Genomic Analyses, Gene Expression and Antigenic Profile of the Trans-Sialidase Superfamily of *Trypanosoma cruzi* Reveal an Undetected Level of Complexity

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Abstract

The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease, a highly debilitating human pathology that affects millions of people in the Americas. The sequencing of this parasite’s genome reveals that trans-sialidase-trans-sialidase-like (TcS), a polymorphic protein family known to be involved in several aspects of *T. cruzi* biology, is the largest *T. cruzi* gene family, encoding more than 1,400 genes. Despite the fact that four TcS groups are well characterized and only one of the groups contains active trans-sialidases, all members of the family are annotated in the *T. cruzi* genome database as trans-sialidase. After performing sequence clustering analysis with all TcS complete genes, we identified four additional groups, demonstrating that the TcS family is even more heterogeneous than previously thought. Interestingly, members of distinct TcS groups show distinctive patterns of chromosome localization. Members of the TcSGroupII, which harbor proteins involved in host cell attachment/invasion, are preferentially located in subtelomeric regions, whereas members of the largest and new TcSGroupV have internal chromosomal locations. Real-time RT-PCR confirms the expression of genes derived from new groups and shows that the pattern of expression is not similar within and between groups. We also performed B-cell epitope prediction on the family and constructed a TcS specific peptide array, which was screened with sera from *T. cruzi*-infected mice. We demonstrated that all seven groups represented in the array are antigenic. A highly reactive peptide occurs in sixty TcS proteins including members of two new groups and may contribute to the known cross-reactivity of *T. cruzi* epitopes during infection. Taken together, our results contribute to a better understanding of the real complexity of the TcS family and open new avenues for investigating novel roles of this family during *T. cruzi* infection.

Introduction

The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease, a debilitating illness that is a major cause of morbidity and mortality in several Latin America countries. Approximately 10 million people carry the parasite, which causes 10,000 deaths annually [1]. During its life cycle, *T. cruzi* passes through three developmental stages. In its insect vectors, the parasite multiplies as extracellular epimastigotes, and in the hindgut it differentiates into non-dividing trypomastigotes. These infective forms are excreted in the feces after a blood meal and may contaminate the puncture site or mucous membranes of a mammalian host, where they can invade a variety of cell types. Inside host cells, trypomastigotes differentiate into amastigotes, which, after a limited number of cell divisions, differentiate into trypomastigotes that are released into circulation upon host cell rupture. This form can then infect another mammalian host cell or be taken by the insect vector during the blood meal, where it differentiates as epimastigotes.

The ability of *T. cruzi* to survive in the mammalian host is in part due to the presence of a diverse surface membrane coat. In fact, a remarkable feature of the *T. cruzi* genome is the massive expansion of genes that encode polymorphic surface proteins, which include the trans-sialidase and trans-sialidase-like superfamilies (hereafter called TcS), MASP (mucin-associated surface protein), and TcMUC mucins [2]. The TcS is the largest *T. cruzi* gene family, which has more than 1,400 genes, half of which are apparently functional. One of the most well-studied members of the TcS superfamily is the trans-sialidase (TcTS) enzyme. *T. cruzi* is unable to synthesize sialic acids de novo [3], a sugar modification present in
The TcS gene family is highly polymorphic, and only a few members have critical residues necessary for catalytic activity [8]. So far, four groups of TcS have been described based on sequence similarity and functional properties. Group I contains active trans-sialidases, namely TCNA and SAPA (shed acute-phase antigen), and TS-epi proteins expressed in the trypomastigote and epimastigote forms, respectively. Group II comprises members of the gp85 surface glycoproteins TSA-1, SA85, gp90, gp82 and ASP-2, which have been implicated in host cell attachment and invasion. FL-160, a representative of group III, is a complementary regulatory protein that inhibits the alternative and classical complement pathways. TSc13, whose function is unknown, is the representative of group IV and is included in the TcS superfamily because it contains the conserved VTVxNVxLYNR motif, which is shared by all known TcS members [8-13].

The TcS family was identified in the 1980s and, after the publication of the T. cruzi genome [2], no comprehensive analysis of its sequences has been performed. Here, by analyzing all the full-length predicted TcS proteins present in the T. cruzi genome, we identified four new groups. The TcS groups were characterized based on presence of key TcS motifs, chromosomal localization, expression profile and antigenic properties. Implications of the TcS diversity for T. cruzi biology are discussed.

Materials and Methods

Sequence diversity of the T. cruzi TcS family

Genome information and sequences were retrieved from TriTrypDB (http://TriTrypDB.org). Only complete TcS sequences totaling 508 sequences were analyzed. The DNA and the translated sequences were aligned using ClustalW 2.0 software with the default parameters [14]. These alignments were used to calculate the total (mean) nucleotide and protein diversity using MEGA5 [15] with three different methods: p-distance (nucleotide and protein sequences), Kimura-2-parameter (nucleotide sequences) and Poisson correction (protein sequences). The diversity error was estimated using bootstrap resampling with 1,000 replications.

Spatial projection and hierarchical clustering

To identify the clusters formed by the TcS protein sequences and by the 3’ sequences flanking the TcS coding regions (300 nucleotides downstream to the stop codons), we calculated the pairwise distance and generated the distance matrixes. The distances between the sequences were generated using the package PHYLIP [16,17]. To provide a visual representation of each distance matrix, we used the multidimensional scaling (MDS) plot with two dimensions (2D). The K-means method [18] was used to define ten clusters. The MDS, hierarchical clustering, statistical analyses and graphing were performed using the R software platform [19].

TcS cluster distribution on T. cruzi chromosomes and protein representation

To define the chromosomal distribution of the TcS groups, we used as reference the genome assembly reported in [20], where pairs of homologous chromosomes were arbitrarily built as having the same size. The chromosomal coordinates of the TcS genes, regardless from each homologous chromosome they are derived, were retrieved from the TriTrypDB (http://TriTrypDB.org) and plotted on the chromosomes. The colors of each coding region were the same as the colors used in the MDS protein clusters. The relative positions in the chromosomes were calculated by dividing the start codon coordinate of each gene by the total length of the chromosome. The values found were used to produce a histogram and to compare the distribution of each cluster and the pseudogenes on the chromosomes.

