Parasitol Res (2011) 108:459–466
DOI 10.1007/s00436-010-2087-4

ORIGINAL PAPER

Sequencing and analysis of chromosomal extremities of *Trypanosoma rangeli* in comparison with *Trypanosoma cruzi* lineages

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Received: 9 July 2010 / Accepted: 16 September 2010 / Published online: 1 October 2010

Abstract The aim of this study was to investigate the genetic variability of sequences present in the chromosome ends of *Trypanosoma rangeli* strains defined by the presence (+) or absence (−) of KP1 minicircles, and to compare the mean terminal restriction fragment (TRF) lengths to those of *Trypanosoma cruzi* populations representative of groups TcI, TcII, TcIV, and TcVI. Southern blots containing *Rsa*I-digested genomic DNA of *T. rangeli* KP1(+) strains, *T. rangeli* KP1(−) strains, and *T. cruzi* strains were probed with the previously described subtelomeric sequences (170 bp) of *T. rangeli* and with telomeric hexamer repeats. Mean TRF length analysis showed that the chromosome ends of *T. rangeli* are distinctly organized, with TRFs ranging from 1.3 to 9 kb for KP1(+) strains and from 0.3 to 5.0 kb for KP1(−) strains. In *T. cruzi*, TRF length ranged from 0.2 to 9 kb and no association with the genotype of the parasite could be established. Sequence analysis of the 170-bp amplicons revealed the occurrence of sequence polymorphisms in the subtelomeric region between and within KP1(+) and KP1(−) strains. The GTT triplet was detected in all KP1(+) strains, except for strain Cas4, but not in any of the KP1(−) strains. The dendrogram constructed by alignment of all *T. rangeli* strains showed the division into two main groups, mainly related to the presence or absence of the KP1 minicircle. In conclusion, the present results extend the genotype differences demonstrated by kDNA and karyotype analysis in *T. rangeli* to the chromosome ends of the parasite.

Abbreviations
TRF Terminal restriction fragment

Introduction

Trypanosoma rangeli infects humans and domestic and wild animals in Central and South America and is found in the same geographic areas as *Trypanosoma cruzi*, the etiological agent of Chagas' disease (D'Alessandro and Saravia 1999). Different molecular methods have been used for the genetic characterization of *T. rangeli* (Vallejo et al. 2002; Marquez et al. 2007; Cabrine-Santos et al. 2009). *T. rangeli* populations can be divided into two groups, called KP1(+) and KP1(−), according to the presence or absence of KP1 minicircles in their kDNA, respectively (Vallejo et al. 2002).

Telomeres are responsible for the maintenance of chromosome integrity. Eukaryotic telomeres share characteristic features such as the same 5′-TTAGGG-3′ sequence found in all trypanosomatids and most vertebrates and the heterogeneity in terminal restriction fragments (TRFs) between chromosomes of the same cell and between species (Wincker et al. 1996; Freitas-Junior et al. 1999; Conte and Cano 2005; Chiurillo et al. 2000; El-Sayed et al. 2005; Lira et al. 2007). Analysis of telomeric and subtelomeric regions has contributed to the understanding of the biology of different trypanosomatid species. The

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subtelomeric regions contain genes that encode proteins related to the virulence and escape mechanisms of these parasites, such as the gp85 and gp90 proteins of *T. cruzi* (Freitas-Junior et al. 1999; Chiurillo et al. 1999, 2002) and the variable surface glycoproteins of *Trypanosoma brucei* (Borst and Rudenko 1994; Vanhamme et al. 2001). In addition, the subtelomeric regions of *T. cruzi*, *T. brucei*, and *Leishmania major* contain specific sequences that can be used as molecular markers (Fu and Barker 1998; Chiurillo et al. 2000, 2001, 2002; El-Sayed et al. 2005).

The telomeric region of the chromosomes of *T. rangeli* comprises a typical hexamer repeat and a final overhang of nine nucleotides (5′-CCCTAACCC-OH-3′) similar to that of *T. cruzi* and *T. brucei*, but different from the telomeric region of *Leishmania donovani* and *L. major* (Chiurillo et al. 2002). However, despite a similarity of 62% to 67% in the nucleotide sequence and of 50% to 56% in G+C content with *T. cruzi*, this region is species specific (Chiurillo et al. 2002; Añez-Rojas et al. 2005). In addition, the subtelomeric region of *T. rangeli* contains genes of the gp85/TS superfamily that presents sialidase activity (Añez-Rojas et al. 2005) and might be involved in attachment of the parasite to the salivary glands of the vector (Peña et al. 2009). Furthermore, analysis of the telomeric and subtelomeric regions of *T. rangeli* may contribute to the elucidation of the mechanisms that are responsible for the karyotype variability observed in this parasite (Henriksson et al. 1996; Cabrine-Santos et al. 2009).

