, M. CAMPANER, A. JUNQUEIRA, J. COURA, N. AÑEZ, J. SHAW, J. STEVENS & M. TEIXEIRA
2004. Phylogeny, taxonomy and grouping of *Trypanosoma rangeli* isolates from man, triatomines and sylvatic mammals from widespread geographical origin based on SSU and ITS ribosomal sequences. *Parasitology*, 129(5):549-561.

DAVILA, A., D. LORENZINI, P. MENDES, T. SATAKE, G. SOUSA, L. CAMPOS, C. MAZZONI, G. WAGNER, P. PIRES, E. GRISARD, M. CAVALCANTI & M. CAMPOS
2005. GARSA: genomic analysis resources for sequence annotation. *Bioinformatics,* 21(23):4302-4303.

DE OLIVEIRA, M. A. & W. DE SOUZA
2001. An electron microscopic study of penetration by *Trypanosoma rangeli* into midgut cells of *Rhodnius prolixus*. *J. Invertebr. Pathol*., 77(1):22-26.

DE SCORZA, C., S. URDANETA-MORALES & F. TEJERO
1986. *Trypanosoma* (*Herpetosoma*) *rangeli* Tejera, 1920: preliminary report on histopathology in expe-rimentally infected mice. *Rev. Inst. Med. Trop. Sao Paulo,* 28(5):371-378.

DE STEFANI MARQUEZ, D., C. RODRIGUES-OTTAIANO, R. MONICA OLIVEIRA, A. PEDROSA, M. CABRINE-SANTOS, E. LAGES-SILVA & L. RAMIREZ
2006. Susceptibility of different triatomine species to *Trypanosoma rangeli* experimental infection. *Vector Borne Zoonotic Dis.,* 6(1):50-56.

DIAS NETO, E., R. CORREA, S. VERJOVSKI-ALMEIDA, M. BRIONES, M. NAGAI, W. DA SILVA, M. ZAGO, S. BORDIN, F. COSTA, G. GOLDMAN, A. CARVALHO, A. MATSUKUMA, G. BAIA, D. SIMPSON, A. BRUNSTEIN, P. DE OLIVEIRA, P. BUCHER, C. JONGENEEL, M. O'HARE, F. SOARES, R. BRENTANI, L. REIS, S. DE SOUZA & A. SIMPSON

2000. Shotgun sequencing of the human transcriptome with ORF expressed sequence tags. *Proc. Natl. Acad. Sci.,* 97(7):3491-3496.

EGER-MANGRICH, I., M. DE OLIVEIRA, E. GRISARD, W. DE SOUZA & M. STEINDEL

2001. Interaction of *Trypanosoma rangeli* Tejera, 1920 with different cell lines in vitro. *Parasitol. Res*., 87(6):505-509.

EICHLER, S. & G. SCHAUB

2002. Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. *Exp. Parasitol.,* 100(1):17-27.

EL-SAYED, N. M., P. MYLER, D. BARTHOLOMEU, D. NILSSON, G. AGGARWAL, A. TRAN, E. GHEDIN, E. WORTHEY, A. DELCHER, G. BLANDIN, S. WESTENBERGER, E. CALER, G. CERQUEIRA, C. BRANCHE, B. HAAS, A. ANUPAMA, E. ARNER, L. ASLUND, P. ATTIPOE, E. BONTEMPI, F. BRINGAUD, P. BURTON, E. CADAG, D. CAMPBELL, M. CARRINGTON, J. CRABTREE, H. DARBAN, J. DA SILVEIRA, P. DE JONG, K. EDWARDS, P. ENGLUND, G. FAZELINA, T. FELDBLYUM, M. FERELLA, A. FRASCH, K. GULL, D. HORN, L. HOU, Y. HUANG, E. KINDLUND, M. KLINGBEIL, S. KLUGE, H. KOO, D. LACERDA, M. LEVIN, H. LORENZI, T. LOUIE, C. MACHA-DO, R. MCCULLOCH, A. MCKENNA, Y. MIZUNO, J. MOTTRAM, S., NELSON, S. OCHAYA, K. OSOEGAWA, G. PAI, M. PARSONS, M. PENTONY, U. PETTERSSON, U., POP, M., RAMIREZ, J. L., RINTA, S. ROBERTSON, D. SALZBERG, J. SANCHEZ, A. SEYLER, R, SHARMA, J. SHETTY, A. SIMPSON, E. SISK, M. TAMMI, R. TARLETON, S. TEIXEIRA, S. VAN AKEN, C. VOGT, P. WARD, B. WICKSTEAD, J. WORTMAN, O. WHITE, C. FRASER, K. STUART& B. ANDERSSON