FRIP coordinates were found using the motif xRxP as a query. Only those occurrences located before the Asp-box and/or closer to the N-terminal extremity were considered. The Asp-box was found using the motif SxDxGxTW as a query, allowing up to 1 mismatch, and the TcS signature motif was searched using the VTVxNVxLYNR sequence as a query. In all query motifs, x represents any amino acid. The motifs were searched using the software PatMatch [21]. The signal peptide and the GPI anchor additional site were predicted using the software SignalP [22] and GPI-SOM [23], respectively. Repetitive sequences were identified using the AA-repeatFinder developed by our group (http://gicab.decom.cefetmg.br/bio-web). Only repeats with more than 10 amino acids were reported. The figures depicting the TcS genome distribution and the protein sequences were constructed using Perl (Practical Extraction and Report Language) scripts and the Bio:Graphics module, part of the Bioperl toolkit (http://www. bioperl.org).

Parasite cultures and RNA extraction

Epimastigotes of the CL Brener clone of T. cruzi were maintained in a logarithmic growth phase at 28°C in liver infusion trypose (LIT) medium supplemented with 10% fetal bovine serum. Amastigote and trypomastigote forms were obtained from infected L6 cells grown in Dulbecco’s Modified Eagle Medium supplemented with 5% fetal bovine serum, at 37°C and 5% CO₂, as described [24]. Total RNA was isolated using the RNasy kit (Qiagen).

Real-time RT-PCR

Primers specific for each cluster were designed using Allele ID 7 (Premier Biosoft, Demo version), and the primer specificity was verified using e-PCR and the entire parasite genome as a template. The primers selected are listed in Table S1. Real-time PCR reactions were performed in an ABI 7500 sequence detection system (Applied Biosystems). Reactions in triplicate were prepared containing 1 mM forward and reverse primers, SYBR Green Supermix (Bio-Rad), and each diluted template cDNA. Standard curves were performed for each experiment for each pair of primers using serially diluted T. cruzi CL Brener genomic DNA and were used in the calculation of the relative quantity (Rq) values for each sample. qRT-PCRs for the constitutively expressed GAPDH gene were performed to normalize the expression of the TcS genes. Results were analyzed with an ANOVA test, and graphics were constructed in GraphPad Prism 5.0 (GraphPad Inc.).

Epitope prediction, spot peptide array and immunoblot

The 508 complete TcS proteins were submitted for linear B-cell epitope prediction using the Bepipred algorithm [25]. Peptides with 15 amino acids and with prediction scores above 1.3 were selected. Peptides with 70% identity over 70% of the peptide length with T. cruzi proteins other than TcS were excluded. For synthesis, we selected those peptides with higher occurrences within a group and with higher prediction scores. The peptides
synthesized are listed in Table S2. The peptides were covalently synthesized in pre-activated cellulose membranes according to the SPOT synthesis technique [26]. Membranes were blocked with 5% BSA and 4% sucrose in PBS and were incubated for one hour and 30 minutes with diluted sera (1:500) in blocking solution. After washing, the membrane was incubated with secondary antibody IgG (Sigma) diluted 1:2000 in blocking solution and, after a second washing, revealed by ECL Plus Western blotting (GE Healthcare). The spots were visualized by fluorescence scanning. The membrane was submitted to the same experimental conditions using sera from uninfected mice. Densitometry measures and analysis of each peptide was performed using Image Master Platinum (GE), and the relative density (Rd) cut-off for positivity was determined as 2.0. Graphics were constructed in GraphPad Prism 5.0 (GraphPad Inc).

**Ethics Statement**

All animal procedures were approved by the animal care ethics committee of the Federal University of Minas Gerais (Protocol # 143/2009).

**Results**

Sequence clustering reveals eight groups of the trans-sialidase/trans-sialidase-like superfamily (TcS) of *T. cruzi*

Despite the fact that four TcS groups were previously described [8,13,27], and only one group corresponds to the active trans-sialidase proteins, a much larger number of members of this gene family was annotated in public databases as trans-sialidases. To sort out which members correspond to the previously defined groups and to eventually identify new groups, we performed cluster analysis on all predicted TcS proteins identified in the CL Brener genome, excluding those annotated as partial and/or pseudogenes. A total of 508 TcS members were used to perform pairwise alignments resulting in a distance matrix that was used to generate a multidimensional scaling (MDS) plot (Figure 1). K-means method was then used to define ten clusters or groups (Figure 1A). Clustering with larger numbers of groups resulted in the fragmentation of previous clusters, without shuffling the members among them, indicating the robustness of the clustering of the family in ten groups (data not shown). Three members were located far from the others in the spatial distribution and therefore are the most divergent members of the family. One of them, Tc00.1047053505699.10, is the only representative of the group shown in black, and the Tc00.1047053509265.120 and Tc00.1047053507699.230 formed the brown group. Manual inspection of these three proteins revealed that their N-terminal regions are longer or shorter compared to the other TcS sequences: Tc00.1047053505699.10 contains an extra 260 amino acids at its N-terminal, whereas Tc00.1047053509265.120 and Tc00.1047053507699.230 have a deletion of approximately 160 and 430 amino acids, respectively, in their N-terminal region. The truncated sequences of these two proteins were due to the location of these genes in contig ends. Because gene prediction regarding the initial start codon could not be corrected for these three anomalous sequences, both black and brown groups were excluded from further analysis. The list of proteins belonging to each group is available in the supporting material (Table S3).

Protein and DNA sequences of the eight remaining groups were then aligned and the intra-cluster diversity was calculated using the p-distance, the Kimura-2-parameter and the Poisson correction methods, as described in the material and methods section. The groups are formed from different numbers of members and show distinct diversity indexes (Table 1). Groups labeled in red and dark green are the largest groups, with 227 and 117 members, respectively, totaling 68% of the TcS members. No clear correlation between the number of members and the diversity indexes was found. For instance, small groups (blue and orange) have similar diversity indexes of the largest ones (Table 1).