These data indicate that the telomeric and subtelomeric regions can be used as a target for the investigation of the genetic and biological diversity of *T. rangeli*. The aim of the present study was to evaluate sequence conservation in the chromosome ends of *T. rangeli* characterized as KP1(+) and KP1(−). In addition, mean TRF lengths were determined and compared to those of *T. cruzi* populations representative of the discrete typing units (DTUs) TcI, TcII, TcIV, and TcVI.

Materials and methods

T. cruzi and *T. rangeli* strains and culture conditions

All strains were maintained in liver infusion tryptose medium at 28°C by weekly passages (Table 1). *T. rangeli* medium was supplemented with 3% (*v*/*v*) human urine (Ferreira et al. 2007). The *T. rangeli* strains were previously characterized using the S35/S36/KP1 primers (Vallejo et al. 2002) and the *T. cruzi* strains were identified by a combination of PCR amplification of the 24S-α rDNA genes (Souto et al. 1996; Brisse et al. 2000), spliced leader genes, and intergenic region of spliced leader (Burgos et al. 2007), according to the nomenclature previously proposed (Zingales et al. 2009).

DNA purification and analysis

Total genomic DNA of *T. rangeli* and *T. cruzi* was prepared according to Lages-Silva et al. (2001). Samples containing approximately 500 ng genomic DNA were digested with *Rsa*I (New England Biolabs). The fragments were separated by electrophoresis on 1% agarose gel and transferred to a nylon membrane (Sambrook et al. 1989).

T. rangeli plasmid DNA used for sequencing was purified according to the alkaline lysis method (Sambrook et al. 1989). The 170-bp subtelomeric region of *T. rangeli* was amplified by PCR using the TrF3 (5′-CCC CAT ACA AAA CAC CCT T-3′) and TrR8 (5′-TGG AAT GAC GGT GCG GCG AC-3′) primers (Chiurillo et al. 2003).

Probe labeling and hybridization assays

Telomeric hexamer repeats (GGGTTA) from *T. brucei* (Van der Ploeg et al. 1984) and the 170-bp fragment of the subtelomeric region of *T. rangeli* (Chiurillo et al. 2003) were radiolabeled with [α-^{32}P] dCTP by the random priming method (Feinberg and Vogelstein 1983) using Ready-To-Go labeling beads (GE Healthcare). The blots were hybridized as described elsewhere (Sambrook et al. 1989) and washed in 2× SSPE at 67°C and in 2× SSPE/0.5% SDS at room temperature. The membrane was exposed to X-ray films and developed. The membrane was reused after stripping with 0.5% SDS at 95°C and confirmation of the absence of signals.

T. cruzi and *T. rangeli* terminal restriction fragment analysis

The mean TRF lengths of the *T. cruzi* and *T. rangeli* chromosomes were determined by comparison with the size of fragments obtained by hybridization of the telomeric probe of *T. brucei* on blots containing genomic DNA samples of *T. cruzi* and *T. rangeli* digested with *Rsa*I using the Quantity One 4.6.3 software.

Cloning and sequencing of subtelomeric fragments

The 170-bp subtelomeric fragments of *T. rangeli* were cloned into the pCR 4-TOPO vector and the recombinant vector was transformed into *Escherichia coli* according to manufacturer instructions (Invitrogen). The transformed cells were cultured on LB agar containing ampicillin (100 μg/ml) for 18 h at 37°C. Five clones obtained from each strain were inoculated into LB/ampicillin medium and cultured at 180 rpm for 18 h at 37°C for the extraction of plasmid DNA. The plasmid DNA was sequenced with an ABI 3100 sequencer (Perkin Elmer) using the BigDye kit (Applied Biosystems). The sequences generated were confirmed by sequencing of both strands of the recombi-

