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The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*☆

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Abstract

This paper presents a re-evaluation of the taxonomic position and evolutionary relationships of *Trypanosoma* (*Herpetosoma*) *rangeli* based on the phylogenetic analysis of ssrRNA sequences of 64 *Trypanosoma* species and comparison of mini-exon sequences. All five isolates of *T. rangeli* grouped together in a clade containing *Trypanosoma* (*Schizotrypanum*) *cruzi* and a range of closely related trypanosome species from bats [*Trypanosoma* (*Schizotrypanum*) *dionisii*, *Trypanosoma* (*Schizotrypanum*) *vespertilionis*] and other South American mammals [*Trypanosoma* (*Herpetosoma*) *leeuwenhoeki*, *Trypanosoma* (*Megatrypanum*) *minasense*, *Trypanosoma* (*Megatrypanum*) *conorhini*] and an as yet unidentified species of trypanosome from an Australian kangaroo. Significantly *T. rangeli* failed to group with (a) species of subgenus *Herpetosoma*, other than those which are probably synonyms of *T. rangeli*, or (b) species transmitted via the salivarian route, although either of these outcomes would have been more consistent with the current taxonomic and biological status of *T. rangeli*. We propose that use of the names *Herpetosoma* and *Megatrypanum* should be discontinued, since these subgenera are clearly polyphyletic and lack evolutionary and taxonomic relevance. We hypothesise that *T. rangeli* and *T. cruzi* represent a group of mammalian trypanosomes which completed their early evolution and diversification in South America.

Keywords: Evolution; Mini-exon; Phylogenetics; Spliced leader; 5S rRNA; SsrRNA; *Trypanosoma cruzi*; *Trypanosoma rangeli*

1. Introduction

Within the genus *Trypanosoma*, the taxonomic position and evolutionary relationships of *Trypanosoma rangeli* are controversial [1–3]. Like *Trypanosoma cruzi*, *T. rangeli* infects humans and a wide range of other mammals in South America and is transmitted by triatomine bugs. However, *T. rangeli* multiplies as trypomastigotes within the bloodstream rather than as amastigotes within tis-

☆ Note: Nucleotide sequence data reported in this paper are available in the EMBL, Genbank® and DDJB databases under the following accession numbers: SsrRNA—*Trypanosoma conorhini* USP, AJ012411; *Trypanosoma leeuwenhoeki* CH 250, AJ012412; *Trypanosoma minasense* LSTM, AJ012413; *Trypanosoma rangeli* Choachi, AJ012414; *T. rangeli* Macias, AJ012415; *T. rangeli* PG, AJ012416; *T. rangeli* San Agustin, AJ012417; *Trypanosoma* sp. (*Rousettus aegyptiacus*), AJ012418. 5S rRNA and spliced leader (mini-exon)—*T. rangeli* RGB, AJ012419; *T. leeuwenhoeki* CH 250, AJ012420.

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sue cysts in the mammalian host, and the infective metacyclics develop in both anterior (salivary glands) and posterior (hindgut) sites in the bug [2]. On morphological and behavioural criteria, *T. rangeli* is generally accepted as belonging to subgenus *Herpetosoma* [1, 2], although Añez [4] allied it to the *Salivaria* and argued for the creation of a new subgenus, *Tejeraia*.

Biochemical characterisation has produced further conflicting information on the affinities of *T. rangeli*. In a study of β-tubulin gene sequences on a limited number of isolates, Amorim et al. [5] found *T. rangeli* to be more closely related to *Trypanosoma brucei* than to *T. cruzi*. Murthy et al. [6] showed that the mini-exon repeat units of *T. rangeli* and *T. cruzi* were substantially different in both size and sequence, and that the repeat unit served as a species-specific DNA probe for *T. rangeli*. More recently, Steindel et al. [7] showed that *T. rangeli*, *T. cruzi* and a range of bat trypanosomes form three genetically distinct groups on the basis of isoenzymes and random amplification of polymorphic DNA (RAPD) analysis, although the lack of suitable outgroup taxa limits interpretation of such findings.