2005. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science,* 309(5733):409-415.

FILIPPINI, A., R. TAFFS, T. AGUI & M. SITKOVSKY

1990. Ecto-ATPase activity in cytolytic T-lymphocytes. Protection from the cytolytic effects of extracellular ATP. *J. Biol. Chem*., 265(1):334-340.

FONSECA, F. V., A. FONSECA DE SOUZA, A. , MARIANO, P. ENTRINGER, K. GONDIM & J. MEYER-FERNANDES

2006. *Trypanosoma rangeli:* characterization of a Mg-dependent ecto ATP-diphosphohydrolase activity. *Exp. Parasitol*., 112(2):76-84.

FURUYA, T., L. ZHONG, J. MEYER-FERNANDES, H. LU, S. MORENO & R. DOCAMPO

1998. Ecto-protein tyrosine phosphatase activity in *Trypanosoma cruzi* infective stages. *Mol. Biochem. Parasitol*., 92(2):339-348.

GARCIA, E., D. CASTRO, I. RIBEIRO, T. TOMASSINI & P. AZAMBUJA

2006. *Trypanosoma rangeli*: effects of physalin B on the immune reactions of the infected larvae of *Rhodnius prolixus*. *Exp. Parasitol.*,112(1):37-43.

GARCIA, E. S., E. MACHADO & P. AZAMBUJA

2004a. Effects of eicosanoid biosynthesis inhibitors on the prophenoloxidase-activating system and microaggregation reactions in the haemolymph of *Rhodnius prolixus* infected with *Trypanosoma rangeli*. *J. Insect Physiol*., 50(2-3):157-165.

2004b. Inhibition of hemocyte microaggregation reactions in *Rhodnius prolixus* larvae orally infected with *Trypanosoma rangeli*. *Exp. Parasitol.*, 107:(1-2)31-38.

GOMES, S. A., D. FEDER, E. GARCIA & P. AZAMBUJA

2003. Suppression of the prophenoloxidase system in *Rhodnius prolixus* orally infected with *Trypanosoma rangel*i. *J. Insect Physiol*., 49(9):829-837.

GOMES, S. A., D. FEDER, N. THOMAS, E. GARCIA & P. AZAMBUJA

1999. *Rhodnius prolixus* infected with *Trypanosoma rangeli*: In vivo and in vitro experiments. *J. Invertebr. Pathol.,* 73(3):289-293.

GOMES, S. A., A. FONSECA DE SOUZA, B. SILVA, T. KIFFER-MOREIRA, J. SANTOS-MALLET, A. SANTOS, & J. MEYER-FERNANDES

2006. *Trypanosoma rangeli*: Differential expression of cell surface polypeptides and ecto-phosphatase activity in short and long epimastigote forms. *Exp. Parasitol*., 112(4):253-262.

GOMEZ, I.

1967. New observations on the pathological actions of *Trypanosoma rangeli* Tejera, 1920 on *Rhodniu prolixus*, 1859. *Rev. Inst. Med. Trop. Sao Paulo*, 9(1):5-10.

GOTTLIEB, M. & D.M. DWYER

1981. *Leishmania donovani*: surface membrane acid phosphatase activity of promastigotes. *Exp. Parasitol.,* 52(1):117-128.

