

Fig. S1. k-mer spectra of (A) *T. melophagium* and (B) *T. theileri* using a k-mer size of 21. The statistics presented in these images represent the lowest estimate size, the highest estimate was used in Table 1 as these had a greater model fit.

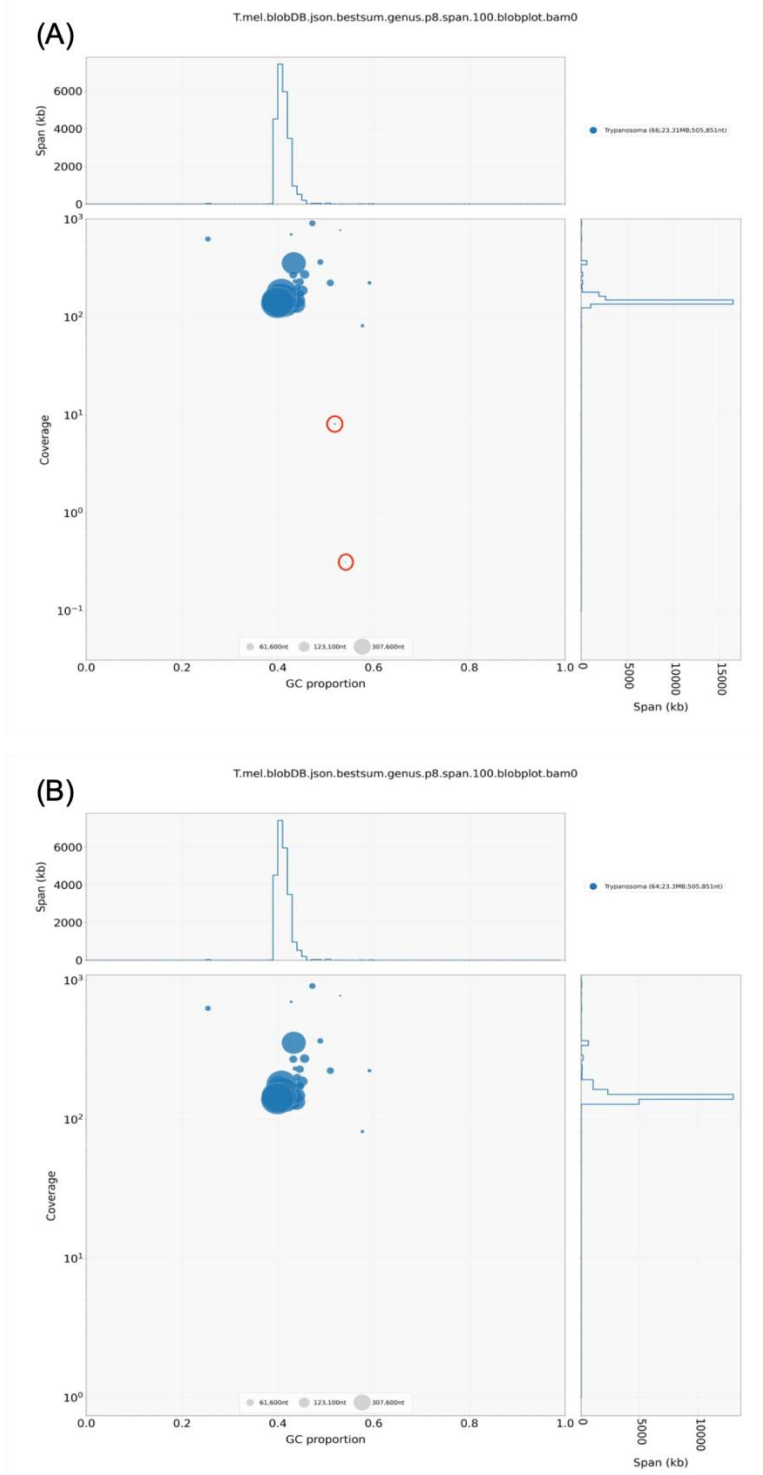


Fig. S2. Coverage and blast annotation for (A) the polished assembly and (B) the manually trimmed assembly.