![Figure 1. Multidimensional scaling (MDS) plot of the TcS protein sequences.](https://journals.plos.org/plosone/article/content/6/10/e25914)

The pairwise alignments of the 508 TcS complete members were performed and the distance matrix was used to generate a multidimensional scaling (MDS) plot. K-means method was used to define the clusters or groups. (A) Pattern of dispersion of all 508 TcS protein sequences resulting in 10 TcS groups. (B) Pattern of dispersion of 508 TcS protein sequences in eight TcS groups. Previously characterized TcS sequences were mapped on the MDS. TcSgroup1 - blue; TcSgroup11 - dark green; TcSgroup11 - light blue; TcSgroupV - magenta; TcSgroupV - red; TcSgroupV - gray; TcSgroupVII - orange and TcSgroupVIII - purple.

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We next mapped on the MDS plot the TcS proteins representative from each of the four previously known groups (Figure 1B). As expected, the characterized TcS members mapped into different MDS clusters. TCNA, SAPA and TS-epi, all active trans-sialidase proteins belonging to the previously defined group I, clustered together in the blue group (hereafter named TcSgroupI). From a total of 19 TcSgroupI members, 11 have the critical catalytic residues (Figure S1). GP92, GP90, TcS-11_SAA5-1.1 and ASP-2, all representatives of the previously defined group II, mapped onto the dark green cluster (TcSgroupII). Finally, FL-160 and Ts13, which belong to sialidase groups III and IV, mapped onto the light blue (TcSgroupIII) and magenta (TcSgroupIV) clusters, respectively. None of the TcS proteins previously characterized mapped onto the clusters that are red (the largest TcS group), gray, orange or purple, hereafter named TcSgroupV, VI, VII and VIII, respectively.

Identifying key sialidase signature motifs in the eight MDS clusters

To characterize each of the eight groups, we initially searched for all the key signature motifs as they are described in the literature [8,13] and mapped them into the MDS plot (Figure S2). The canonical VTVxNVxLYNR motif was found in only 328 of the 508 TcS sequences used in this study. This result prompted us to investigate whether the other proteins annotated as TcS have a degenerate form of this motif or do not have this motif at all. To investigate whether TcSgroupII as well as sequences from the other four new groups have a degenerate form of the Asp box, we searched for a degenerated version of this motif, as described in the material and methods section. This search increased the number of positive Asp box sequences to 383. Only one additional degenerate position was found, resulting in the consensus SxDxGxxW. Although the majority of them have one (220) or two Asp boxes (154), a few (9) have three. Considering this new consensus motif, the Asp box is found in a large majority of the members from TcSgroupI (blue, 17 of 19 members), TcSgroupII (dark green, 14/17), and TcSgroupIV (magenta, 24/25) and is also present in the new groups TcSgroupV (red, 188/227) and TcSgroupVI (gray, 36/39). On the other hand, as previously described, it is missing in TcSgroupIII (light blue) and has only a few occurrences in the new groups TcSgroupVII (orange, 14/17) and TcSgroupVIII (purple, 3/46) (Figure 2).

The FRIP motif was searched using the pattern xRxP (where x is any amino acid). Because this is a small and degenerate sequence, we considered only those occurrences that are before the Asp-box and/or closest to the N-terminal region [30]. A total of 205 TcS proteins contain the FRIP motif, which is found in the majority of the members of TcSgroupI (blue, 68%), TcSgroupII

| Table 1. Diversity indexes of nucleotide, protein and 3’ flanking sequences of the TcS family. |
|-----------------------------------------------|
| **Number of members** | DNA p-distance | DNA K2p | Protein p-distance | Protein Poisson correction |
|-----------------------|----------------|--------|-------------------|---------------------------|
| TcSgroupI Blue        | 19              | 0.371/0.004 | 0.690/0.029 | 0.494/0.009 | 0.881/0.027 |
| TcSgroupII Dark green | 117             | 0.264/0.004 | 0.340/0.007 | 0.419/0.010 | 0.558/0.018 |
| TcSgroupIII Light blue| 15              | 0.209/0.005 | 0.263/0.008 | 0.366/0.010 | 0.492/0.023 |
| TcSgroupIV Magenta    | 25              | 0.179/0.003 | 0.226/0.005 | 0.250/0.008 | 0.320/0.012 |
| TcSgroupV Red         | 227             | 0.252/0.004 | 0.316/0.006 | 0.396/0.009 | 0.513/0.015 |
| TcSgroupVI Gray       | 39              | 0.246/0.004 | 0.312/0.007 | 0.394/0.009 | 0.513/0.016 |
| TcSgroupVII Orange    | 17              | 0.298/0.004 | 0.425/0.009 | 0.448/0.009 | 0.651/0.020 |
| TcSgroupVIII Purple   | 46              | 0.215/0.004 | 0.270/0.006 | 0.335/0.009 | 0.453/0.013 |
| TcS family            | 508             | 0.413/0.004 | 0.662/0.011 | 0.574/0.090 | 0.912/0.023 |
| 3’ UTR                | 495             | 0.573/0.007 | 1.086/0.029 | -             | -             |

P-distance was used to measure the diversity of the coding regions, proteins and 3’ flanking sequences, with kimura-2-parameters and Poisson correction only to DNA coding and protein sequences, respectively.

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Therefore, as previously described, this motif is a signature of the TcS family that is found in all its members. As shown in Figure 2, although variations on the VTVxNVxLYNR motif are observed, the motif is highly conserved within each cluster.

We also searched for the Asp box motif found in bacterial and viral sialidases [28], using as query the SxDxGxTW sequence, where x is any amino acid. A total of 135 sequences have this motif, of which 133 belong to the previously described TcS groups I (blue), II (dark green) and IV (magenta) (Figure 2). The two other sequences having this motif belong to the TcSgroupV (red) and TcSgroupVI (gray). This result is in agreement with previous reports showing that TcSgroupIII (light blue) does not have this motif [29]. To investigate whether TcSgroupIII as well as sequences from the other four new groups have a degenerate form of the Asp box, we searched for a degenerated version of this motif, as described in the material and methods section. This search increased the number of positive Asp box sequences to 303.

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Figure 2. Prototype of each TcS group. The motifs are shown only when they occur in the majority of the proteins within the group. The Asp-box and VTVxNVxLYNR logos are shown above each motif. The numbers within parentheses indicate the number of occurrences of a given motif. The length of the proteins within the groups may vary. Graphical representations are not to scale.

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(light blue, 87%), TcSgroupIV (magenta, 88%), TcSgroupVII (orange, 76%) and TcSgroupVIII (purple, 97%).