GREWAL, M. S.

1957. Pathogenicity of *Trypanosoma rangeli* Tejera, 1920 in the invertebrate host. *Exp. Parasitol*., 6(2):123-130.

GRISARD, E. C., D. CAMPBELL & A. ROMANHA

1999a. Mini-exon gene sequence polymorphism among *Trypanosoma rangeli* strains isolated from distinct geographical regions. *Parasitology,* 118 (Pt 4):375-382.

GRISARD, E. C., M. STEINDEL, A. GUARNERI, I. EGER-MANGRICH, D. CAMPBELL & A. ROMANHA

1999b. Characterization of *Trypanosoma rangeli* strains isolated in Central and South America: an overview. *Mem. Inst. Oswaldo Cruz,* 94(2):203-209.

GUEVARA, P., M. DIAS, A. ROJAS, G. CRISANTE, M. ABREU-BLANCO, E. UMEZAWA, M. VAZQUEZ, M. LEVIN, N. AÑEZ & J. RAMIREZ

2005. Expression of fluorescent genes in *Trypanosoma cruzi* and *Trypanosoma rangeli* (Kinetoplastida: Trypanosomatidae): its application to parasite-vector biology. *J. Med. Entomol.*, 42(1):48-56.

GUHL, F., C. JARAMILLO, J. CARRANZA & G. VALLEJO
2002. Molecular characterization and diagnosis of *Trypanosoma cruzi* and *T. rangeli*. *Arch. Med. Res*., 33(4):362-370.

GUHL, F.& G. VALLEJO
2003. *Trypanosoma* (*Herpetosoma*) *rangeli* Tejera, 1920: an updated review. *Mem. Inst. Oswaldo Cruz,* 98(4):435-442.

GURGEL-GONÇALVES, R., E. RAMALHO, M. DUARTE, A. PALMA, F. ABAD-FRANCH, J. CARRANZA & C. CUBA CUBA
2004. Enzootic transmission of T*rypanosoma cruzi* and *T. rangeli* in the Federal District of Brazil. *Rev. Inst. Med. Trop. Sao Paulo*, 46(6):323-330.

HECKER, H., M. SCHWARZENBACH & W. RUDIN
1990. Development and interactions of *Trypanosoma rangeli* in and with the reduviid bug *Rhodnius prolixus*. *Parasitol. Res.*, 76(4):311-318.

HENRIKSSON, J., A. SOLARI, M. RYDAKER, O, SOUSA, & U. PETTERSSON
1996. Karyotype variability in *Trypanosoma rangeli*. *Parasitology,* 112 (Pt 4):385-391.

HERBIG-SANDREUTER, A.
1955. Experimental study of the life cycle of *Trypanosoma rangeli* Tejera 1920 in warm-blooded animals and in *Rhodnius prolixus*. *Acta Trop*., 12(3):261-264.

HERNANDEZ-RIVAS, R., S. MARTINEZ-CALVILLO, M. ROMERO & R. HERNANDEZ
1992. *Trypanosoma cruzi* 5S rRNA genes: molecular cloning, structure and chromosomal organization. *FEMS Microbiol. Lett.,* 71(1):63-67.