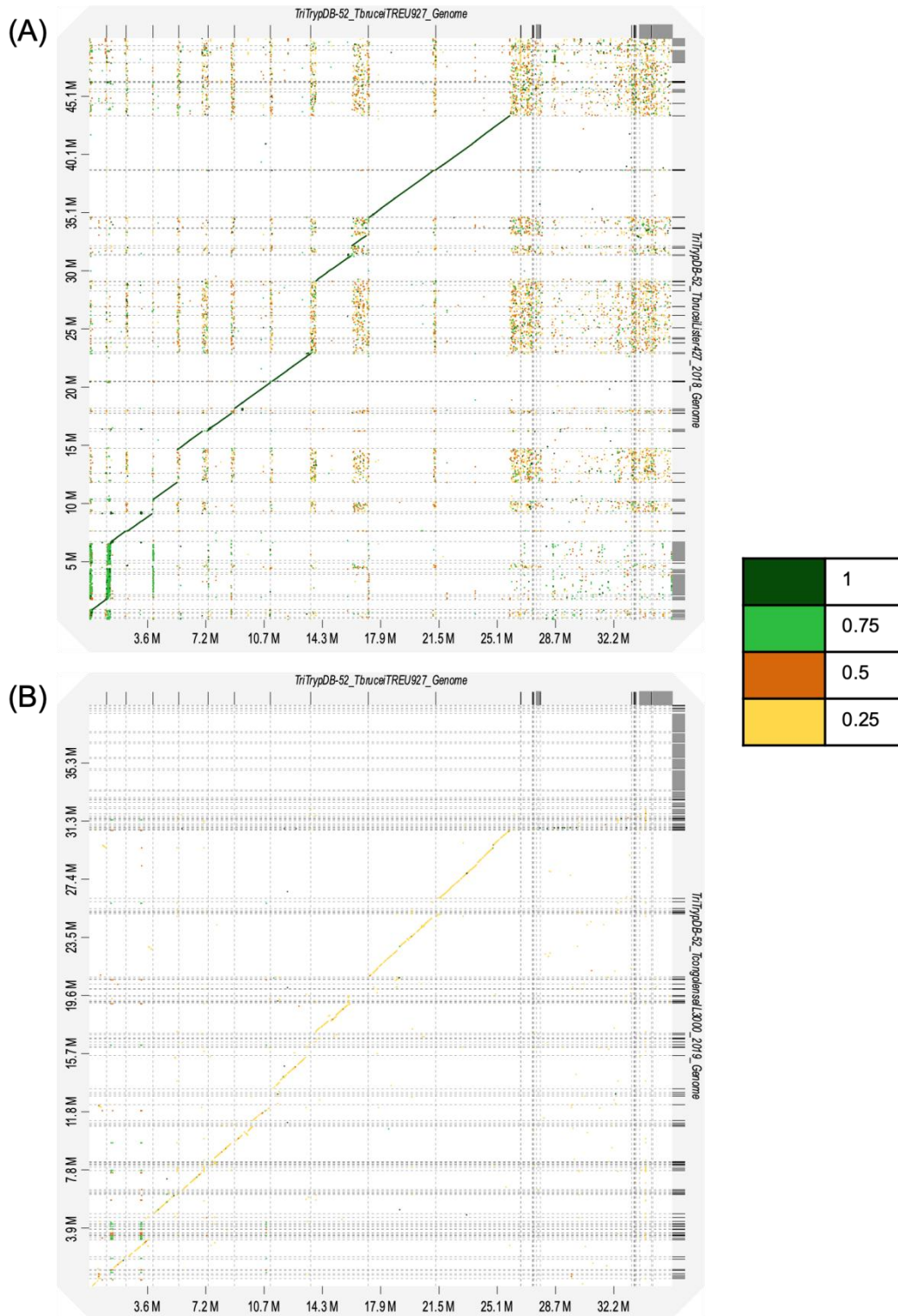


Fig. S3. Synteny plot of (A) *T. brucei* TREU927/4 and *T. brucei* Lister 427 (2018) and (B) *T. brucei* TREU927/4 and *T. congolense* IL3000 2019 genome sequences. The legend refers to the identity between the sequences.

