

S1 Table. Morphometry of *T. simiae* 1K1N trypomastigotes in tsetse midgut, proventriculus and proboscis.

The mean $\pm$ SE in μm is top line in each box with the range below. L = total cell length; W = maximum cell width; KPost, NPost = distances from the cell posterior to the kinetoplast or nucleus respectively; KAnt and NAnt, distances from the cell anterior to the kinetoplast or nucleus respectively, were derived by subtracting KPost or NPost from the total cell length; KNuc = distance between the kinetoplast and nucleus; NL, NW, NArea = nucleus length, width and area respectively; CellArea = area of cell body. Total no. of cells = 443.

Morphotype	N	L	W	KPost	NPost	KAnt	NAnt	KNuc	NL	NW	NArea	CellArea
Mid/posterior midgut trypomastigotes	104	25.41 $\pm$ 0.39 15.82-34.80	1.91 $\pm$ 0.04 1.30-3.19	4.19 $\pm$ 0.18 0.73-8.07	8.59 $\pm$ 0.23 3.65-15.24	21.21 $\pm$ 0.30 13.71-29.71	16.81 $\pm$ 0.31 9.71-26.27	3.52 $\pm$ 0.14 0.73-7.08	2.75 $\pm$ 0.05 1.45-4.06	1.25 $\pm$ 0.02 0.81-1.79	3.16 $\pm$ 0.06 1.71-4.88	33.18 $\pm$ 0.65 18.16-49.03
Anterior midgut trypomastigotes	115	31.37 $\pm$ 0.46 20.04- 48.56	2.19 $\pm$ 0.04 1.40-4.15	5.64 $\pm$ 0.19 1.39-14.79	10.30 $\pm$ 0.21 4.26-18.25	25.73 $\pm$ 0.38 16.41-36.48	21.07 $\pm$ 0.44 13.62-33.10	3.81 $\pm$ 0.14 0.23-6.68	3.06 $\pm$ 0.05 1.79-5.16	1.23 $\pm$ 0.05 0.49-2.28	3.44 $\pm$ 0.07 2.08-5.80	43.56 $\pm$ 0.74 27.47-79.92
Proventricular trypomastigotes	108	37.48 $\pm$ 0.31 28.16-47.21	1.87 $\pm$ 0.03 1.17-2.88	7.19 $\pm$ 0.20 2.00-14.05	10.22 $\pm$ 0.24 3.58-15.29	30.30 $\pm$ 0.21 25.33-36.83	27.26 $\pm$ 0.22 22.25-33.41	2.31 $\pm$ 0.08 0.32-4.22	3.66 $\pm$ 0.05 2.60-5.06	1.09 $\pm$ 0.02 0.67-1.62	3.63 $\pm$ 0.07 2.29-6.62	43.89 $\pm$ 0.59 25.65-59.44
Proboscis trypomastigotes	116	30.44 $\pm$ 0.29 19.97-40.26	2.57 $\pm$ 0.04 1.39-3.84	3.22 $\pm$ 0.18 0.32-12.93	4.74 $\pm$ 0.17 2.01-13.40	27.22 $\pm$ 0.27 17.35-34.60	25.70 $\pm$ 0.25 16.34-32.53	0.99 $\pm$ 0.05 0.00-2.34	2.98 $\pm$ 0.05 1.24-4.37	1.27 $\pm$ 0.02 0.81-1.96	3.47 $\pm$ 0.08 0.87-6.33	39.52 $\pm$ 0.67 27.28-59.73

S2 Table. Loadings for principal components PC1, PC2 and PC3.

Variables contributing most to each principal component are in bold.

Variable	PC1	PC2	PC3
CellArea	0.184	0.132	0.219
NK	-0.079	0.253	-0.060
logLength	0.115	0.221	0.297
logWidth	0.148	-0.176	0.082
logNL	0.214	0.033	-0.100
logNW	0.163	-0.160	0.005
logNPost	-0.107	0.196	0.333
logNArea	0.224	-0.013	-0.072
logNAnt	0.178	0.115	-0.154
log KPost	0.045	-0.102	0.531
logKAnt	0.080	0.290	-0.192

S3 Table. Morphometry of *T. simiae* trypanosomes with a single nucleus in tsetse proboscis, including putative sexual stages.