IVENS, A., C. PEACOCK, E. WORTHEY, L. MURPHY, G. AGGARWAL, M. BERRIMAN, E. SISK, M. RAJANDREAM, E. ADLEM, R. AERT, A. ANUPAMA, Z. APOSTOLOU, P. ATTIPOE, N. BASON, C. BAUSER, A. BECK, S. BEVERLEY, G. BIANCHETTIN, K. BORZYM, G. BOTHE, C. BRUSCHI, M. COLLINS, E. CADAG, L. CIARLONI, C. CLAYTON, R. COULSON, A. CRONIN, A. CRUZ, R. DAVIES, J. DE GAUDENZI, D. DOBSON, A. DUESTERHOEFT, G. FAZELINA, N. FOSKER, A. FRASCH, A. FRASER, M. FUCHS, C. GABEL, A. GOBLE, A. GOFFEAU, D. HARRIS, C. HERTZ-FOWLER, H. HILBERT, D. HORN, Y. HUANG, S. KLAGES, A. KNIGHTS, M. KUBE, N. LARKE, L. LITVIN, A. LORD, T. LOUIE, M. MARRA, D. MASUY, K. MATTHEWS, S. MICHAELI, J. MOTTRAM, S. MULLER-AUER, H. MUNDEN, S, NELSON, H. NORBERTCZAK, K. OLIVER, S. O'NEIL, M. PENTONY, T. POHL, C. PRICE, B. PURNELLE, M. QUAIL, E. RABBINOWITSCH, R. REINHARDT, M. RIEGER, J. RINTA, J. ROBBEN, L. ROBERTSON, J. RUIZ, S. RUTTER, D. SAUNDERS, M. SCHAFER, J. SCHEIN, D. SCHWARTZ, K. SEEGER, A. SEYLER, S. SHARP, H. SHIN, D. SIVAM, R. SQUARES, S. SQUARES, V. TOSATO, C. VOGT, G. VOLCK-AERT, R. WAMBUTT, T. WARREN, H. WEDLER, J. WOOD-WARD, S. ZHOU, W. ZIMMERMANN, D. SMITH, J. BLACK-WELL, K. STUART, B. BARRELL & P. MYLER
2005. The genome of the kinetoplastid parasite, *Leishmania major*. *Science,* 309(5733): 436-442.

KIRLEY, T. L.
1997. Complementary DNA cloning and sequencing of the chicken muscle ecto-ATPase. Homology with the lymphoid cell activation antigen CD39. *J. Biol. Chem*., 272(2):1076-1081.

KOERICH, L. B., P. EMMANUELLE-MACHADO, K. SANTOS, E. GRISARD & M. STEINDEL
2002. Differentiation of *Trypanosoma rangeli*: high production of infective Trypomastigote forms in vitro. *Parasitol. Res.,* 88(1):21-25.

MACHADO, E. M., P. AZAMBUJA & E. GARCIA
2006. WEB 2086, a platelet-activating factor antagonist, inhibits prophenoloxidase-activating system and hemocyte microaggregation reactions induced by *Trypanosoma rangeli* infection in *Rhodnius prolixus* haemolymph. *J. Insect. Physiol.*, 52(7):685-692.

MACHADO, P. E., I. EGER-MANGRICH, G. ROSA, L. KOERICH, E. GRISARD & M. STEINDEL
2001. Differential susceptibility of triatomines of the genus *Rhodnius* to *Trypanosoma rangeli* strains from different geographical origins. *Int. J. Parasitol*., 31(5-6): 632-634.

MAIA DA SILVA, F., A. RODRIGUES, M. CAMPANER,C. TAKATA, M. BRIGIDO, A. JUNQUEIRA, J. COURA, G. TAKEDA, J. SHAW & M. TEIXEIRA
2004. Randomly amplified polymorphic DNA analysis of *Trypanosoma rangeli* and allied species from human, monkeys and other sylvatic mammals of the Brazilian Amazon disclosed a new group and a species-specific marker. *Parasitology,* 128(3):283-294.

MARGOLIS, R. N., M. SCHELL, S. TAYLOR & A. HUBBARD
1990. Hepatocyte plasma membrane ECTO-ATPase (pp120/HA4) is a substrate for tyrosine kinase activity of the insulin receptor. *Biochem. Biophys. Res. Commun*., 166(2):562-556.

MARINKELLE, C. J., G. VALLEJO, F. GUHL & N. DE SANCHEZ
1985. Differentiation between *Trypanosoma cruzi and T. rangeli* in the intestine of the vector *Rhodnius prolixus*, based on the behavior of these flagellates with regard to the lytic activity of complement. *Rev. Latinoam. Microbiol*., 27(1):21-25.