To attempt to resolve the position of *T. rangeli*, we have used a molecular phylogenetic approach. A single isolate of *T. rangeli* included in our previous phylogeny based on ssrRNA sequences was placed unequivocally in a clade containing *T. cruzi* and other representatives of subgenus *Schizotrypanum* [8]. To substantiate this rather surprising result, we now present additional mini-exon sequence data confirming the identity of this isolate as *T. rangeli*. Phylogenetic analysis of ssrRNA sequences from *T. rangeli* isolates of diverse origins and a broad range of potentially related trypanosome species from South American mammals further clarifies the evolutionary relationships of *T. rangeli*.

2. Materials and methods

2.1. Trypanosomes

Four *T. rangeli* isolates from the Trypanosomatid Culture Collection, Department of Parasitology, University of Sao Paulo, were included in this study. All were confirmed as *T. rangeli* on the basis of growth and morphology in culture (LIT medium), and in triatomines (*Rhodnius prolixus*), and the absence of invasion of and multiplication within LLCMK2 cells in vitro. Two of the isolates had also been further characterised by mini-exon sequence (San Augustin [6]) and/or by isoenzymes and RAPD analysis (Macias and San Augustin [7]). Isolates of *Trypanosoma leeuwenhoeki*, *Trypanosoma conorhini*, *Trypanosoma minasense* and an undescribed species of trypanosome from an African bat (*Rousettus aegypticus*) were also included in this study (Table 1).

2.2. Sequence analysis

A 2-kb DNA fragment containing the ssrRNA gene was amplified by PCR using conserved primers [8, 12] from purified DNA (*T. rangeli*, *T. conorhini*) or from DNA lysates prepared from small numbers (10^6–10^7) of cultured or cryopreserved purified trypanosomes [*T. leeuwenhoeki*, *T. minasense* and *Trypanosoma* sp. (bat)] [13]. Polymerase chain reaction fragments were sequenced in both directions at intervals of approximately 300 bp using suitable internal primers [12] on a Perkin–Elmer ABI 377 automated sequencer.

The approximately 800 bp mini-exon repeat units of *T. rangeli* isolate RGB and *T. leeuwenhoeki* were amplified by PCR using conserved primers [6] and cloned into a plasmid vector (pGEMT, Promega) before sequencing using flanking primers in the vector. Insufficient DNA was available to analyse the mini-exon repeats of *T. minasense* and *Trypanosoma* sp. (bat).

2.3. Sequence alignment

SsrRNA sequences were aligned primarily on the basis of their secondary structure [14]. Subsections of the alignment, between regions of high homology, were sub-aligned using the program Clustal W [15], before final adjustments were made by eye. The eight new sequences were aligned to the 55 analysed previously [8], together

Table 1
Details of *Trypanosoma cruzi* clade taxa[a]

Subgenus	Species	Clade ref. No.[b]	Sample	Host		Location	Ref.[c]	Accession No.
Schizotrypanum	*T. cruzi*	1	Sylvio-X10 cl.1	Human	*Homo sapiens*	Brazil	[8]	AJ009147
	T. cruzi	1	VINCH 89	Triatomine bug	*Triatoma infestans*	Chile	[8]	AJ009149
	T. cruzi	1	CAN III cl. 1	Human	*H. sapiens*	Brazil	[8]	AJ009148
	T. cruzi	1	—	?		Mexico	[9]	M31432
	T. cruzi	1	No. 3/Peru	?		Peru	[10]	X53917
	T. c. marinkellei	2	B7	Bat	*Phyllostomum discolor*	Brazil	[8]	AJ009150
	T. dionisii	3	P3	Pipistrelle bat	*Pipistrellus pipistrellus*	England	[8]	AJ009151
	T. dionisii	3	PJ	Pipistrelle bat	*P. pipistrellus*	Belgium	[8]	AJ009152
	T. vespertilionis	7	P14	Pipistrelle bat	*P. pipistrellus*	England	[8]	AJ009166
Herpetosoma	*T. rangeli*	4	RGB (Basel)	Dog	*Canis familiaris*	Venezuela	[8]	AJ009160
	T. rangeli	4	PG	Human	*H. sapiens*	Panama	USP	
	T. rangeli	5	San Agustin	Human	*H. sapiens*	Colombia	USP	
	T. rangeli	5	Choachi	Triatomine bug	*Rhodnius prolixus*	Colombia	USP	
	T. rangeli	5	Macias	Triatomine bug	*R. prolixus*	Venzuela	USP	
	T. leeuwenhoeki	4	CH 250	Sloth	*Choloepus hoffmanni*	Colombia	IOC	
Megatrypanum	*Trypanosoma* sp.	9	H25	Kangaroo	*Macropus giganteus*	Australia	[8, 11]	AJ009168
	Trypanosoma sp.	6	—	Bat	*Rousettus aegyptiacus*	Gabon	LSTM	
	T. conorhini	8	—	Rat	*Rattus rattus*	Brazil	USP	
	T. minasense	5	—	Squirrel monkey	*Saimiri boliviensis*	South America	LSTM	