To identify repetitive regions on TcS sequences, we used the AA-repeat finder program (http://gicab.decom.cefetmg.br/bio-web). Only repeats with more than 10 amino acids were considered. We found that repeats are more frequent in the TcSgroupI (blue) and TcSgroupIV (magenta) clusters. These two groups have the largest repetitive regions, which encompass up to 884 amino acids. In fact, although we identified new repeats in these two groups, the largest repeats are those corresponding to the known DSSAH(S/G)TPSTP(A/V) repeat found in TS APA and the TcTs13 EPKSA-repeat. On the other hand, TcSgroupV (red), TcSgroupVI (gray) and TcSgroupVII (orange) groups have only 1, 2.5 and 6% of their members, respectively, with repetitive domains whereas no repeat was found in members of the TcSgroupIII (light blue). All repeats identified in this study are shown in Table S4.

In the prototype representation of the eight TcS groups shown in Figure 2, it is possible to identify three patterns of motif occurrence. The TcSgroupI (blue) and TcSgroupIV (magenta) clusters have the most complex structure, with the FRIP, Asp box and VTVxNVxLYNR motifs and the C-terminal repeats, although the sequences of the VTVxNVxLYNR motif and the C-terminal tandem repeats are distinct. TcSgroupII (dark green), TcSgroupV (red) and TcSgroupVI (gray) clusters contain the Asp box and VTVxNVxLYNR motifs. TcSgroupIII (light blue), TcSgroupVII (orange) and TcSgroupVIII (purple) clusters only have the FRIP and VTVxNVxLYNR motifs, which have a consensus sequence that is group-specific. This pattern of motif occurrence is in agreement with the space distribution of the TcS consensus sequence that is group-specific. This pattern of motif occurrence is in agreement with the space distribution of the TcS sequences. We investigated whether there is any bias on the chromosomal distribution of the TcS groups in the MDS (Figure 1). TcS groups I and IV that have all motifs are centered in the MDS, whereas TcSgroups II, V and VI are clustered in the bottom and TcSgroups III, VII and VIII are clustered in the left top region. A graphical representation for each of the 508 TcS proteins can be found in Figure S3.

Mapping the TcS groups on T. cruzi chromosomes

It is known that TcS genes can be found in T. cruzi subtelomeric regions or in internal positions in the chromosomes that are associated with other genes that encode surface proteins [2]. Subtelomeric regions are defined here as sequences extending from the telomeric hexamer repeats to the first nonrepetitive sequence. We investigated whether there is any bias on the chromosome localization of the TcS clusters. Figure 3 shows the chromosomal distribution of the TcS groups. A total of 60 complete TcS genes (not including partial or pseudogenes) can be found associated with the subtelomeric regions. One of them belongs to the brown cluster, which, as mentioned above, was excluded from our analysis. The majority of the subtelomeric TcS genes (36 members, 61%) belongs to TcSgroupII (dark green), 7 members from TcSgroupIV (magenta) and 10 from TcSgroupVIII (purple) (Figures 3 and 4). No TcSgroupIII (light blue) or TcSgroupVI (gray) genes are located at these regions. Interestingly, with one exception, all members of the largest TcS cluster (TcSgroup V, red) are at internal locations in the chromosomes (Figures 3, 4A and 4B). We have also found that the subtelomeric regions are enriched for TcS pseudogenes (Figure 4C), which is in agreement with the hypothesis that these regions were subject to intense rearrangement [54].

Expression profile of the TcS genes belonging to distinct groups

To characterize the expression profile of TcS genes belonging to distinct groups, we have performed real-time RT-PCR using member-specific primers, designed as described in the material and methods section. The expression of 12 TcS genes derived from six TcS groups was evaluated throughout the three parasite developmental stages using GAPDH mRNA levels, whose expression is constitutive throughout the parasite life cycle, for internal normalization (Figure 5). As a control, we used primers to amplify the cDNAs from the alpha-tubulin and amastin genes, whose mRNA levels we have previously shown to be up-regulated in epimastigotes and amastigotes, respectively [24,31]. The majority of the TcS transcripts are expressed in trypomastigotes and/or amastigotes forms. Interestingly, within a group, the expression profile may be highly variable. For example, the TcS5 gene that belongs to TcSgroupII is highly expressed in trypomastigote forms, whereas the TcS27 from the same group shows a much lower level of expression in the trypomastigote and amastigote forms and is barely detected in epimastigotes. Also, TcS9 and TcS33 from TcSgroupV are more expressed in trypomastigotes and amastigotes; however, TcS34, which is from the same group, is scarcely expressed in all the development stages. The new groups also display a variable expression profile. A very low level of expression was verified for the two genes analyzed from TcSgroupV in all the developmental stages (Figure 5) as well as in the blood trypomastigotes (data not shown). On the other hand, the gene TcS32 from TcSgroupVII is more expressed in the trypomastigotes. The two members of TcSgroupVIII show a variable expression profile, with TcS24 more expressed in trypomastigotes and TcS25 more expressed in amastigotes.

Analyzing sequence conservation of the 3’ flanking region of TcS groups

It is well established that, in Trypanosomatids, the 3’UTR regions are involved in post-transcriptional control mechanisms that confer stage-specific gene expression. To investigate whether the 3’ flanking sequences of TcS genes that belong to the same groups are conserved, we performed pairwise alignments of the 300 nt downstream of the stop codon of the TcSs, and the distance matrix was used to generate the MDS projection. We decided to analyze 300 nt downstream of the stop codon because this is the mean average length of the T. cruzi 3’UTRs [32]. The sequences were then color-coded according to the protein clusters showed in Figure 1. TcS genes already characterized as well as those genes whose expression levels were analyzed by real-time RT-PCR (Figure 5) were then mapped onto the MDS projection (Figure 6). We could not find a very clear association between the protein and the 3’ flanking region distances. For example, members of the TcSgroupV (red) form a robust cluster at the protein level and are much more variable according to the analysis of the 3’ flanking region. Also, the 3’ flanking regions of TcSgroupI (dark green) members are scattered in three MDS areas. On the other hand, the 3’ flanking regions of the TcSgroupVIII (purple) members clustered together, which suggests that similar mechanisms may control the expression of some of their genes. Interestingly, the 3’ flanking regions of SAPA and TCNA, both active trans-sialidase enzymes expressed in the trypomastigate forms (TcSgroupI), are clustered very close. Also, the 3’ flanking region of the TS-epi, an active trans-sialidase that is expressed in the epimastigote stage that also belongs to TcSgroupI, is located farther away from the SAPA and TCNA sequences. Moreover, the 3’ flanking region of gp90 and gp82, both expressed in the metacyclic trypomastigotes, and ASP-2, expressed in the amastigote stage, all belong to TcSgroupII and are very close in the MDS projection. Interestingly, although TcS35-11_SA85-1.1 and TcTs13 are expressed in the trypomastigote stage, they are divergent at the protein level (Figure 1) and belong to different TcS groups (II and
IV, respectively); they have similar 3′ flanking regions, which suggests that similar mechanisms for gene regulation may act on both genes.

