

Supplementary Table 1: Prevalence of Trypanosome single and multiple infection in tsetse samples analyzed per country

Region	Country	Prevalence of trypanosome species/subspecies and mixed infection (%)*										
		Tc	Tv	Tz	Tsg	TcTv	TcTz	TcTsg	TvTz	TvTsg	TzTsg	TcTvTz
East and south Africa	Ethiopia	6.10 ^{ab}	0.65 ^{ab}	1.09 ^a	10.46 ^{ab}	0 ^{ab}	0 ^{ab}	0.65 ^{ab}	0 ^{ab}	0.44 ^{ab}	0.65	0.00
	Kenya	13.00 ^b	5.65 ^{ac}	0.60 ^a	15.87 ^{ab}	2.08 ^b	0.50 ^a	6.05 ^b	0.20 ^a	0.10 ^b	0.40	0.00
	Democratic R. of Congo	0.00 ^{ab}	2.86 ^{ab}	0.00 ^a	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00	0.00
	Mozambique	3.00 ^a	1.00 ^{acd}	0.00 ^a	3.00 ^{ab}	0.00 ^a	0.00 ^{ab}	0.00 ^a	0.00 ^a	1.00 ^{ab}	0.00	0.00
	South Africa	3.99 ^a	0.57 ^{bc}	3.04 ^a	7.03 ^{ab}	0.19 ^a	0.00 ^a	0.38 ^a	0.00 ^a	0.00 ^b	0.00	0.00
	Eswatini	0.00 ^{ab}	0.00 ^{ab}	0.00 ^a	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^{ab}	0.00	0.00
	Tanzania	14.20^b	2.07 ^{ab}	1.18 ^a	10.95 ^{ab}	0.00 ^a	0.30 ^a	6.21 ^b	0.30 ^a	1.78 ^{ab}	0.89	0.00
	Uganda	2.86 ^b	0.95 ^{ab}	0.95 ^a	3.81 ^{ab}	0.00 ^a	0.00 ^a	0.48 ^a	0.00 ^a	0.00 ^{ab}	0.00	0.00
	Zambia	4.76 ^b	0.48 ^{abd}	0.95 ^a	30.95^{ab}	0.00 ^{ab}	0.00 ^{ab}	9.05^b	0.00 ^{ab}	0.00 ^{ab}	0.00	0.00
	Zimbabwe	7.11 ^b	0.47 ^{bc}	1.90 ^a	39.81 ^{ab}	0.00 ^a	0.00 ^a	4.27 ^a	0.00 ^a	0.00 ^b	0.00	0.00
	Subtotal (Average%)	8.38	2.43	1.25	14.13	0.70	0.19	3.71	0.10	0.32	0.32	0.00
West Africa	Burkina Faso	2.55 ^a	12.14 ^a	2.68 ^a	1.41 ^{ac}	0.26 ^a	0.53 ^a	0.04 ^a	0.70 ^a	1.19 ^a	0.26	0.09
	Ghana	1.71 ^a	14.10^a	19.66^b	0.00 ^{ac}	0.00 ^a	10.68^b	0.00 ^a	12.39^b	0.00 ^b	0.00	1.71
	Guinea	0.00 ^a	2.23 ^{bc}	0.00 ^a	0.00 ^{ac}	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^b	0.00	0.00
	Mali	0.27 ^a	5.49 ^{ac}	1.10 ^a	0.00 ^{ac}	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^b	0.00	0.00
	Senegal	0.55 ^a	8.96 ^{acd}	1.28 ^a	1.83 ^{ac}	0.00 ^a	0.00 ^a	0.00 ^a	1.65 ^a	0.00 ^b	0.00	0.00
		Subtotal (Average%)	1.77	10.37	3.16	1.13	0.16	0.99	0.03	1.45	0.72	0.16
	Total (Average%)	4.78	6.75	2.29	7.06	0.41	0.63	1.71	0.83	0.54	0.23	0.09

* Tc: *T. congolense* savannah, *T. congolense* Kilifi; *T. congolense* forest, Tsg: *T. simiae*; *T. simiae* Tsavo; *T. godfreyi*, Tv: *T. vivax*, Tz: *T. b. brucei*, *T. b. gambiense*, *T. b. rhodensiense*, *T. evansi*,

Supplementary Table 2: Prevalence of Trypanosome single and multiple infection in tsetse samples analyzed per tsetse species