[a] Details of other ssrRNA sequences used in this paper are given in Ref. [8].
[b] Clade reference number refers to superscript numbers in Figs. 1 and 2.
[c] Numbered references provide original isolation details of each trypanosome stock. Source of other stocks are: USP, Universidade de São Paulo; IOC, Instituto Oswaldo Cruz, Rio de Janeiro; LSTM, Liverpool School of Tropical Medicine.

with an isolate of *Trypanosoma cyclops*, a primate trypanosome from southeast Asia [16].

The mini-exon repeat unit sequences of *T. rangeli* RGB and *T. leeuwenhoeki* were compared with that of *T. rangeli* isolate Bg-60 (Accession No. X62675 [6]) and *T. cruzi* isolates CL (Accession No. U57984 [17]) and Tulahuen (Accession No. X00632 [18]) from the EMBL database. Again, sequences were aligned using Clustal W, prior to final adjustments by eye. All alignments are available from J.R. Stevens (e-mail: j.r.stevens@exeter.ac.uk) on request.

2.4. Phylogenetic analysis

Analysis of two data sets was performed. For maximum parsimony analysis the number of taxa necessitated the use of an heuristic search strategy to find the most parsimonious trees. The default options of PAUP were used: 10 random addition sequences, TBR branch swapping, all minimal trees saved and zero branch lengths collapsed; all characters were assigned equal weight. The full data set of 64 ssrRNA sequences was analysed to establish the phylogenetic position of the new isolates relative to other *Trypanosoma* species. *Trypanosoma* sequences were compared with a range of outgroup taxa (*Trypanoplasma borreli*, *Crithidia* spp. , *Leishmania* spp.) and the tree was rooted on *Bodo caudatus*. The suitability of free-living bodonid taxa as outgroups for phylogenetic studies of trypanosomatids has been established by a number of studies using a range of ribosomal and protein coding genes (e.g. [8] and references therein).

A second analysis on ssrRNA sequences from a subset of 21 taxa, including *T. rangeli* and related species, was performed, allowing a finer degree of phylogenetic resolution to be obtained. As previously, a standard alignment [8] was used, reduced from 1809 to 1798 nucleotides following the deletion of superfluous blanks in the reduced data set. The type species, *Trypanosoma lewisi* and *Trypanosoma theileri*, of the two sister subgenera within the Stercorarian section, *Herpetosoma* and *Megatrypanum*, were included as outgroups. Preliminary phylogenetic analyses (not presented) also supported the use of these taxa as suitable outgroup species.

Bootstrapped maximum parsimony analyses with 100 replicates were performed for both the full data set (64 taxa) and the *T. cruzi* clade subset (21 taxa; Table 1); each bootstrap replicate was based on a single random addition sequence, using the default PAUP parameters given previously. The 21 taxa subset was also analysed by maximum likelihood analysis; starting trees were derived by both parsimony and neighbour joining. Transition/transversion ratios were estimated from the data in preliminary runs and then fixed for the full analysis. All phylogenetic analyses were performed using PAUP* (test version 4.0d64) written by David L. Swofford.

Mini-exon sequence homology was determined for *T. cruzi*, *T. leeuwenhoeki* and *T. rangeli* (see Sequence alignment) by pairwise comparison of aligned sequences.

3. Results

3.1. Identity of Trypanosoma rangeli isolates

Comparison of mini-exon repeat unit and/or ssrRNA sequences of the five isolates of *T. rangeli* demonstrated unequivocally that all were closely related (Table 2 and 3).