Table 1 Parasite strains and their genotypes, host, and geographic origin

Species	Strain	Host	Lineage	Origin
T. rangeli	P02	*Didelphis albiventris*	KP1(+)	Brazil
	P07	*Didelphis albiventris*	KP1(+)	Brazil
	P18	*Didelphis albiventris*	KP1(+)	Brazil
	P19	*Didelphis albiventris*	KP1(+)	Brazil
	P21	*Didelphis albiventris*	KP1(+)	Brazil
	Cas4	*Rhodnius prolixus*	KP1(+)	Colombia
	CH	*Rhodnius prolixus*	KP1(+)	Colombia
	SO18	*Rhodnius pallescens*	KP1(−)	Colombia
	SO28	*Rhodnius pallescens*	KP1(−)	Colombia
	SO29	*Rhodnius pallescens*	KP1(−)	Colombia
	SO48	*Rhodnius pallescens*	KP1(−)	Colombia
	LDG	*Homo sapiens*	KP1(−)	Colombia
	Gal60	*Didelphis marsupialis*	KP1(−)	Colombia
	G5	*Rhodnius pallescens*	KP1(−)	Colombia
T. cruzi	Rom	*Homo sapiens*	TcII	Brazil
	VIC	*Homo sapiens*	TcII	Brazil
	JG	*Homo sapiens*	TcII	Brazil
	CL Brener	*Triatoma infestans*	TcVI	Brazil
	RN1	*Homo sapiens*	TcII	Brazil
	RN2	*Homo sapiens*	TcII	Brazil
	1008	*Homo sapiens*	TcII	Brazil
	PV	*Homo sapiens*	TcIV	Brazil
	Mut	*Panstrongylus megistus*	TcI	Brazil
	Alv	*Panstrongylus megistus*	TcI	Brazil
	AQ-2	*Triatoma sordida*	TcI	Brazil

nant clones and deposited in the GenBank database (accession numbers HM126609 to HM126627). Only sequences of good quality were used for subsequent analysis.

Computer analysis of the sequences

The sequences generated were compared with *T. rangeli* sequences deposited in the GenBank database using the BLASTN program (Basic Local Alignment Search Tool). The sequences were aligned between the clones of each strain using the ClustalW2 program (www.ebi.ac.uk/Tools/clustalw2/index.html) and the most representative clone was chosen for the alignment of all strains.

The neighbor joining method (Saitou and Nei 1987) was used to calculate the distance between pairs of sequences in the multiple alignments and to generate the distance matrix. The latter was used for the construction of the dendrogram by the unweighted pair-group method with arithmetic mean, with a bootstrap of 100 pseudoreplicates (Sneath and Sokal 1973).

Results

Mean TRF length analysis of *T. rangeli* and *T. cruzi* strains

Samples of *T. cruzi* and *T. rangeli* genomic DNA were *Rsa*I digested (Fig. 1a). Hybridization of the telomeric probe of *T. brucei* to digested *T. rangeli* DNA revealed distinct patterns in the two subpopulations of the parasite. In KP1(+) strains, the probe hybridized to fragments ranging from 1.3 to 9 kb, whereas in KP1(−) strains the probe hybridized to fragments of 0.3–5 kb (Fig. 1b). The length of the TRFs of the *T. cruzi* strains ranged from 0.2 to 9 kb and no association was observed with any of the genotypes of the parasite (Fig. 1b).

When hybridizing the subtelomeric *T. rangeli* probe to digested DNA of this parasite and of *T. cruzi*, only hybridization with restriction fragments of *T. rangeli* was observed, a finding confirming the specificity of this sequence. In addition, the hybridization pattern observed differed between KP1(+) and KP1(−) strains, with the identification of fragments of 1.3 to 9 kb in the former and of 0.3 to 5 kb in the latter (Fig. 1c).