MATTHEWS, K. R.
2005. The developmental cell biology of *Trypanosoma brucei. J. Cell. Sci.,* 118(2):283-290.

MEDINA-ACOSTA, E., A. FRANCO, A. JANSEN, M. SAMPOL, N. NEVES, L. PONTES-DE-CARVALHO, G. GRIMALDI JUNIOR & V. NUSSENZWEIG
1994. Trans-sialidase and sialidase activities discriminate between morphologically indistinguishable trypanosomatids. *Eur. J. Biochem*., 225(1):333-339.

MEIRELLES, R. M., A. HENRIQUES-PONS, M. SOARES & M. STEINDEL
2005. Penetration of the salivary glands of *Rhodnius domesticus* Neiva & Pinto, 1923 (Hemiptera: Reduviidae) by *Trypanosoma rangel*i Tejera, 1920 (Protozoa: Kinetoplastida). *Parasitol. Res.*, 97(4): 259-269.

MEJIA, J. S., F. MORENO, C. MUSKUS, I. VELEZ & R. TITUS
2004. The surface-mosaic model in host-parasite rela-tionships. *Trends Parasitol.,* 20(11):508-511.

MELLO, C. B.,E. GARCIA, N. RATCLIFFE & P. AZAMBUJA
1995. *Trypanosoma cruz*i and *Trypanosoma rangel*i: interplay with haemolymph components of *Rhod-nius prolixus*. *J. Invertebr. Pathol.*, 65(3):261-268.

MILETTI, L. C., L. KOERICH, L. PACHECO, M. STEINDEL, & B. STAMBUK
2006. Characterization of D-glucose transport in *Trypanosoma rangeli*. *Parasitology,* 133(6):721-727.

MURTHY, V. K., K. DIBBERN & D.CAMPBELL
1992. PCR amplification of mini-exon genes differentiates *Trypanosoma cruzi* from *Trypanosoma rangeli*. *Mol. Cell. Probes*., 6(3):237-243.

OSORIO, Y., B. TRAVI, G. PALMA & N. SARAVIA
1995. Infectivity of *Trypanosoma rangeli* in a promo-nocytic mammalian cell line. *J. Parasitol*., 81(5):687-693.

PALAU, M. T., A. MEJIA, U. VERGARA & C. ZUNIGA
2003. Action of *Trypanosoma rangeli* in infections with virulent *Trypanosoma cruzi* populations. *Mem. Inst. Oswaldo Cruz*, 98(4):543-548.

PLESNER, L.
1995. Ecto-ATPases: identities and functions. *Int. Rev. Cytol.*, 158:141-214.

PONTES-DE-CARVALHO, L. C., S. TOMLINSON & V. NUSSENZWEIG
1993. *Trypanosoma rangeli sialidase* lacks trans-sialidase activity. *Mol. Biochem. Parasitol.*, 62(1):19-25.

PUERTA, C., P. CUERVO, M. THOMAS & M. LOPEZ
2000. Molecular characterization of the histone H2A gene from the parasite *Trypanosoma rangeli*. *Parasitol. Res.*, 86(11):916-922.

RAMIREZ, L. E., E. LAGES-SILVA, F. ALVARENGA-FRANCO, A. MATOS, N. VARGAS, O. FERNANDES & B. ZINGALES
2002. High prevalence of *Trypanosoma rangeli* and *Trypanosoma cruzi* in opossums and triatomids in a formerly-endemic area of Chagas disease in Southeast Brazil. *Acta Trop*., 84(3):189-198.

REMALEY, A. T., S. DAS, P. CAMPBELL, G. LAROCCA, M. POPE & R. GLEW
1985a. Characterization of *Leishmania donovani* acid phosphatases. *J. Biol. Chem*., 260(2):880-886.