The mini-exon repeat unit comprises a short (39 bp) exon, an intron of approximately 100 bp and a spacer region of variable length, which has been shown to contain a copy of the 5S rRNA gene in *T. rangeli* [6]. The exon sequence is usually completely conserved and the intron less so, while the spacer is highly variable among species and useful as a species-specific probe [6]. Here, the mini-exon repeats of *T. rangeli* isolates RGB and Bg-60 and *T. leeuwenhoeki* were highly homologous, even within the spacer region (Table 2); this is due in part to the presence of a 5S rRNA gene within the spacers of both species. The 5S sequences of *T. rangeli* RGB and *T. leeuwenhoeki* are identical, differing from that identified in the Bg-60 isolate [6] by a GC insertion and a C to T transition at the 3′ end; a 5S sequence is absent from the mini-exon repeat

Table 2
Matrix of nucleotide similarities (%) between mini-exon repeats

	Trypanosoma rangeli RGB	*T. rangeli* Bg-60	*Trypanosoma leeuwenhoeki*	*Trypanosoma cruzi* CL
T. rangeli Bg-60	**91** (96, 90)[a]			
T. leeuwenhoeki	**97** (97, 97)	**90** (96, 89)		
T. cruzi CL	**66** (66, 63)	**63** (70, 59)	**64** (66, 61)	
T. cruzi Tulahuen	**56** (62, 52)	**54** (66, 48)	**55** (62, 51)	**80** (92, 76)

[a] Figures in bold are overall similarity of the repeat unit, followed by similarities of intron and spacer, respectively, in parentheses. The 39 bp exon was completely conserved in all cases. 5S rRNA sequences in *T. rangeli* and *T. leeuwenhoeki* occupied approximately 14% of the spacer region (125 bp).

unit of *T. cruzi* [6, 17, 18]. The total length of the mini-exon repeat was 955 bp in *T. leeuwenhoeki*, 919 bp in *T. rangeli* RGB and 858 bp in *T. rangeli* Bg-60. Most of the variation in length is accounted for by large deletion/insertion events in the spacer region between the 3′ end of the 5S rRNA gene and the start of the mini-exon. *Trypanosoma leeuwenhoeki* has a 27 bp insertion in this region relative to *T. rangeli* RGB, while in turn *T. rangeli* RGB has insertions of 19 bp and 45 bp in this region relative to *T. rangeli* Bg-60. Comparison of mini-exon repeat units from other trypanosome species shows that spacer conservation is restricted to very closely related species. For example, homology between *T. rangeli*/*T. leeuwenhoeki* and *T. cruzi* spacer regions is at a much lower level than that between *T. rangeli* and *T. leeuwenhoeki* (Table 2).

The ssrRNA sequences of all *T. rangeli* isolates, *T. leeuwenhoeki* and *T. minasense* were 100% homologous (Table 3), while homology with *T. cruzi* isolates was consistently 97–98%.

Table 3
Matrix of pairwise differences (lower) and percentage homology (upper) between ssrRNA sequences of 19 species within the *Trypanosoma cruzi* clade

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *T. cruzi* × 10	—	100	100	100	100	100	97	97	97	97	97	97	97	98	98	97	97	98	98
2. *T. cruzi* VINCH	3	—	100	100	100	100	97	97	97	97	97	97	97	98	98	97	98	98	98
3. *T. cruzi* Can III	2	3	—	100	100	100	97	97	97	97	97	97	97	99	99	97	98	98	98
4. *T. cruzi* (M31432)	3	6	5	—	100	100	97	97	97	97	97	97	97	98	98	97	97	97	98
5. *T. cruzi* (X53917)	5	4	3	8	—	100	97	98	98	97	98	97	98	99	99	97	98	98	98
6. *T. c. marinkellei*	7	6	7	10	6	—	97	97	97	97	97	97	97	99	99	97	98	98	98
7. *T. rangeli* RGB	58	59	58	61	54	57	—	100	100	100	100	100	100	98	98	98	98	99	98
8. *T. rangeli* Choachi	55	56	55	58	51	54	3	—	100	100	100	100	100	98	98	98	98	99	98
9. *T. rangeli* Macias	55	56	55	58	51	54	3	0	—	100	100	100	100	98	98	98	98	99	98
10. *T. rangeli* PG	58	59	58	61	54	57	0	3	3	—	100	100	100	98	98	98	98	99	98
11. *T. rangeli* San Ag	55	56	55	58	51	54	3	0	0	3	—	100	100	98	98	98	98	99	98
12. *T.leeuwenhoeki*	58	59	58	61	54	57	0	3	3	0	3	—	100	98	98	98	98	99	98
13. *T. minasense*	55	56	55	58	51	54	3	0	0	3	0	3	—	98	98	98	98	99	98
14. *T. dionisii* PJ	33	32	31	36	30	31	45	44	44	45	44	45	44	—	100	97	98	98	98
15. *T. dionisii* P3	33	32	31	36	30	31	45	44	44	45	44	45	44	0	—	97	98	98	98
16. *Trypanosoma* sp. (bat)	60	61	62	63	61	61	46	43	43	46	43	46	43	57	57	—	98	98	98
17. *T. vespertilionis*	53	52	51	56	50	50	44	41	41	44	41	44	41	49	49	49	—	99	98
18. *T. conorhini*	50	49	48	43	47	46	27	26	26	27	26	27	26	39	39	36	21	—	99
19. *Trypanosoma* sp. (kangaroo)	49	48	47	52	46	46	38	37	37	38	37	38	37	41	41	49	42	29	—