The mean $\pm$ SE in μm is top line in each box with the range below. L = total cell length; W = maximum cell width; K1Post, K2Post, NPost = respectively the distances from the cell posterior to kinetoplast 1 or 2 (if present), or nucleus; K1Ant, K2Ant, NAnt, respectively the distances from the cell anterior to kinetoplast 1 or 2 (if present), or nucleus, were derived by subtracting K1Post, K2Post, NPost from the total cell length; K1Nuc, K2Nuc = distance between kinetoplast 1 or 2 (if present) and the nucleus; K1K2 = distance between kinetoplasts K1 and K2; NL, NW, NArea = nucleus length, width and area respectively; CellArea = area of cell body.

Morphotype	N	L	W	K1Post	K2Post	NPost	K1Ant	K2Ant	NAnt	K1Nuc	K2Nuc
Epimastigote 1K1N	315	28.64 $\pm$ 0.33 14.23-48.42	2.45 $\pm$ 0.03 1.15-4.22	11.93 $\pm$ 0.34 1.85-31.27	-	10.04 $\pm$ 0.33 0.97-28.46	16.71 $\pm$ 0.24 7.53-32.97	-	18.61 $\pm$ 0.23 8.50-33.80	-0.24 $\pm$ 0.02 -1.62-0.00	-
Dividing epimastigote 2K1N	36	28.00 $\pm$ 0.88 15.52-40.77	3.26 $\pm$ 0.08 2.18-4.22	11.40 $\pm$ 0.85 4.42-28.44	8.39 $\pm$ 0.87 3.20-26.46	6.20 $\pm$ 0.91 0.59-24.97	15.60 $\pm$ 0.68 9.44-26.42	19.60 $\pm$ 0.69 12.32-28.38	21.80 $\pm$ 0.73 14.27-29.08	2.83 $\pm$ 0.57 0.00-16.36	0.64 $\pm$ 0.19 0.51-6.10
Epimastigote-Trypomastigote 2K1N	18	28.69 $\pm$ 1.27 18.41-38.85	2.78 $\pm$ 0.09 1.95-3.41	12.52 $\pm$ 1.72 5.77-26.21	7.98 $\pm$ 1.68 1.27-21.90	8.61 $\pm$ 1.70 1.95-22.37	16.17 $\pm$ 1.17 6.56-23.63	20.71 $\pm$ 1.13 10.89-27.45	20.08 $\pm$ 1.21 10.15-27.55	-1.16 $\pm$ 0.23 - 3.95-0.00	0.51 $\pm$ 0.11 -0.36-1.54
Meiotic divider 2K1N	129	26.71 $\pm$ 0.25 21.03-33.88	3.50 $\pm$ 0.04 2.34-4.57	10.49 $\pm$ 0.18 5.01-15.76	6.72 $\pm$ 0.13 3.75-11.63	0.84 $\pm$ 0.11 0.00-8.75	16.22 $\pm$ 0.22 10.36-21.86	19.99 $\pm$ 0.22 13.73-25.91	25.87 $\pm$ 0.25 18.85-33.23	4.15 $\pm$ 0.13 0.19-4.15	0.77 $\pm$ 0.08 0.65-0.76
Meiotic intermediate 3K1N	13?										
Gametes total 1K1N and 2K1N	93	23.32 $\pm$ 3.75 13.50-31.59	2.00 $\pm$ 0.63 1.09-4.73	4.78 $\pm$ 2.30 0.73-16.01	-	2.60 $\pm$ 1.49 0.36-10.40	18.53 $\pm$ 3.42 6.45-27.43	-	20.72 $\pm$ 3.27 12.47-29.31	-0.70 $\pm$ 1.94 -13.02-0.00	-
Gamete 1K1N	81	23.16 $\pm$ 3.52 13.50-31.01	1.89 $\pm$ 0.48 1.09-3.29	4.53 $\pm$ 1.66 0.73-12.19	-	2.59 $\pm$ 1.46 0.69-10.40	18.63 $\pm$ 3.10 10.83-27.43	-	20.57 $\pm$ 3.10 12.47-29.31	-0.38 $\pm$ 0.64 -3.35-0.00	-
Gamete 2K1N	12	24.37 $\pm$ 5.09 16.86-31.59	2.73 $\pm$ 0.98 1.60-4.73	6.52 $\pm$ 4.53 1.82-16.01	2.47 $\pm$ 1.60 0.46-5.08	2.67 $\pm$ 1.69 0.36-5.00	17.86 $\pm$ 5.27 6.45-26.01	21.91 $\pm$ 4.29 16.04-29.96	21.71 $\pm$ 4.26 16.20-28.63	-2.91 $\pm$ 4.74 -13.02-0.00	0.00 $\pm$ 1.03 -2.68-1.63