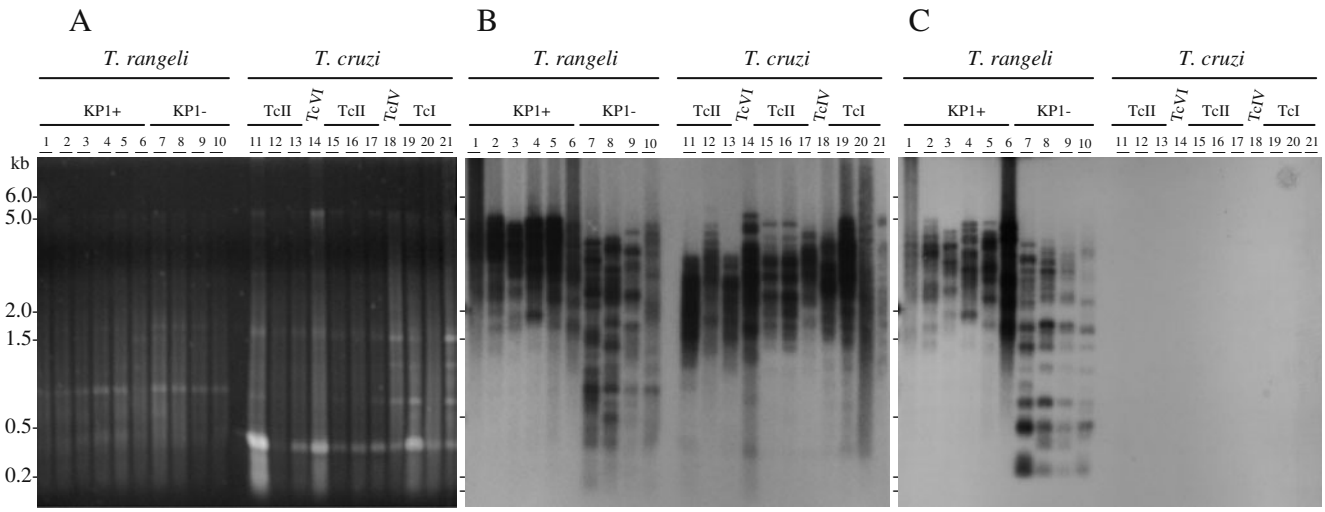

Fig. 1 *Trypanosoma cruzi* and *T. rangeli* terminal restriction fragment analysis. **a** Ethidium-bromide-stained agarose gel containing *Rsa*I-digested genomic DNA of *T. cruzi* and *T. rangeli*. **b** Hybridization of the *T. brucei* telomeric (GGGTTA) probe to the blot of the gel shown in (**a**). **c** Hybridization of the *T. rangeli* subtelomeric probe to the blot of the gel shown in (**a**). *Lanes*: MM, 100-bp molecular marker; (*1*) TrP02; (*2*) TrP07; (*3*) TrP18; (*4*) TrP19; (*5*) TrP21; (*6*) TrCas4; (*7*) TrSO18; (*8*) TrSO28; (*9*) TrSO29; (*10*) TrSO48; (*11*) TcRom; (*12*) TcVIC; (*13*) TcJG; (*14*) TcCL Brener; (*15*) TcRN1; (*16*) TcRN2; (*17*) Tc1008; (*18*) TcPV; (*19*) TcMut; (*20*) TcAlv; (*21*) TcAQ-2

Analysis of *T. rangeli* subtelomeric sequences

Sequence analysis of the 26 KP1(+) clones and 25 KP1(−) clones of *T. rangeli* (Table 2) with the BLASTN program showed that the sequences analyzed presented 80% to 96% identity with the *T. rangeli* telomeric sequences deposited in the database. Alignment of the sequences generated from the different strains revealed sequence polymorphisms in the subtelomeric region between *T. rangeli* strains and between recombinant clones of the same strain, with the observation of nucleotide substitutions in some regions and insertions or deletions (indel events) in others (Fig. 2). Twelve (46.2%) of the 26 clones of the seven *T. rangeli* KP1(+) strains presented an insertion of three nucleotides (GTT) at position 144–146 (Fig. 2). However, no GTT triplet was detected in any of the three clones of strain Cas4 (Table 2). Similarly, this triplet was not observed in any of the sequenced clones of the seven KP1(−) strains.