3.2. Phylogenetic analysis of 64 taxa

The ssrRNA sequences were subjected to phylogenetic analysis. The evolutionary relationships of 56 of the taxa presented in Fig. 1 have been discussed elsewhere [8, 16], so only those results relating to the eight new taxa are described here. Analysis of the complete data set (64 taxa) placed all five *T. rangeli* isolates together in a single, well-supported clade (*T. cruzi* clade; bootstrap >91%; Fig. 1). This clade also contains *T. cruzi* (five isolates), a range of other subgenus *Schizotrypanum* species from bats (four isolates), *T.* (*Herpetosoma*) *leeuwenhoeki* from a sloth and four subgenus *Megatrypanum* species from a rat (*T. conorhini*), a squirrel monkey (*T. minasense*), an African bat (*Trypanosoma* sp.) and an Australian kangaroo (*Trypanosoma* sp.) (Table 1). All isolates, except the three from Old World bats and that from a kangaroo, were isolated from South American hosts.

3.3. Phylogenetic analysis of subset of 21 taxa

The ssrRNA sequences of the 19 taxa contained within the above clade, plus two outgroup taxa, were re-analysed separately; the results are presented in Fig. 2. Pairwise differences between the sequences, providing a measure of absolute genetic difference between taxa, are presented in Table 3.

All taxa are well separated from the outgroup species, *T.* (*Herpetosoma*) *lewisi* and *T.* (*Megatrypanum*) *theileri*, in terms of both relative genetic distance and bootstrap support (98%) for the relationships defined. This confirms the distinct genetic nature of taxa within this clade. For *T. cruzi* and *T. rangeli* where several isolates were sequenced, a high degree of genetic homogeneity is apparent. All *T. cruzi* isolates form a homogeneous cluster which, together with *Trypanosoma cruzi marinkellei*, are well-separated from all other taxa in terms of both relative genetic distance and bootstrap support (100%). Likewise, *T. rangeli* and two closely related species—*T. leeuwenhoeki*, which is classified by Hoare [1] as being allied to *T. rangeli*, and *T. minasense*, examples of which have also been classified as *T.* (*Herpetosoma*) *saimiri* (Rodhain, cited Hoare [1])—also form a genetically distinct grouping within the clade (bootstrap support 84%).