Antigenicity of the TcS groups

Because the antigenicity of some members of the sialidase family was already reported [33,34], we decided to investigate whether other peptides derived from the TcS family are also antigenic. To this end, we have performed linear B-cell epitope prediction on all 508 complete members of the TcS family. A total of 40 peptides with 15 residues, high prediction scores and high occurrences within the TcS group were synthesized in a solid support by the spot synthesis technique and screened with sera from animals infected with T. cruzi. The list of all peptides used in this study is shown in the Table S2. As shown in Figure 7, 11 TcS peptides derived from distinct groups displayed antigenic properties based on a cut-off signal well above background. In agreement with previous studies, peptides corresponding to the SAPA (D5 and D8) [33] and to the TsTc13 repeats (B5) [34] are highly antigenic. We have also identified new epitopes specific to the previously characterized TcS groups I and IV (D9 and D10, and B10, respectively). At least one peptide from each of the new TcS groups V, VI, VII and VIII - was recognized by sera of infected animals (A1, C3, A10 and B4, and A5, respectively). The peptide C3 occurs in the largest number of members (60 in total) from the new TcS groups V and VI and from the previously characterized TcS groups II and IV.

Discussion

The TcS superfamily, the largest T. cruzi multigene family [2], was described more than 20 years ago, and, after the T. cruzi genome release, no comprehensive analysis of the diversity of this gene family was reported. Here, by analyzing all the 508 TcS complete genes present in the T. cruzi CL Brener genome [2], we demonstrated that this family displays an even greater variability.
than previously thought, as shown by means of the diversity indexes and the MDS projection. Based on their pattern of dispersion, we identified eight groups of TcS sequences, four of which were never described before (Figure 1). The distances among the clusters are consistent with the level of similarity and function of the previously described TcS sequences. All proteins that display trans-sialidase activity clustered together (TcSgroupI, blue). Another cluster was formed (TcSgroupII, dark green) from TcS proteins that have no trans-sialidase activity but that are capable of binding to β-galactose, laminin [35], fibronectin [36], collagen [37,38], and cytokeratin [39] and are involved in cell adhesion and invasion. The third TcS group encompasses proteins
involved in the regulation of the complement system (CRP - complement regulatory proteins). Previously characterized members of this group are the CRPs [29,30], which include the FL-160 [41]. Recently, using data from the Trypanosoma cruzi CL Brener genome project, Beucher and Norris (2008) identified CRP paralogs based on sequence similarity with a functional characterized CRP (GenBank accession number AAB49414). Also, these authors divided the CRPs into two groups, HSG (high similarity group, with more than 80% identity with AAB49414) and LSG (low-similarity group, with sequence identity between 54 and 62% with AAB49414) [29]. Here we could verify that all HSGs, and excluding two exceptions, the LSGs, fell into TcSgroupIII (light blue). These two members, which do not belong to TcSgroupIII, were clustered within TcSgroupVII (orange). In fact, they are the two most divergent sequences of the LSG subgroup [29] and correspond to members of the TcSgroupVII that are closest to the TcSgroupIII (Figure S4). Further investigation is necessary to verify whether these two proteins as well as other members of the TcSgroupVII have complement regulatory activity. Finally, a member of the TcSgroupIV that was previously described corresponds to the TsTc13 family, whose function is unknown. Based on the pattern of dispersion of the TcS groups in the MDS projection and the occurrence and sequence of key TcS motifs, we hypothesize that the new groups V and VI and the previously described TcSgroupII are more related among each other when compared to the other groups. The same is valid for the new groups VII and VIII and the TcSgroup III. For instance, TcS groups II, V and VI are the only ones that do not have the FRIP motif and their consensus sequences of the VTVxNVxLYNR motif are very similar. Also, TcS groups III, VII and VIII share the same pattern of motif occurrence and are clustered in a similar region in the MDS projection.

**Trypanosoma brucei** genome encodes active trans-sialidases expressed in the insect form of the parasite [42]. Although no active trans-sialidase was identified in Trypanosoma rangeli, sialidases/sialidase-like proteins similar to TcS groups I, II and III were found, and several of these members are expressed in the epimastigote and trypomastigote forms of the parasite [43,44]. The evolution of the TcS family suggests a gene ancestor encoding an active trans-sialidase expressed in insect forms of the genus Trypanosoma and several rounds of duplication and diversification would give rise to trans-sialidases expressed in mammalian forms [45]. Later in evolution T. rangeli, would have lost the active trans-sialidase, retaining the sialidase activity. These evidences along with the centered location of TcSgroupI in the MDS projection suggest that extensive expansion and sequence diversification of trans-sialidases similar to TcSgroupI would have originated other groups and functions.

Although the TcS family displays a high degree of sequence variation (Table 1), several motifs are conserved. The most conserved is the VTVxNVxLYNR motif, which is located upstream from the carboxyl terminus of all the TcS full-length members (Figure 2). Recently, it has been demonstrated that a version of this motif (VTVTVFLYRNLPLN), referred to as the FLY motif, may act as a virulence factor [46,47]. BALB/c mice administered with FLY-synthetic peptide are more susceptible to *T. cruzi* infection, displaying increased systemic parasitaemia and mortality [47]. Also, it has been shown that the FLY motif binds to endothelial cells of the heart, suggesting that it might contribute to the parasite tropism to this organ [10]. We identified the exact sequence of the FLY peptide in 28 members of TcSgroupII. Because a very similar version of this motif (ATVAVFVFLYRNLPLN, in which mismatches are indicated in bold and are underlined) is also found in 23 members of TcSgroupIV, we speculate that, as several TcSgroupII members, this group may also participate in host cell attachment/invasion.