Table 2 Frequency of the GTT triplet in the sequences generated from a conserved fragment (170 bp) of the subtelomeric region of *Trypanosoma rangeli* clones

Strain	Lineage	Number of clones analyzed	Number of clones with GTT
P02	KP1(+)	4	3
P07	KP1(+)	2	1
P18	KP1(+)	5	4
P19	KP1(+)	5	1
P21	KP1(+)	5	1
CAS4	KP1(+)	3	0
CH	KP1(+)	2	2
SO18	KP1(–)	3	0
SO28	KP1(–)	4	0
SO29	KP1(–)	4	0
SO48	KP1(–)	4	0
LDG	KP1(–)	5	0
GAL60	KP1(–)	4	0
G5	KP1(–)	1	0

On the basis of this finding, two representative clones of each KP1(+) strain, except for Cas4, one with and one without the GTT triplet, were selected for multiple alignment of the strains. The dendrogram constructed by alignment of all strains showed polymorphism among all sequences generated. Despite this polymorphism, the strains could be divided into two main groups. One group comprised exclusively clones of *T. rangeli* KP1(+) strains and the other included all clones of the KP1(−) strains and three KP1(+) clones (P19-9F, Cas4-3F, and P07-1R; Fig. 3).

Discussion

The combination of telomeric and subtelomeric probes for hybridization to digested DNA revealed variability in the chromosome ends of different *T. rangeli* and *T. cruzi* strains. TRF heterogeneity has also been reported for *T. cruzi* (Freitas-Junior et al. 1999; Chiurillo et al. 1999), as well as for *Leishmania* and *T. brucei* (Fu and Barker 1998;

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P02-1R     CACGGATTGCATTCGTCGAGCACTCAATACAAACAGTCTCACAAACCAAAAACCACCTTACCGCACAAAA 70
P02-3R     CACGGATTGCATTGGCTGAGCGCTCAATACAAACAGTCTCACAAACCAAAACACGCTTTACCGCAGAAAA 70
P07-1R     CACGGATTGCATTGGCTGAACACTCAATACAAACAGTCCCCGAAACCAAAAACCACATTACCGCACAAAA 70
P07-2R     CATGGATTGCATTCGTCGAGCACTCAACACAAACTGTCTCACAAACCAAAAACCACCTTACCGCACAAAA 70
P18-6R     CACGGATTGCATTCGTCGAGCACTCAATACAAACAGTCTCACAAACCAAAACCCGCTTTACCGCAGAAAA 70
P18-9F     CACGGATTGCATTCGTCGAGCATCCAATACAAACAGTCTCACAAACCAAAACACGCTTTACCGCAGAAAA 70
P19-7R     CACGGATTGCATTCGTCGAGCACTCAATACAAACAGTCTCACAAACCAAAACCCGCTTTACCGCAGAAAA 70
P19-9F     CACGGATTGCATTGGCTGAACACTCAATACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
P21-1R     CACGGATTGCATTCGTCGAGCACTCAATACAAACAGTCTCACAAACCAAAAACCACCTTACCGCACAAAA 70
P21-7F     CACGGATTGCATTGGCTGAGCGCTCAATACAAACAGTCTCACAAACCAAAACACGCTTTACCGCAGAAAA 70
Cas4-3F    TACGGATTGCATTGGCTGAACACTCAATACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
CH-1R      CACGGATTGCATTCGTCGAGCACTCAATACAAACAGTCTCACAAACCAAAAACCACCTTACCGCACAAAA 70
SO18-3F    CACGGATTGCATTGGCTGAACACCCAATACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
SO28-4R    CACGGATTGCATTGGCTGAACACTCAATACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
SO29-2R    CACGGATTGCATTGGCTGAACACCCAACACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
SO48-2R    CATGAATTGCATTGGCTGAACACTCAATACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
LDG-1R     CATGGATTGCATTGGCTGAACACTCAATACAAACAGTCTCACAAACCAAAACTCACTTCACCGCACAAAA 70
Gal60-4R   CACGGATTGCATTGGCTGAACACCCAACACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
G5-3R      CACGGATTGCATTGGCTGAGCACTCAACACAAACAGTCTCACAAACCAAAACTCACTTTACCGCACAAAA 70
            * * ******** *  ** *   *** ****** *** *  *********  * * * ****** ****