Species	Prevalence of trypanosome species/subspecies and mixed infection (%)*										
	Tc	Tv	Tz	Tsg	TcTv	TcTz	TcTsg	TvTz	TvTsg	TzTsg	TcTvTz
<i>G. austeni</i>	4.91 ^a	0.87 ^a	2.60 ^a	7.23 ^a	0.29 ^a	0.29 ^a	0.58 ^a	0.00 ^a	0.00 ^a	0.00	0.00 ^a
<i>G. brevipalpis</i>	2.00 ^a	0.86 ^{ab}	2.29 ^a	4.29 ^{ab}	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.29 ^a	0.00	0.00 ^a
<i>G. f. fuscipes</i>	2.73 ^{ab}	4.37 ^{ab}	0.55 ^a	7.10 ^{ab}	1.09 ^{ab}	0.55 ^{ab}	0.55 ^a	0.00 ^{ab}	0.00 ^a	0.00	0.00 ^a
<i>G. medicorum</i>	9.09 ^{ab}	11.69 ^{ab}	13.64 ^{ab}	0.00 ^a	1.30 ^b	1.30 ^{ab}	0.00 ^a	0.65 ^{ab}	0.00 ^a	0.00	1.30 ^b
<i>G. m. morsitans</i>	9.76 ^{ab}	0.54 ^{ac}	1.08 ^{ac}	22.76 ^{ac}	0.00 ^{ad}	0.00 ^a	5.15 ^a	0.00 ^a	1.36 ^a	0.27	0.00 ^a
<i>G.m. submorsitans</i>	5.25 ^{abc}	11.37 ^{ab}	0.58 ^{ac}	0.00 ^{ad}	0.00 ^a	0.87 ^{ab}	0.00 ^a	0.00 ^{ab}	0.00 ^a	0.00	0.00 ^a
<i>G. pallidipes</i>	10.68 ^b	3.20 ^a	0.87 ^{ac}	16.54 ^b	1.03 ^{ab}	0.27 ^a	5.10 ^b	0.16 ^a	0.22 ^a	0.49	0.00 ^a
<i>G. p. gambiensis</i>	0.46 ^{ac}	8.86 ^b	1.89 ^{ac}	1.94 ^{ac}	0.00 ^{ac}	0.09 ^a	0.05 ^a	1.06 ^a	1.20 ^{ab}	0.28	0.00 ^{ac}
<i>G. p. palpalis</i>	0.00 ^{abc}	2.86 ^{ab}	0.00 ^{abc}	0.00 ^{abc}	0.00 ^{abcd}	0.00 ^{ab}	0.00 ^{ab}	0.00 ^a	0.00 ^a	0.00	0.00 ^a
<i>G. tachinoides</i>	2.25 ^{ac}	12.92 ^{bc}	4.96 ^b	0.00 ^{ac}	0.37 ^{ac}	2.81 ^b	0.00 ^a	2.81 ^b	0.00 ^{ac}	0.00	0.37 ^{ad}
Total (Average%)	4.78	6.75	2.29	7.06	0.41	0.63	1.71	0.83	0.54	0.23	0.09

* Tc: *T. congolense* savannah, *T. congolense* Kilifi; *T. congolense* forest, Tsg: *T. simiae*; *T. simiae* Tsavo; *T. godfreyi*, Tv: *T. vivax*, Tz: *T. b. brucei*, *T. b. gambiense*, *T. b. rhodensiense*, *T. evansi*,

Supplementary Table 3: Prevalence of Trypanosome single and multiple infection in tsetse samples analyzed per tsetse species and per country

Species	Country	Prevalence of trypanosome species/subspecies and mixed infection (%)*										
		Tc	Tv	Tz	Tsg	TcTv	TcTz	TcTsg	TvTz	TvTsg	TzTsg	TcTvTz
<i>G. austeni</i>	Mozambique	6.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	South Africa	6.19	0.44	3.54	10.18	0.44	0.00	0.88	0.00	0.00	0.00	0.00
	Eswatini	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Tanzania	0.00	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>G. brevipalpis</i>	Mozambique	0.00	2.00	0.00 ^a	2.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00
	South Africa	2.33	0.67	2.67 ^b	4.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>G. f. fuscipes</i>	Kenya	2.25	6.74	0.00 ^a	11.24	2.25	1.12	0.00 ^a	0.00	0.00	0.00	0.00
	Uganda	3.19	2.13	1.06 ^b	3.19	0.00	0.00	1.06 ^b	0.00	0.00	0.00	0.00
<i>G. medicorum</i>	Burkina Faso	9.09	11.69	13.64^a	0.00	1.30	1.30	0.00 ^a	0.65	0.00	0.00	1.30
<i>G. m. morsitans</i>	Kenya	2.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ^a	0.00	0.00
	Tanzania	18.52	1.23	1.23	18.52	0.00	0.00	6.17	0.00	6.17^b	1.23 ^b	0.00
	Zambia	12.5	0.00	1.56	21.88	0.00	0.00	12.5	0.00	0.00 ^a	0.00 ^a	0.00
	Zimbabwe	7.91	0.72	1.44	39.57	0.00	0.00	4.32	0.00	0.00 ^a	0.00 ^a	0.00
<i>G.m. submorsitans</i>	Burkina Faso	5.25	11.37	0.58	0.00	0.00	0.87	0.00	0.00	0.00	0.00	0.00
<i>G. pallidipes</i>	Ethiopia	6.10	0.65 ^a	1.09	10.46	0.00	0.00	0.65	0.00 ^a	0.44	0.65	0.00
	Kenya	15.23	6.12 ^{ab}	0.72	17.99	2.28	0.48	7.31	0.24 ^a	0.12	0.48	0.00
	Tanzania	15.21	1.84 ^a	0.46	10.14	0.00	0.46	7.37	0.46 ^{ab}	0.46	0.92	0.00
	Uganda	2.59	0.00 ^{ac}	0.86	4.31	0.00	0.00	0.00	0.00 ^{ac}	0.00	0.00	0.00
	Zambia	1.37	0.68 ^a	0.68	34.93	0.00	0.00	7.53	0.00 ^a	0.00	0.00	0.00
	Zimbabwe	5.56	0.00 ^{ac}	2.78	40.28	0.00	0.00	4.17	0.00 ^{ab}	0.00	0.00	0.00
<i>G.p. palpalis</i>	Democratic R. of Congo	0.00	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table 3 cont.: Prevalence of Trypanosome single and multiple infection in tsetse samples analyzed per tsetse species and per country