Two other motifs, FRIP (RxRP) and Asp box, can be found in various groups of the TcS family. The FRIP motif, which is closest to the N-terminal, is involved in binding the carbohydrate group of sialic acid [48]. This motif is found not only in TcSgroupI, but also in the majority of the members of the TcS groups III, IV, VII and VIII (Figure 2). Although this motif is involved in binding sialic acid, it has been shown that enzymatically inactive members of the sialidase family in *T. cruzi* still preserve carbohydrate binding properties [49,50]. The Asp box follows the FRIP motif and can be repeated up to five times in the sequences of viral, bacterial, trypanosomatid and mammalian sialidases. Although its function is unknown, it is worth noting that the Asp box occurs in secreted proteins and in proteins that act on, or interact with, carbohydrates [51]. Recently, it has been shown that at least some inactive trans-sialidases act as lectin-like proteins able to interact with the carbohydrate portion of glycoconjugates, only if they are sialylated [52]. The authors hypothesized that these inactive trans-sialidase proteins could bind to host surfaces that are rich in sialyl-donor glycoconjugates (functioning as anchors), facilitating the active enzyme to more efficiently undertake the sialyl-transferring activity. Here, we have shown that, in addition to TcSgroupI, members of TcSgroupIV have both FRIP and Asp box motifs.
Figure 7. Antigenic profile of TcS peptides. The top panel shows a representative result of immunoblot employing a SPOT synthesis membrane and pools of sera from T. cruzi-infected mice (A) and from control uninfected mice (B). The reaction was revealed with secondary anti-total IgG antibody. The bottom panel shows the relative intensity of the signal of each spot estimated based on a comparison of the reactivity in immunoblots with sera from T. cruzi-infected mice to the background levels, determined by reactivity with sera from uninfected mice. A signal was scored as reactive when relative intensity (RI) $\geq 2$. The peptides analyzed for each TcS group are as follows: TcS group I, D5–D10; TcS group III, C9-D4; TcS group IV, B5-C1, C3; TcS group V, A1, C2, C3, C7; TcS group VI, C2–C8; TcS group VII, A9-B4; TcS group VIII, A2–A8.

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members of the two new TcS groups V and VI. Also, similar but
occurs in the largest number of proteins (60 in total) including
member. Specifically, the highly reactive peptide C3 (Figure 7)
nine of the 14 reactive peptides are found in more than one TcS
derived from distinct groups of the TcS family (Figure 7). Besides
sequencing approaches will clarify these questions.
change during the parasite infection. High-throughput RNA
and whether the repertoire and/or the level of expressed genes may
unclear what the proportion of the total TcS repertoire is expressed
other than the 3
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is possible that cis-acting regulatory elements present in regions
pattern of expression is very distinct (Figures 5 and 6). In this case, it
trypomastigotes, have almost identical 3
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TCNA genes, which are both active trans-sialidases expressed in
On the other hand, the 3
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similarity in their 3
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implied in host cell attachment/invasion, and T. cruzi has the
ability to infect a broad range of host cells. Therefore, it is possible
that the large repertoire of peptides derived from TcSgroupII may
contribute to this phenomenon.
Co-expression of several members of the TcS family has been
described in the mammalian stages of the parasite [59]. Here, we
show that the levels of expression are not homogeneous between
and within the TcS groups (Figure 5). It is well known that the
3’UTRs are implicated in the control of the gene expression of several
T. cruzi genes that are regulated during the life cycle. Although we have not mapped the 3’UTRs of the genes selected for
expression analysis, for a few genes, it was possible to find a
correlation between the expression profile and the sequence
similarity in their 3’ flanking regions. For example, SAPA and
TCNA genes, which are both active trans-sialidases expressed in
trypomastigotes, have almost identical 3’ flanking regions (Figure 6).
On the other hand, the 3’ flanking sequences of the genes TcS8 and
TcS25 are quite similar (75% identity) despite the fact that their
pattern of expression is very distinct (Figures 5 and 6). In this case, it
is possible that cis-acting regulatory elements present in regions
other than the 3’UTR may modulate their expression. It is also
unclear what the proportion of the total TcS repertoire is expressed
and whether the repertoire and/or to the level of expressed genes may
change during the parasite infection. High-throughput RNA
sequencing approaches will clarify these questions.
We have also investigated the antigenic profile of peptides
derived from distinct groups of the TcS family (Figure 7). Besides
the known epitopes derived from the repetitive sequences of the
SAPA and TcTS13 proteins, new B-cell epitopes were identified in
members from both previously described and new TcS groups.
Nine of the 14 reactive peptides are found in more than one TcS
member. Specifically, the highly reactive peptide C3 (Figure 7)
occurring in the largest number of proteins (60 in total) including
members of the two new TcS groups V and VI. Also, similar but
not identical sequences of this peptide were found in more than
150 TcS members. The cross-reaction among several epitopes and
the sequence variability of the TcS family might contribute to the
simultaneous presence of B-cell related epitopes during an
infection. In fact, it has been proposed that cross-reactivity among
the T. cruzi epitopes could be an evasion mechanism that drives
the immune system into a series of spurious and non-neutralizing
antibody responses [62]. In this regard, it has been shown that
subtle differences at amino acid positions in or around the active
site of the TcS proteins that have trans-sialidase activity might
delay the immune response and avoid inhibiting the complete
enzymatic makeup of the parasite [63]. This scenario may
represent an evolutionary pressure driving the diversification of
TcSgroupI, which harbors the active trans-sialidases. Whether a
similar mechanism is involved in the diversification of the other
TcS groups remains to be addressed.
The diversity of the TcS family may be even greater than
reported here since the current assembly of the CL Brener genome
is fragmented [2,20], and therefore additional TcS genes may not
be part of the dataset analyzed in this study. Nevertheless, based
on the nearly complete repertoire of TcS sequences, we can now
design probes and antibodies specific for each group, to be
employed in more assertive strategies to investigate the role of this
complex family during T. cruzi infection.