REMALEY, A. T., R. GLEW, D. KUHNS, R. BASFORD, A. WAGGONER, L. ERNST & M. POPE
1985b. *Leishmania donovani*: surface membrane acid phosphatase blocks neutrophil oxidative metabolite production. *Exp. Parasitol.*, 60(3):331-341.

SALDAÑA, A., SAMUDIO, F., MIRANDA, A., HERRERA, L. M., SAAVEDRA, S. P., CACERES, L., BAYARD, V.& CALZADA, J. E.
2005. Predominance of *Trypanosoma rangeli* infection in children from a Chagas disease endemic area in the west-shore of the Panama canal. Mem Inst Oswaldo Cruz 100(7):729-731.

SCHOTTELIUS, J. & V. MULLER
1984. Interspecific differentiation of *Trypanosoma cruzi*, *Trypanosoma conorhini* and *Trypanosoma rangeli* by lectins in combination with complement lysis. *Acta Trop.*, 41(1):29-38.

SNOEIJER, C., G. PICCHI, B. DAMBROS, M. STEINDEL, S. GOLDENBERG, S. FRAGOSO, D. LORENZINI & E. GRISARD
2004. *Trypanosoma rangeli* Transcriptome Project: Generation and analysis of expressed sequence tags. Kinetoplastid. *Biol. Dis.,* 3(1):1.

SOUSA, O. E., M. LOMBARDO & A. SALDAÑA
2005. *Trypanosoma rangeli* sialidase: lack of activity in stocks from Panama and other regions. *Mem. Inst. Oswaldo Cruz*, 100(3):241-243.

STEINDEL, M., E. DIAS NETO, C. PINTO, E. GRISARD, C. MENEZES, S. MURTA, A. SIMPSON & A. ROMANHA
1994. Randomly amplified polymorphic DNA (RAPD) and isoenzyme analysis of *Trypanosoma rangeli* strains. *J. Eukaryot. Microbiol*., 41(3):261-267.

STEINDEL, M., E. NETO, C. DEMENEZES, A. ROMANHA & A. SIMPSON
1993. Random Amplified Polymorphic DNA Analysis of *Trypanosoma cruzi* Strains. *Molecular and Biochemical Parasitology,* 60(1):71-80.

STEINDEL, M., J. PINTO, H. TOMA, R. MANGIA, R. RIBEIRO-RODRIGUES & A. ROMANHA
1991. *Trypanosoma rangeli* (Tejera, 1920) isolated from a sylvatic rodent (*Echimys dasythrix*) in Santa Catarina Island, Santa Catarina State: first report of this trypanosome in southern Brazil. *Mem. Inst. Oswaldo Cruz,* 86(1):73-79.

STEVENS, J. R., M. TEIXEIRA, L. BINGLE & W. GIBSON
1999. The taxonomic position and evolutionary relationships of *Trypanosoma rangeli. Int. J. Parasitol.*, 29(5):749-757.

STURM, N. R., W. DEGRAVE, C. MOREL & L. SIMPSON
1989. Sensitive detection and schizodeme classification of *Trypanosoma cruzi* cells by amplification of kinetoplast minicircle DNA sequences: use in diagnosis of Chagas' disease. *Mol. Biochem. Parasitol*., 33(3):205-214.

SZOOR, B., J. WILSON, H. MCELHINNEY, L. TABERNERO & K. MATTHEWS
2006. Protein tyrosine phosphatase TbPTP1: A molecular switch controlling life cycle differentiation in trypanosomes. *J. Cell. Biol*., 175(2):293-303.

TOALDO, C., M. STEINDEL, M. SOUSA & C. TAVARES
2001. Molecular karyotype and chromosomal localization of genes encoding beta-tubulin, cysteine proteinase, hsp 70 and actin in *Trypanosoma rangeli. Mem. Inst. Oswaldo Cruz*, 96(1):113-121.

TOBIE, E. J.
1964. Increased Infectivity of a Cyclically Maintained Strain of *Trypanosoma rangeli to Rhodnius prolixus* and Mode of Transmission by Invertebrate *Host. J. Parasitol.,* 50:593-598.