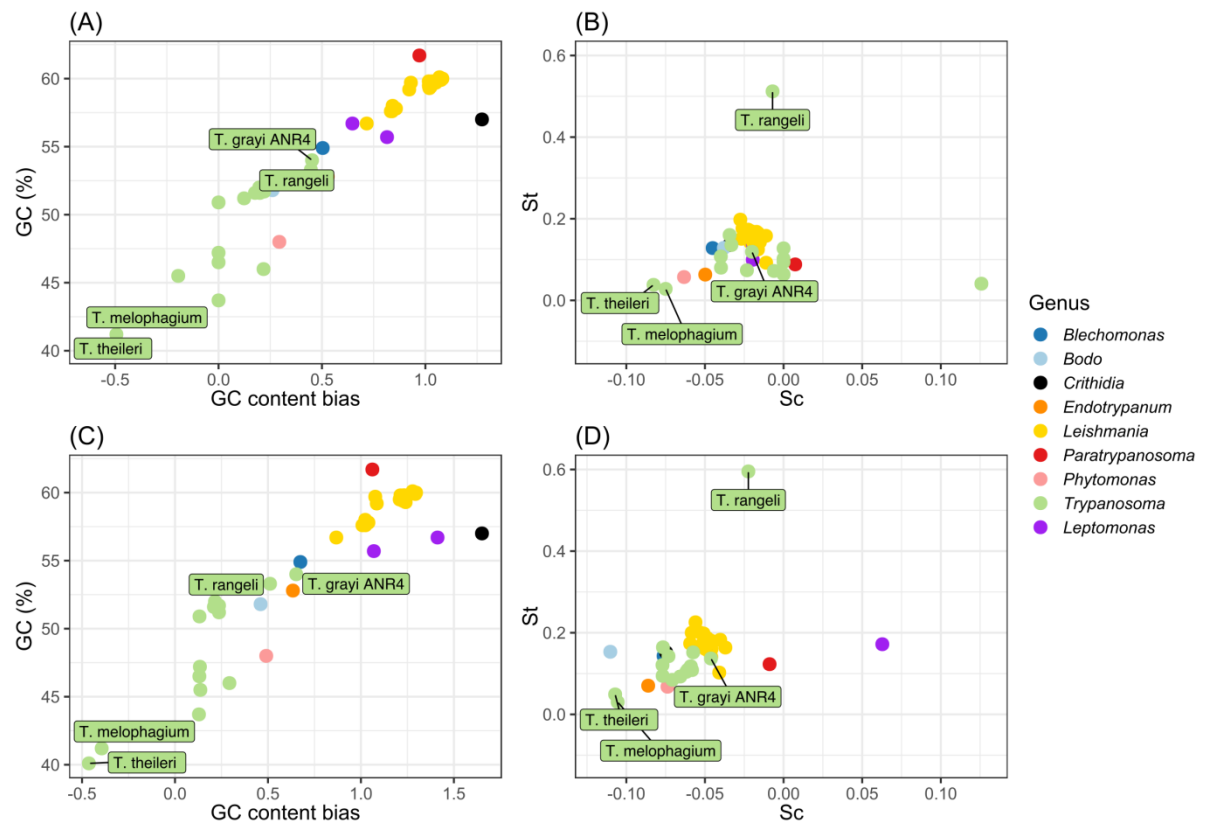


Fig. S4. (A) GC content across the whole genome and GC content bias in a every CDS. (B) Selection acting on translational efficiency (St) and selection acting on nucleotide cost (Sc). (C) GC content across the whole genome and GC content bias in CDS of a trypanosomatid universal single copy orthologue which is essential in every life cycle stage in *T. brucei* (n=158). (D) Selection acting on translational efficiency (St) and selection acting on nucleotide cost (Sc) in trypanosomatid universal single copy orthologues which is essential in every life cycle stage in *T. brucei* (n=158).