P02-1R     CCTTAAGTGTCCCAAATGAAAAATGGCCGCGCAGTGTTGTTGCGGATAGTTAAGGTT 127
P02-3R     ACTTAAGTGTCCTAAATGAAAAATGGCCGCGCCGTGTTGTTGCAAACAGTTAAG--- 124
P07-1R     ACTTAAGTGTCCAGAATGAAAATTGGCCACGCAGCGTTGTTGCAGACAGTTAAGGTT 127
P07-2R     CCTTAAGTGTCCAGAAAAAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
P18-6R     ACTTAAGTGTCCTAAATGAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAGGTT 127
P18-9F     ACTTAAGTGTCCTAAATGAAAAATGGCCGCGCCGTGTTGTTGCAAACAGTTAAG--- 124
P19-7R     ACTTAAGTGTCCTAAATGAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAGGTT 127
P19-9F     ACTTAAGTGCCCAGAATGAAAAATGGCCGCGCAGTGTTGTTGCAAACAGTTAAG--- 124
P21-1R     CCTTAAGTGTCCCAAATGAAAAATGGCCGCGCAGTGTTGTTGCGGATAGTTAAGGTT 127
P21-7F     ACTTAAGTGTCCTAAATGAAAAATGGCCGCGCCGTGTTGTTGCAAACAGTTAAG--- 124
Cas4-3F    CCTTAAGTGTCCAGAATGAAAAATGCCCGCGCAGCTTTGTTGCCGAGAGTCAAG--- 124
CH-1R      CCTTAAGTGTCCCAAATGAAAAATGGCCGCGCAGTGTTGTTGCGGATAGTTAAGGTT 127
SO18-3F    ACTCAAGTGTCTAAAATGAAAAATGGTCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
SO28-4R    ACTTAAGTGCCCAGAATGAAAAATGGCCGCGCAGTGTTGTTGCAAACAGTTAAG--- 124
SO29-2R    ACTCAAGTGTCCAGAATGAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
SO48-2R    ACTCAAGTGTCTAAAATGAAAAATGGTCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
LDG-1R     ACTCAAGTGTCTAAAATGAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
Gal60-4R   ACTCAAGTGTCCAGAATGAAAAATGGTCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
G5-3R      ACTCAAGTGTCCAGAATGAAAAATGGCCGCGCAGTGTTGTTGCAGACAGTTAAG--- 124
            ** ***** *   **  **** **  * *** *  *******  * *** ***
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Fig. 2 ClustalW alignment of KP1(+) and KP1(−) *T. rangeli* subtelomeric sequences. Gaps are indicated by *dashes* and *asterisks* indicate nucleotides in a column that are identical in all sequences of the alignment. An indel event can be observed at the 3′-end of some sequences (GTT). Primers sequences were removed from the sequences used in the alignment

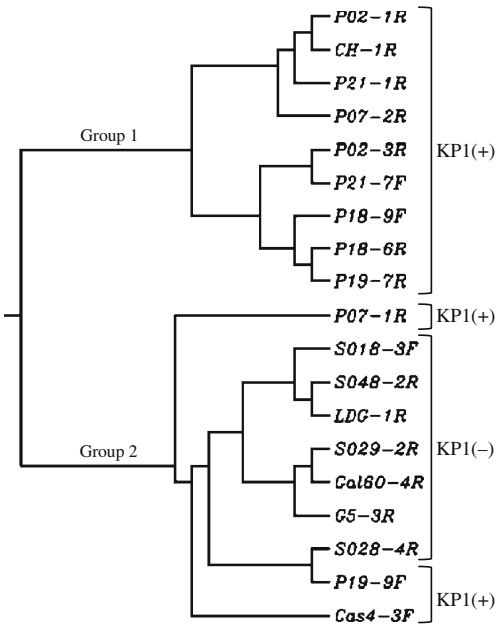

Fig. 3 Dendrogram obtained by multiple sequence alignment of the subtelomeric sequences of *T. rangeli*

Chiurillo et al. 2000; El-Sayed et al. 2005; Conte and Cano 2005). This fact might be explained by variation in the number of telomeric hexamer repeats or by the presence of sequence polymorphisms that create or destroy restriction sites for the endonucleases used (Freitas-Junior et al. 1999; Vargas et al. 2004).

Despite variations in TRF length between the different *T. rangeli* strains, an association could be established between the hybridization pattern observed and the KP1(+) and KP1(−) genotypes, a fact suggesting that the organization of these regions differs between these two subpopulations. In *T. cruzi*, no association was observed between TRF length and the different parasite groups studied. Some authors suggest the division of *T. cruzi* into two groups as a function of telomere length (Freitas-Junior et al. 1999). However, no such association was observed by Vargas et al. (2004) who analyzed TRFs from three *T. cruzi* I strains and six *T. cruzi* II strains, which were recently reclassified as belonging to the TcII, TcV, and TcVI DTUs Zingales et al. (2009), in agreement with the present findings.