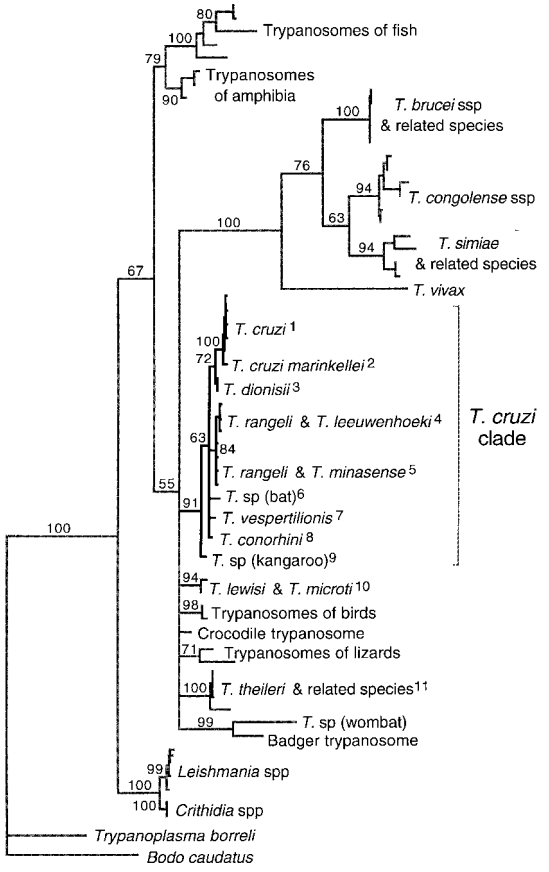

Fig. 1. Phylogram constructed by bootstrapped (100 replicates) maximum parsimony analysis of 64 kinetoplastid ssrRNA sequences. The tree is derived from the eight most parsimonious trees of length = 1129 (RI = 0.845, CI = 0.588), based on an alignment of 1809 nucleotide sites. Bootstrap values for all major nodes are given and all branches receiving bootstrap support values >50% are shown; relationships failing to achieve this level of support are shown as polytomies (i.e. branch points at which three or more branches arise from the ancestral line). The "*T. cruzi* clade" of 19 taxa is bracketed, and full details of these taxa (minor clades 1–9 indicated by superscript figures) are given in Table 1; see Fig. 2 for further analysis. The type species, *T. lewisi* and *T. theileri*, of the subgenera *Herpetosoma* and *Megatrypanum*, are classified in minor clades 10 and 11, respectively. See Refs. [8] and [16] for details of the additional 45 taxa presented.

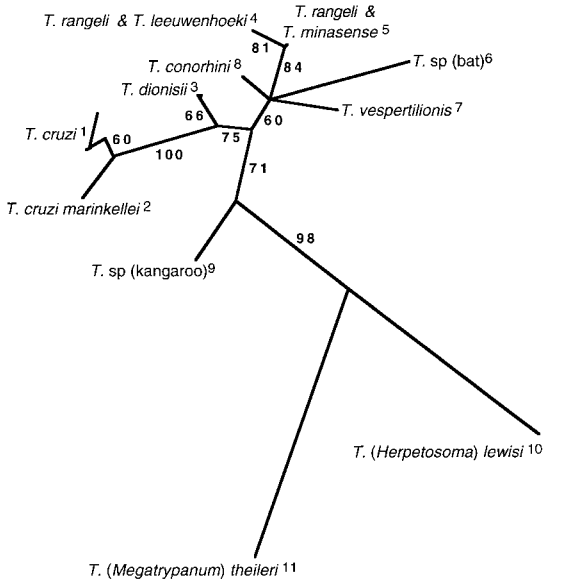

Fig. 2. Phylogram constructed by bootstrapped (100 replicates) maximum parsimony analysis of the 19 *Trypanosoma* spp. ssrRNA sequences from the "*T. cruzi* clade" in Fig. 1, together with outgroup taxa, *T. lewisi* (Acc. No. AJ009156) and *T. theileri* (Acc. No. AJ009164). The tree is derived from the four most parsimonious trees of length = 81 (RI = 0.884, CI = 0.827), based on the standard alignment reduced from 1809 to 1798 bp following the deletion of superfluous blanks. Bootstrap values for all major nodes are given and all branches receiving bootstrap support values >50% are shown; relationships failing to achieve this level of support are shown as polytomies (i.e. branch points at which three or more branches arise from the ancestral line). Full details of all taxa are given in Table 1.

As in the full analysis, the polyphyletic nature of the three Stercorarian subgenera *Schizotrypanum*, *Herpetosoma* and *Megatrypanum*, as classically defined, is evident. The kangaroo trypanosome is placed at the periphery of the clade in both analyses.

Maximum-likelihood analysis of the 21 taxa yielded a consensus tree almost identical to that produced by parsimony analysis, with the following exception. *Trypanosoma* sp. from a bat (group 6) and *Trypanosoma vespertilionis* (group 7) clustered together in 100% of all trees; their position relative to all other taxa within the phylogram was unaltered.