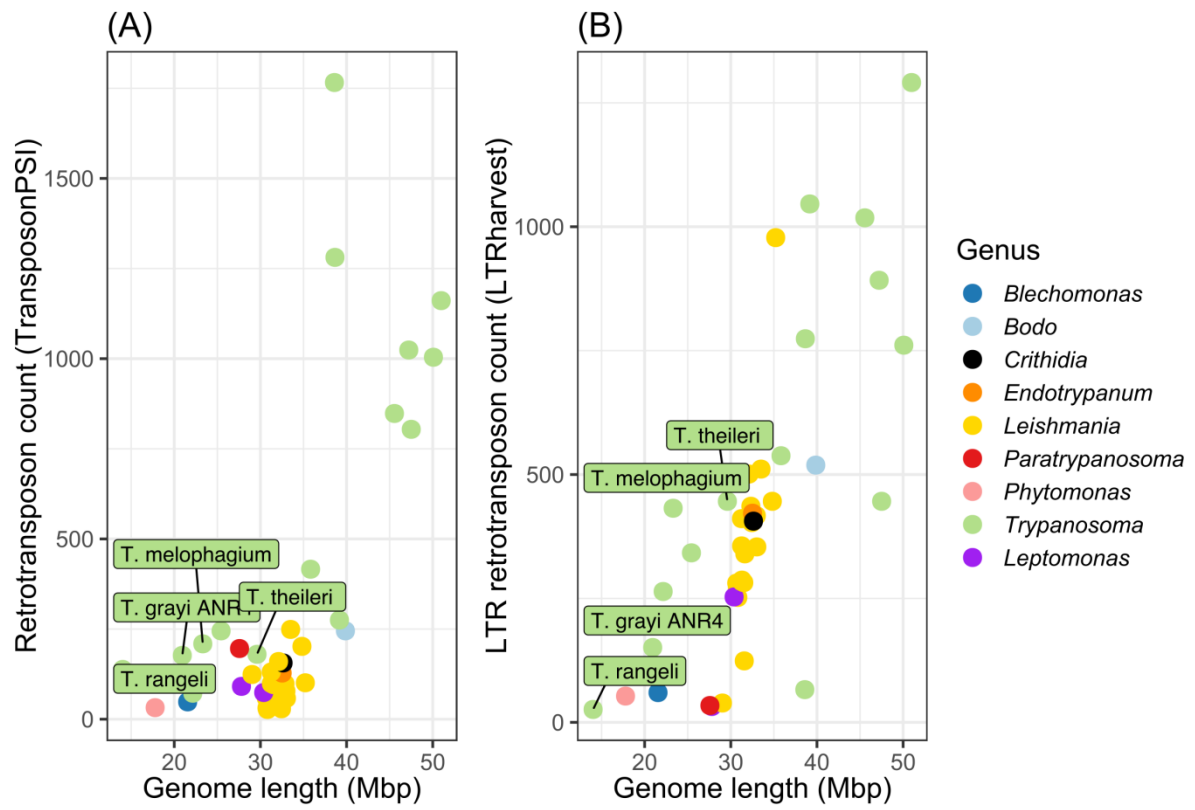


Fig. S5. (A) Retrotransposon (B) long terminal repeat (LTR) retrotransposon counts compared to genome length.

Table S1. Software and any options used.

Tool	Version	Flag	Available at
Guppy	4.0.15	--flowcell FLO-MIN106 --kit SQK-RAD004	https://community.nanoporetech.com/
PycoQC	2.5.0.3		https://github.com/arslanslide/pycoQC
Trimmomatic	0.39	-SLIDINGWINDOW 4:20 -MINLEN 50	https://github.com/timflutre/trimmomatic
GenomeScope	1	-Kmer 21 -Max kmer cov. 1000	http://qb.cshl.edu/genomescope/
Jellyfish	2.2.10	-C -m 21 -s 1000000000	https://github.com/gmarcais/Jellyfish
Wtdbg2	3	-x ont -g 30m -A -t 10	https://github.com/ruanjue/wtdbg2
Minimap2	2.17-r941	-ax map-ont	https://github.com/lh3/minimap2
Racon	v1.4.13	-m 8 -x -6 -g -8 -w 500	https://github.com/isovic/racon
Medaka	V5	-m r941_min_high_g360	https://github.com/nanoporetech/medaka
BWA-MEM	0.7.17-r1188		https://github.com/lh3/bwa
Pilon	1.24	--genome --diploid --bam --bam	https://github.com/broadinstitute/pilon
Blast	2.6.0+	blastn -task megablast -outfmt '6 qseqid	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/

		staxids bitscore std' -max_target_seqs 1 -max_hsps 1 -evaluate 1e-25 or 1e-5	
DIAMOND	2.0.5.14 3	Blastx --sensitive --max-target-seqs 1 --evaluate 1e-25	http://www.diamondsearch.org
BlobTools	1.1.1	-r genus	https://github.com/DRL/blobtools
Scaffold_stats.pl	N/A		https://github.com/sujaikumar/assemblage
BUSCO	V5	-l euglenozoa_odb10	https://busco.ezlab.org/
RepeatModeler	N/A	-database - LTRStruct -pa 8	http://www.repeatmasker.org/RepeatModeler/
RepeatMasker	N/A	-lib consensi.fa.classified -pa 8 -xsmall -nolow -gff	http://www.repeatmasker.org/RMDownload.html
BRAKER2	2.1.6	--prot_seq --bam --softmasking --etpmode --gff3	https://github.com/Gaius-Augustus/BRAKER
D-Genies	1.2.0	-Minimap2	http://dgenies.toulouse.inra.fr/run
tRNAscan-SE	2.0.9	-E	http://lowelab.ucsc.edu/tRNAscan-SE/
CodonMuse	0.1.0	-f -tscan	https://github.com/easeward/CodonMuSe

TransposonPSI	1.0.0	-nuc	http://transposonpsi.sourceforge.net
gt suffixerator	1.6.2	-tis -suf -lcp -des -ssp -sds -dna	http://genometools.org/tools/gt_ltrharvest.html
gt LTRharvest	1.6.2		http://genometools.org/tools/gt_ltrharvest.html
OrthoFinder	2.5.2	-S diamond_ultra_sens	https://github.com/davidemms/OrthoFinder
InterProScan	5.52-86.0	--dp --goterms -appl SignalP-EUK-4.1, Pfam -f TSV	https://www.ebi.ac.uk/interpro/
KinFin	1.0.3		https://github.com/DRL/kinfin
iTOL	6		https://itol.embl.de
R	3.6.1		https://www.r-project.org/
ggplot2	3.3.3		https://cran.r-project.org/web/packages/ggplot2/index.html
ggrepel	0.9.1		https://cran.r-project.org/web/packages/ggrepel/index.html

Table S2.

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Table S3.

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Table S4.

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Table S5.

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