Multiple alignment of the *T. rangeli* sequences generated showed the existence of variability not only between

KP1(+) and KP1(−) strains, but also within these groups. This was demonstrated by dendrogram analysis in which clones P07-1R, P19-9F, and Cas4-3F (KP1+) were grouped to the branch that comprised all KP1(−) strains, in addition to the occurrence of the GTT triplet in some KP1(+) clones but not in others. Interestingly, the latter polymorphism has also been observed among clones of *T. rangeli* strain DOG82 (Chiurillo et al. 2002). The implications of the presence or absence of this triplet in the subtelomeric region of some clones are unknown. Polymorphism in the organization of the telomeric and subtelomeric regions among clones of the same strain has also been observed in other trypanosomatid species such as *L. donovani* (Chiurillo et al. 2000), *L. major*, and *Leishmania braziliensis* (Fu and Barker 1998).

In a previous study from our group, we demonstrated that, despite karyotype variability, the *T. rangeli* strains studied formed two divergent branches that coincided with genotypes KP1(+) and KP1(−) (Cabrine-Santos et al. 2009). Sequence analysis of the mini-exon, SSU rDNA, and CatL-like genes permitted the division of the parasite populations into five lineages (A, B, C, D, and E), with the strains from Triângulo Mineiro (Brazil) used in that study belonging to lineage A (Maia-da-Silva et al. 2004a, b; 2007; 2009; Ortiz et al. 2009). In addition, other strains belonging to lineage A were associated with genotype KP1(+) and lineages C and D with genotype KP1(−) (Maia-da-Silva et al. 2009; Vallejo et al. 2009). There are no studies of this kind involving lineages B and E. In the present investigation, both TRF analysis and sequence analysis of the subtelomeric region of *T. rangeli* demonstrated the division of the strains into two main groups, which might be related, at least in part, to the presence or absence of the KP1 minicircle in the strains. This finding shows that the KP1(+) and KP1(−) lineages of the parasite present peculiar characteristics and suggests that the KP1 minicircle is a good marker for strain differentiation. However, other markers should be used for strain characterization as done for *T. cruzi* because of the existence of polymorphisms within the KP1(+) and KP1(−) groups as demonstrated in the present study and by the data reported above. The true population structure of this parasite and its biological implications are still unclear. However, despite the wide diversity it was possible to associate the circulating parasite lineage with the vector found in the region (Maia-da-Silva et al. 2007, 2009; Salazar-Antón et al. 2009). Furthermore, the findings suggest that control of parasite transmission between different *Rhodnius* species might be achieved by mechanisms that impair the penetration or persistence of genotype-specific strains in the salivary gland of the triatomine vectors (Sanchez et al. 2005; Pulido et al. 2008; Salazar-Antón et al. 2009).

In conclusion, polymorphisms in the subtelomeric sequences of *T. rangeli* permitted the division of strains into groups associated with the KP1(+) and KP1(−) genotypes of the parasite. However, analysis of the subtelomeric sequences showed a greater variability among parasite lineages than these groups defined based on kDNA. Mean TRF length analysis showed that the chromosome ends of *T. rangeli* KP1(+) and KP1(−) strains are organized distinctly. The present results extend the genotype differences demonstrated by kDNA and karyotype analysis in *T. rangeli* to the chromosome ends of the parasite. Further studies investigating these regions may reveal novel features of *T. rangeli* genetics and biology.

Acknowledgments The Colombian *T. rangeli* strains were kindly provided by Dr. Jaime Moreno, Departamento de Biologia, Faculdad de Ciências Exactas y Naturales, Universidade de Antioquia, Colombia. *T. cruzi* clone CL Brener was kindly provided by Dr. Égler Chiari, Departamento de Parasitologia, Universidade Federal de Minas Gerais, Brazil. We thank Dr. Angela Kaysel Cruz, Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil, for permitting the use of the hybridization equipment and sequencing facilities, and Tânia Paula Aquino Defina for technical assistance with the processing of DNA sequences.

This study was supported by Fundação de Ensino e Pesquisa de Uberaba (FUNEPU, grant 2007/1112), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant 476175/2007-0), and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG, grant APQ-01787-09). B.F.S. was a recipient of an undergraduate fellowship from FAPEMIG. All experiments were conducted according to Brazilian laws.

Author disclosure statement No competing financial interests exist.

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