4. Discussion

The results of this study indicate a close evolutionary relationship between *T. rangeli* and *T. cruzi*. *Trypanosoma rangeli* is placed firmly within a clade containing *T. cruzi* and a range of closely related trypanosome species from bats, other South American mammals and an as yet unidentified species of trypanosome from an Australian kangaroo. Bootstrap support for the clade is high (91%) and the robustness of this grouping was confirmed by re-analysis of a 19 taxon subset, using *T. lewisi* and *T. theileri*, the type species of the two sister subgenera, *Herpetosoma* and *Megatrypanum*, as outgroups. Significantly, *T. rangeli* failed to group with other trypanosome species transmitted via saliva in either analysis, indicating that transmission route may not be an important evolutionary character.

In both analyses, *Trypanosoma* sp. (kangaroo) was firmly within, but at the periphery of, the clade. The phylogenetic association of this Australian trypanosome with a broad range of South American species substantiates the hypothesis that this clade may have evolved in ancestral marsupials before the separation of South America and Australia [8]. The present diversity of life-cycles and transmission strategies among these trypanosomes implies substantial diversification from a hypothesised common ancestor. Both bat trypanosomes and *T. conorhini* have also become more geographically widespread. It is easy to envisage how bat trypanosomes became widely dispersed in a highly mobile host able to occupy diverse ecological niches. Similarly, *T. conorhini* appears to have spread globally by association with the ubiquitous rat [1].

Despite being unequivocally in the same clade, genetic distances between *T. cruzi* and *T. rangeli* isolates are some of the largest in the clade. Pairwise nucleotide differences between *T. cruzi* and *T. rangeli* ssrRNA sequences (Table 2) are consistently some of the greatest in the group, suggesting distinct evolutionary pathways within the clade. This concurs with the obvious life-cycle and morphological differences between the two species. The five isolates of *T. rangeli* were grouped together in a robust subgroup with

species from a sloth (*T. leeuwenhoeki*) and from a squirrel monkey (*T. minasense*) (bootstrap 84%). Within this grouping two isolates of *T. rangeli* (PG and RGB) and *T. leeuwenhoeki* were almost identical to each other by both ssrRNA and mini-exon sequence, clustering together with 81% bootstrap support. The ssrRNA sequence of *T. rangeli* PG has a single phylogenetically uninformative guanine deletion relative to that of *T. rangeli* RGB (Table 2), while the mini-exon intron and spacer show some minor variations. Similarly, a high degree of genetic homogeneity between *T. rangeli* isolates has been reported previously in mini-exon repeat unit sequences [6], underlining the overall homogeneity of this species. The genetic similarity of *T. rangeli* and *T. leeuwenhoeki* is also supported by morphology and behaviour in culture. Shaw [19] noted that *T. leeuwenhoeki* and certain strains of *T. rangeli* grew well in culture and produced similar infections in mice, in which they were morphologically similar, while Hoare [1], primarily on the basis of morphology, described *T. leeuwenhoeki* and a number of other Latin American trypanosomes as being *rangeli*-like. *Trypanosoma leeuwenhoeki* is restricted to sloths and opossums, while *T. rangeli* infects a range of mammals [2]. We conclude that *T. leeuwenhoeki*, and possibly also *T. minasense*, are host-range variants of *T. rangeli*.

In addition to the evolutionary implications, our findings indicate the need for a major revision of the taxonomy of South American trypanosome species, at both subgenus and species levels. The single clade containing subgenus *Schizotrypanum* also contains species classified in the subgenera *Herpetosoma* or *Megatrypanum*. Within the clade, *T. vespertilionis* is on a separate branch from the other species belonging to subgenus *Schizotrypanum*, and this isolation is even more apparent in the second analysis on the subset of taxa, where *T. vespertilionis* is separated from *T. cruzi* ssp. and *T. dionisii* by relatively large genetic distances. This indicates that subgenus *Schizotrypanum*, as currently defined, may also be polyphyletic, although the exact nature of the phylogenetic relationships between these particular species remains to be resolved.

Species belonging to subgenera *Herpetosoma* or *Megatrypanum* were also found in two (*Herpetosoma*) or three (*Megatrypanum*) well-supported, apparently unrelated, phylogenetic groupings, showing clearly that these two subgenera, as currently defined, are polyphyletic. Thus these subgenera appear to lack evolutionary or taxonomic relevance and we suggest that use of the names *Herpetosoma* and *Megatrypanum* should be discontinued until their status is clarified.

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