TOBIE, E. J.

1965. Biological factors influencing transmission of *Trypanosoma* r*angeli* by *Rhodnius prolixus*. *J. Parasitol.,* 51(5):837-841.

URDANETA-MORALES, S. & F. TEJERO

1985. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: mouse model for high, sustained parasitemia. *J. Parasitol*., 71(4):409-414.

1986. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. Intracellular amastigote stages of reproduction in white mice. *Rev. Inst. Med. Trop. Sao Paulo*, 28(3):166-169.

URREA, D. A., J. CARRANZA, C. CUBA, R. GURGEL-GONÇALVES, F. GUHL, C. SCHOFIELD, O. TRIANA & G. VALLEJO

2005. Molecular characterisation of *Trypanosoma rangeli* strains isolated from *Rhodnius ecuadoriensis* in Peru, *R. colombiensis* in Colombia and *R. pallescens* in Panama, supports a co-evolutionary association between parasites and vectors. *Infect. Genet. Evol.*, 5(2):123-129.

URUENA, C., P. SANTANDER, H. DIEZ, M. MONTILLA, I. ZARANTE, C. THOMAS MDEL, M. LOPEZ & C. PUERTA

2004. Chromosomal localization of the KMP-11 genes in the KP1(+) and KP1(-) strains of *Trypanosoma rangeli*. *Biomedica,* 24(2):200-203.

VALLEJO, G., F. GUHL, J. CARRANZA, L. LOZANO, J. SANCHEZ, J. JARAMILLO, D. GUALTERO, N. CASTANEDA, J. SILVA & M. STEINDEL

2002. kDNA markers define two major *Trypanosoma rangel*i lineages in Latin-America. *Acta Trop*., 81(1):77-82.

VALLEJO, G., F. GUHL, J. CARRANZA, J. , MORENO, O. TRIANA & E. GRISARD

2003. Parity between kinetoplast DNA and mini-exon gene sequences supports either clonal evolution or speciation in *Trypanosoma rangeli* strains isolated from *Rhodnius colombiensis, R. pallescens* and *R. prolixus* in Colombia. *Infect. Genet. Evol.*, 3(1):39-45.

VALLEJO, G. A., A. MACEDO, E. CHIARI & S. PENA

1994. Kinetoplast DNA from *Trypanosoma rangeli* contains two distinct classes of minicircles with different size and molecular organization. *Mol. Biochem. Parasitol.*, 67(2):245-253.

VALLEJO, G. & C. MARINKELLE

1992. Presence of neuraminidase in *Rhodnius prolixus* infected with *Trypanosoma rangeli*. *Rev. Latinoam. Microbiol*., 34(2):91-94.

VARGAS, N., R. SOUTO, J. CARRANZA, G. VALLEJO & B. ZINGALES

2000. Amplification of a specific repetitive DNA sequence for *Trypanosoma rangeli* identification and its potential application in epidemiological inves-tigations. *Exp. Parasitol*., 96(3):147-159.

VERDUN, R. E., N. DI PAOLO, T. URMENYI, E. RONDINELLI, A. FRASCH & D.SANCHEZ

1998. Gene discovery through expressed sequence Tag sequencing in *Trypanosoma cruzi*. *Infect. Immun*., 66(11):5393-5298.

WATKINS, R.

1971. *Trypanosoma rangeli*: effect on excretion in *Rhodnius prolixus. J. Invertebr. Pathol*., 17(1):67-71.

WHITTEN, M., C. MELLO, S. GOMES, Y. NIGAM, P. AZAMBUJA, E. GARCIA & N. RATCLIFFE

2001. Role of superoxide and reactive nitrogen intermediates in *Rhodnius prolixus* (Reduviidae)/*Trypanosoma rangeli* interactions. *Exp. Parasitol*., 98(1):44-57.