Species	Country	Prevalence of trypanosome species/subspecies and mixed infection (%)*										
		Tc	Tv	Tz	Tsg	TcTv	TcTz	TcTsg	TvTz	TvTsg	TzTsg	TcTvTz
<i>G. p. gambiensis</i>	Burkina Faso	0.64	12.09 ^a	3.50	3.39 ^a	0.00	0.21	0.11	1.48	2.86 ^a	0.64	0.00
	Guinea	0.00	2.23 ^b	0.00	0.00 ^b	0.00	0.00	0.00	0.00	0.00 ^b	0.00	0.00
	Mali	0.27	5.49 ^{ab}	1.10	0.00 ^b	0.00	0.00	0.00	0.00	0.00 ^b	0.00	0.00
	Senegal	0.55	8.96 ^a	1.28	1.83 ^b	0.00	0.00	0.00	1.65	0.00 ^b	0.00	0.00
<i>G. tachinoides</i>	Burkina Faso	2.40 ^a	12.59	0.60	0.00	0.48 ^a	0.60	0.00	0.12	0.00	0.00	0.00
	Ghana	1.71 ^b	14.10	19.66	0.00	0.00 ^b	10.68	0.00	12.39	0.00	0.00	1.71
Total (Average%)		4.78	6.75	2.29	7.06	0.41	0.63	1.71	0.83	0.54	0.23	0.09

* Tc: *T. congolense* savannah, *T. congolense* Kilifi; *T. congolense* forest, Tsg: *T. simiae*; *T. simiae* Tsavo; *T. godfreyi*, Tv: *T. vivax*, Tz: *T. b. brucei*, *T. b. gambiense*, *T. b. rhodensiense*, *T. evansi*,

Supplementary Table 5. List of Primers used for PCR and quantitative PCR (qPCR) analyses of microbiome in *Glossina* species

Target Gene	Primer Name	Primer Sequence (Listed 5'- to -3')	Annealing Temperature (°C)	Amplicon Size (bp)	References
fliC (flagellin) (Sodalis)	sod-FliCF	GCA GTT TCA GGA TAC CC	52.5	508	1
	sod-FliCR	GGC GGA AAA TGG TAT AG			
GpCAG133	GpCAG133-F	ATT TTT GCG TCA ACG TGA	52.5	185-205	2
	GpCAG133-R	ATG AGG ATG TTG TCC AGT TT			
fliC (flagellin) (Sodalis)	sodqPCR-FliCF	GAA GCC ACC GAT CCT GTA AC	55	508	3
	sodqPCR-FliCR	CAT CTT TGC CCG TAG AAA TCA C			
ITS 1	ITS 1-CF	CCG GAA GTT CAC CGA TAT TG	60	250-710	4
	ITS 1-BR	TTG CTG CGT TCT TCA ACG AA			
18S rDNA Trypanosomatidae	18S Typ F	CGCCAAGCTAATACATGAACCAA	60	106	5
	18S Tryp R	TAATTTTCATTCATTCGCTGGACG			

Reference list

- Toh, H. *et al.* Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Res.* **16**, 149–156 (2006).
- Baker, M. D. & Krafur, E. S. Identification and properties of microsatellite markers in tsetse flies *Glossina morsitans sensu lato* (Diptera: Glossinidae). *Mol. Ecol. Notes* **1**, 234–236 (2001).
- Weiss, B. L., Maltz, M. & Aksoy, S. Obligate symbionts activate immune system development in the tsetse fly. *J Immunol* **188**, 3395–3403 (2012).
- Njiru, Z. K. *et al.* The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitol. Res.* **95**, 186–192 (2005).
- Deborggraeve, S. *et al.* Molecular dipstick test for diagnosis of sleeping sickness. *J. Clin. Microbiol.* **44**, 2884–2889 (2006).