S3 table contd.

Morphotype	N	K1K2	NL	NW	NArea	CellArea
Epimastigote 1K1N	315	-	2.21 ± 0.03 0.99-4.62	1.24 ± 0.03 0.69-8.94	2.41 ± 0.05 1.00-7.75	33.77 ± 0.50 13.83-64.45
Dividing epimastigote 2K1N	36	2.42±0.40 0.00-10.54	2.53±0.12 1.15-3.80	1.46±0.05 0.97-2.08	3.23±0.20 1.11-5.90	38.56±1.33 22.59-57.38
Epimastigote- Trypomastigote 2K1N	18	3.91 ±0.24 2.93-6.65	2.72 ± 0.13 1.85-3.60	1.55 ± 0.05 1.15-1.98	3.39 ± 0.18 2.35-5.11	39.77 ± 1.65 28.68-56.51
Meiotic divider 2K1N	129	3.32±0.08 1.15-5.41	5.28±0.10 2.62-8.50	2.10±0.03 1.15-3.09	7.31±0.13 3.56-11.94	40.62±0.53 28.23-58.94
Meiotic intermediate 3K1N						
Gametes total 1K1N and 2K1N	93	-	1.79 ± 0.55 0.87-3.25	0.99 ± 0.25 0.51-1.76	1.59 ± 0.61 0.66-3.66	22.93 ± 7.74 10.65-47.08
Gamete 1K1N	81	-	1.81 ± 0.51 0.87-3.25	0.96 ± 0.22 0.51-1.50	1.58 ± 0.57 0.71-3.66	22.22 ± 6.93 11.26-45.31
Gamete 2K1N	12	3.19 ± 3.41 0.59-11.01	1.60 ± 0.76 0.87-3.19	1.16 ± 0.33 0.65-1.76	1.60 ± 0.84 0.66-3.27	27.73 ± 11.06 10.65-47.08

S4 Table. Morphometry of *T. simiae* cells from a time course of development of proventricular trypanosomes *in vitro*.

At Time = 0 hours, *T. simiae* TV008 proventricular trypanosomes were put into wells of culture medium and sampled every 4 hours. The mean $\pm$ SE in μm is top line in each box with the range below. L = total cell length; W = maximum cell width; KPost, NPost = respectively the distances from the cell posterior to kinetoplast, or nucleus; KAnt, NAnt, respectively the distances from the cell anterior to kinetoplast or nucleus, were derived by subtracting K1Post, NPost from the total cell length; KNuc = distance between kinetoplast and the nucleus; NL, NW = nucleus length and width respectively. For *T. simiae* TV008 *PFR1::YFP*, the visible fluorescent paraflagellar rod (PFR) of the flagellum allowed three further measurements: FL, FPost, FAnt = respectively PFR length, distances between cell posterior and PFR posterior end, and distances between cell anterior and PFR anterior end.