Supporting Information
Figure S1 Partial alignment of active trans-sialidase
proteins. FRIP and Asp-box motifs, and critical amino acids
residues involved in trans-sialidase activity are shaded in gray. The
amino acid positions are relative to the first methionine. Only N-
terminal region of the active trans-sialidase proteins is shown.
(DOCX)
Figure S2 Multidimensional scaling plot of the TcS
proteins indicating the presence of characteristic TcS
motifs. TcS proteins with the motifs are represented by red dots.
(A) SXDXGXTW motif; (B) VT VXNVLNRY motif; (C)
SXDXGXTW motif allowing 1 mismatch; (D) sequences with
VT VXNVLNRY motif found in the alignment block of the 505
TcS derived from the eight clusters identified in this study; (E)
FRIP (XRXP) motif. X represents any amino acid.
(DOCX)
Figure S3 Prototype of each TcS protein. The peptide
signal is represented in gray, FRIP in green, Asp-box in blue,
VT VXNVLNRY in red, repeats in black and GPI anchor
addition site in orange.
(TIF)
Figure S4 Divergent CRP – complement regulatory
proteins. Protein sequences involved in the regulation of
complement system identified by Beucher and Norris (2008).
Sequences were mapped on the MDS showed in Figure 1. HSG
sequences (high similarity group) and LSG sequences (low-
similarity group) are indicated by red and black squares,
respectively.
(DOCX)
Table S1 Primers used in the Real-time RT-PCR
reactions.
(DOC)
Table S2 TcS peptides analyzed by immunoblotting.
(DOCX)
Table S3 List of members of each TcS group.
(XLS)
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References

1. World Health Organization (2002) Control of Chagas Disease. Second report of the WHO Expert Committee. WHO Technical Report Series 905.
2. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, et al. (2005) The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease. Science 309: 405-413.
3. Previtali JO, Andrade AF, Pessolani MC, Mendonça-Previtali L (1983) Incorporation of sialic acid into Trypanosoma cruzi macromolecules. A proposal for a new metabolic route. Mol Biochem Parasitol 16: 85-96.
4. Mucci J, Risso MG, Leguizamón MS, Frasch AC, Campetella O (2006) The trans-sialidase from Trypanosoma cruzi triggers apoptosis by target cell sialidation. Cell Microbiol 8: 1086-1095.
5. Vercelli CA, Hidalgo AM, Hyon SY, Argibay PF (2005) Trypanosoma cruzi trans-sialidase inhibits human lymphocyte proliferation by nonapoptotic mechanisms: implications in pathogenesis and transplant immunology. Transplant Proc 37: 4549-457.
6. Frasch AC (2000) Functional diversity in the trans-sialidase and mucin families in Trypanosoma cruzi. Parasitol Today 16: 202-206.
7. Pereira-Chioccola VA, Acosta-Serrano A, Correia de Almeida J, Ferguson MA, Souto-Padron T, et al. (2000) Mucin-like molecules form a negatively charged coat that protects Trypanosoma cruzi trypanosomastigotes from killing by human anti-alpha-galactosyl antibodies. J Cell Sci 113(Pt 7): 1299-1307.
8. Schenkman S, Eichinger D, Pereira ME, Neunserneix V (1994) Structural and functional properties of Trypanosoma cruzi trans-sialidase. Annu Rev Microbiol 48: 499-523.
9. Souza W, Carvalho TM, Barrias ES (2010) Review on Trypanosoma cruzi cell epitopes. Immunome Res 2: 2.
10. Tonelli RR, Giordano RJ, Barbs EM, Torrechiales AC, Kohayashi GS, et al. (2010) Role of the gpl55 trans-sialidase in Trypanosoma cruzi tissue tropism: preferential binding of a conserved peptide motif to the vasculature in vivo. PLoS Neg Trop Dis 4: e1064.
11. Tzepelis F, de Alencar BC, Penido ML, Claser C, Machado AV, et al. (2008) Virus-like particles expressing both Trypanosoma cruzi trans-sialidase and Amyloid-β peptides elicit anti-amyloid responses in a murine model of Alzheimer’s disease. Cell Microbiol 8: 1086-1095.
12. Cross GA, Takle GB (1993) The surface trans-sialidase family of Trypanosoma cruzi. Annu Rev Microbiol 47: 385-411.
13. Campos PC, Bartholomeu DC, DaRocha WD, Cerqueira GC, Teixeira SM (2008) Sequences involved in mRNA processing in Trypanosoma cruzi. Int J Parasitol 38: 1383-1389.
14. Pollevick GD, Alcharnoo JL, Frasch AC, Sánchez DO (1991) The complete sequence of a shed acute-phase antigen of Trypanosoma cruzi. Mol Biochem Parasitol 47: 247-250.
15. Burns JM, Shreffler WG, Rosman DE, Sleath PR, March CJ, et al. (1992) Identification and synthesis of a major conserved antigenic epitope of Trypanosoma cruzi. Proc Natl Acad Sci U S A 89: 1293-1295.
16. Griscald R, Chaumans R, Veiga SS, Colli W, Alves MJ (1994) Trypanosoma cruzi signal peptides: SignalP 3.0. J Mol Biol 340: 783-795.
17. Magdesian MH, Giordano R, Ulrich H, Juliano MA, Juliano L, et al. (2001) Cells expressing Trypanosoma cruzi trans-sialidase bind to laminin in a carbohydrate-independent way. Braz J Med Biol Res 27: 2313-2318.
18. Trujillo J, Taihi A, Velge P, Capron A (1988) Major surface immunogen of Trypanosoma cruzi trypanosomastigotes. Mem Inst Oswaldo Cruz 83 Suppl 1: 502.
19. Velge P, Ouaisi MA, Cornette J, Alcharnoo JL, Capron A (1988) Identification and isolation of Trypanosoma cruzi trans-sialidase collagen-binding proteins: possible role in cell-parasite interaction. Parasitology 107: 253-260.
20. Santana JM, Grellier P, Schrével J, Teixeira AR (1997) A novel binding activity of Trypanosoma cruzi secreted 80 kDa proteinase with specificity for human collagen types I and IV. Biochem J 325(Pt 1): 129-137.
21. Magdesian MH, Giordano R, Ulrich H, Juliano MA, Juliano L, et al. (2001) Trypanosoma cruzi infection: Identification of a parasite ligand and its host cell receptor. J Biol Chem 276: 19382-19389.
22. Norris KA, Baeth B, Cooper NR, So M (1991) Characterization of a Trypanosoma cruzi C3 binding protein with functional and genetic similarities to the human complement regulatory protein, decay-accelerating factor. J Immunol 147: 2240-2247.
23. Van Voorhis WC, Eisen H (1989) Fl-160. A surface antigen of Trypanosoma cruzi that mimics mammalian nervous tissue. J Exp Med 169: 641-652.
24. Engeler M, Reuter G, Schrader R (1992) Purification and characterization of a novel sialidase found in procyclic culture forms of Trypanosoma brucei. Mol Biochem Parasitol 54: 21-30.
25. Griscald EC, Stooch PH, Wagner G, Sincero TC, Rotava G, et al. (2010) Transcriptomic analyses of the avirulent protozoan parasite Trypanosoma rangeli. Mol Biochem Parasitol 174: 18-25.
26. Buschiazzo A, Campetella O, Frasch AC (1997) Trypanosoma rangeli sialidase: cloning, expression and similarity to T. cruzi trans-sialidase. Glycobiology 7: 1167-1173.
27. Briones MR, Eimga CM, Eichinger D, Schenkman S (1995) Trans-sialidase genes expressed in mammalian forms of Trypanosoma cruzi evolved from ancestor genes expressed in insect forms of the parasite. J Mol Evol 41: 120-131.
28. Magdesian MH, Tonelli RR, Feisel MR, Silveira MT, Schumacher RJ, et al. (2007) A conserved domain of the gpl55/trans-sialidase family activates host cell extracellular signal-regulated kinase and facilitates Trypanosoma cruzi infection. Exp Cell Res 313: 210-218.
29. Tonelli RR, Torrechias AC, Jacyn JF, Juliano MA, Colli W, et al. (2011) In vivo infection by Trypanosoma cruzi: the conserved FYL domain of the gpl55/trans-sialidase family potentiates host infection. Parasitology 138: 481-492.
30. Gaskell A, Cremel S, Taylor G (1995) The three domains of a bacterial sialidase: a beta-propelior, an immunoglobulin domain and a galactose-binding jelly-roll. Structure 3: 1197-1205.
31. Cremona ML, Campetella O, Sánchez DO, Frasch AC (1999) Enzymatically inactive members of the trans-sialidase family from Trypanosoma cruzi display beta-galactose-binding activity. Glycobiology 9: 361-367.
32. Todeschini AR, Dias WB, Girard MF, Wieruszeski JM, Mendonça-Previtali L, et al. (2004) Enzymatically inactive trans-sialidase from Trypanosoma cruzi binds sialyl and beta-galactopyranosyl residues in a sequential ordered mechanism. J Biol Chem 279: 5322-5326.