Wildtype *T. simiae* TV008

Time		N	L	W	KPost	NPost	KAnt	NAnt	KNuc	NL	NW
0		35	34.43 $\pm$ 0.66 27.26-40.44	1.52 $\pm$ 0.04 1.15-1.95	7.35 $\pm$ 0.31 2.40-11.47	10.37 $\pm$ 0.34 6.62-14.38	27.08 $\pm$ 0.51 20.59-31.54	24.07 $\pm$ 0.45 18.30-28.06	2.19 $\pm$ 0.11 0.67-3.87	3.24 $\pm$ 0.08 2.54-4.47	1.18 $\pm$ 0.04 0.73-1.66
4		52	33.38 $\pm$ 0.59 25.49-42.39	2.67 $\pm$ 0.08 1.50-4.23	2.56 $\pm$ 0.23 0.51-6.71	5.18 $\pm$ 0.27 2.21-10.34	30.83 $\pm$ 0.53 23.45-40.08	28.20 $\pm$ 0.52 20.66-36.46	2.01 $\pm$ 0.11 0.67-3.87	3.00 $\pm$ 0.07 2.08-4.57	1.47 $\pm$ 0.04 0.95-2.14
8		43	32.21 $\pm$ 0.61 23.07-40.21	3.04 $\pm$ 0.07 2.24-4.11	1.34 $\pm$ 0.12 0.16-3.63	4.05 $\pm$ 0.12 2.76-5.84	30.88 $\pm$ 0.58 22.70-38.18	28.16 $\pm$ 0.56 20.31-35.95	2.04 $\pm$ 0.08 0.87-3.31	3.00 $\pm$ 0.07 1.95-4.12	1.48 $\pm$ 0.05 0.99-2.26
12		43	28.52 $\pm$ 0.64 19.44-35.87	2.96 $\pm$ 0.08 1.75-4.18	1.34 $\pm$ 0.15 0.00-4.52	3.73 $\pm$ 0.17 1.38-7.34	27.18 $\pm$ 0.59 18.79-33.66	24.78 $\pm$ 0.60 16.64-31.37	1.74 $\pm$ 0.11 0.59-3.83	2.93 $\pm$ 0.07 2.05-3.73	1.53 $\pm$ 0.04 0.92-2.21
16		42	28.98 $\pm$ 0.61 17.65-39.20	2.73 $\pm$ 0.08 1.95-4.39	1.88 $\pm$ 0.15 0.00-3.78	4.59 $\pm$ 0.17 2.53-8.30	27.09 $\pm$ 0.58 16.71-35.82	24.39 $\pm$ 0.54 14.69-31.22	1.99 $\pm$ 0.12 0.36-3.92	2.81 $\pm$ 0.13 1.73-6.85	1.48 $\pm$ 0.05 0.92-2.62
20		42	28.72 $\pm$ 0.58 22.53-35.72	2.97 $\pm$ 0.08 1.96-4.55	2.34 $\pm$ 0.11 0.81-3.82	4.50 $\pm$ 0.14 2.26-5.90	26.37 $\pm$ 0.56 19.91-33.76	24.22 $\pm$ 0.56 17.55-30.71	1.35 $\pm$ 0.12 0.00-3.75	3.07 $\pm$ 0.09 2.18-4.77	1.76 $\pm$ 0.06 1.27-2.89
24		44	27.61 $\pm$ 0.54 17.60-33.18	2.99 $\pm$ 0.06 2.18-3.90	2.22 $\pm$ 0.13 0.51-4.37	4.40 $\pm$ 0.16 2.62-7.44	25.39 $\pm$ 0.55 16.63-31.68	23.20 $\pm$ 0.52 14.83-29.22	1.57 $\pm$ 0.12 0.16-3.91	3.07 $\pm$ 0.09 1.62-4.14	1.54 $\pm$ 0.05 0.92-2.17

***T. simiae* TV008 PFR1::YFP**

Time	N	L	W	KPost	NPost	KAnt	NAnt	KNuc	NL	NW
0	65	44.38 ± 0.60 33.20-60.05	1.75 ± 0.03 1.30-2.53	11.89 ± 0.35 5.09-21.61	16.58 ± 0.37 6.66-25.77	32.48 ± 0.40 27.42-40.54	27.79 ± 0.41 21.65-36.16	3.98 ± 0.14 0.49-6.19	3.61 ± 0.08 2.34-5.12	1.16 ± 0.02 0.83-1.69
4	38	43.12 ± 0.95 34.63-53.74	2.03 ± 0.06 1.04-2.84	8.91 ± 0.63 1.31-16.43	12.59 ± 0.73 3.23-22.17	34.21 ± 0.55 25.14-41.22	30.54 ± 0.57 22.37-38.55	2.90 ± 0.17 0.36-5.46	3.79 ± 0.13 2.30-6.34	1.21 ± 0.03 0.92-1.85
8	26	37.72 ± 1.00 27.60-46.35	2.21 ± 0.06 1.62-3.07	5.63 ± 0.63 1.04-12.73	8.71 ± 0.67 4.41-16.51	32.09 ± 0.66 21.86-38.98	29.01 ± 0.66 19.07-34.28	2.54 ± 0.16 1.31-4.21	3.31 ± 0.09 2.48-4.19	1.24 ± 0.03 0.87-1.54
12	14	33.40 ± 1.27 23.57-31.34	2.01 ± 0.1 1.47-2.90	3.30 ± 0.49 0.67-7.15	5.99 ± 0.57 3.12-9.42	30.10 ± 1.46 17.98-40.68	27.42 ± 1.49 14.37-36.89	2.11 ± 0.26 0.83-2.11	3.22 ± 0.09 2.76-3.89	0.99 ± 0.05 0.73-1.40
24	14	35.09 ± 1.08 28.56-43.41	2.13 ± 0.10 1.67-3.13	4.20 ± 0.41 1.73-6.49	6.82 ± 0.49 3.06-8.74	30.89 ± 0.88 25.80-39.27	28.27 ± 0.84 23.95-35.44	2.02 ± 0.21 0.82-3.65	3.69 ± 0.15 2.48-4.65	1.35 ± 0.06 1.03-1.85
30	16	34.38 ± 1.34 27.99-45.06	2.13 ± 0.08 1.67-2.77	5.70 ± 0.36 3.65-9.19	8.07 ± 0.32 5.46-11.08	28.68 ± 1.21 21.84-38.02	26.31 ± 1.17 20.02-35.35	1.60 ± 0.22 0.00-3.65	3.31 ± 0.12 2.32-4.09	1.18 ± 0.05 0.81-1.54
36	4	33.56 ± 5.37 26.22-49.26	2.26 ± 0.37 1.67-3.29	5.61 ± 1.04 3.99-8.54	8.41 ± 1.07 6.47-10.98	27.95 ± 4.46 21.39-40.73	25.15 ± 4.41 19.76-38.28	2.17 ± 0.70 0.65-3.95	4.10 ± 0.32 3.33-4.84	1.21 ± 0.04 1.15-1.30

Time	N	FL	FPost	FAnt
0	65	30.25 ± 0.50 22.25-39.26	14.31 ± 0.35 7.16-23.96	1.84 ± 0.19 0.00-6.91
4	38	31.16 ± 0.57 22.26-38.57	11.19 ± 0.66 2.29-18.99	1.56 ± 0.30 0.00-10.74
8	26	29.45 ± 0.68 21.66-34.89	7.98 ± 0.62 3.15-15.26	1.48 ± 0.29 0.00-4.46
12	14	28.56 ± 1.60 13.74-40.64	5.06 ± 0.63 1.89-9.24	0.71 ± 0.26 0.00-2.82
24	14	29.16 ± 0.88 25.60-36.91	6.44 ± 0.50 3.09-8.94	1.82 ± 0.31 0.00-4.25
30	16	26.65 ± 1.12 22.19-36.40	8.09 ± 0.38 5.96-12.12	1.25 ± 0.41 0.00-4.86
36	4	27.31 ± 4.52 20.30-40.14	8.04 ± 0.98 5.91-10.47	0.83 ± 0.30 0.00-1.39