T. cruzi Trans-Sialidase/Sialidase-Like Family

Author Contributions

Conceived and designed the experiments: LMF SLS DCB. Performed the experiments: LMF SLS GRL TAOM. Analyzed the data: LMF SLS DCB. Contributed reagents/materials/analysis tools: TSR RTG SMRT RTF DCB. Wrote the paper: LMF SLS SMRT DCB.
51. Copley RR, Russell RB, Ponting CP (2001) Sialidase-like Asp-boxes: sequence-similar structures within different protein folds. Protein Sci 10: 285–292.

52. Oppezzo P, Ohaf G, Baraibar MA, Pritsch O, Alzari PM, et al. (2011) Crystal structure of an enzymatically inactive trans-sialidase-like lectin from *Trypanosoma cruzi*. The carbohydrate binding mechanism involves residual sialidase activity. Biochim Biophys Acta 1814: 1154–1161.

53. Scherf A, Lopez-Rubio JJ, Riviere L (2008) Antigenic variation in *Plasmodium falciparum*. Annu Rev Microbiol 62: 445–470.

54. Kim D, Chiurillo MA, El-Sayed N, Jones K, Santos MR, et al. (2005) Telomere and subtelomere of *Trypanosoma cruzi* chromosomes are enriched in (pseudo)genes of retrotransposon hot spot and trans-sialidase-like gene families: the origins of *T. cruzi* telomeres. Gene 346: 153–161.

55. Horn D, Barry JD (2005) The central roles of telomeres and subtelomeres in antigenic variation in African trypanosomes. Chromosome Res 13: 525–533.

56. Chiurillo MA, Peralta A, Ramirez JL (2002) Comparative study of *Trypanosoma rangeli* and *Trypanosoma cruzi* telomeres. Mol Biochem Parasitol 120: 305–308.

57. Cano MI (2001) Telomere biology of trypanosomatids: more questions than answers. Trends Parasitol 17: 425–429.

58. Scherf A, Figueiredo LM, Freitas-Junior LH (2001) Plasmodium telomeres: a pathogen’s perspective. Curr Opin Microbiol 4: 409–414.

59. Atwood JA, Weatherly DB, Minning TA, Bundy B, Cavola C, et al. (2005) The *Trypanosoma cruzi* proteome. Science 309: 473–476.

60. Azuaje FJ, Ramirez JL, Da Silveira JF (2007) *In silico*, biologically-inspired modelling of genomic variation generation in surface proteins of *Trypanosoma cruzi*. Kinetoplastid Biology and Disease 6: 6–17.

61. Azuaje F, Ramirez JL, Da Silveira JF (2007) An exploration of the genetic robustness landscape of surface protein families in the human protozoan parasite *Trypanosoma cruzi*. IEEE Transactions on Nanobioscience 6: 223–228.

62. Pitcovsky TA, Buscaglia CA, Mucci J, Campetella O (2002) A functional network of intramolecular cross-reacting epitopes delays the elicitation of neutralizing antibodies to *Trypanosoma cruzi* trans-sialidase. J Infect Dis 186: 397–404.

63. Ratier L, Urrutia M, Paris G, Zarebski L, Frasch AC, et al. (2008) Relevance of the diversity among members of the *Trypanosoma cruzi* trans-sialidase family analyzed with camelids single-domain antibodies. PLoS One 3: e3524.