Prevalence of *Trypanosoma* and *Sodalis* in wild populations of tsetse flies and their impact on sterile insect technique programmes for tsetse eradication

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The sterile insect technique (SIT) is an environment friendly and sustainable method to manage insect pests of economic importance through successive releases of sterile irradiated males of the targeted species to a defined area. A mating of a sterile male with a virgin wild female will result in no offspring, and ultimately lead to the suppression or eradication of the targeted population. Tsetse flies, vectors of African *Trypanosoma*, have a highly regulated and defined microbial fauna composed of three bacterial symbionts that may have a role to play in the establishment of *Trypanosoma* infections in the flies and hence, may influence the vectorial competence of the released sterile males. *Sodalis* bacteria seem to interact with *Trypanosoma* infection in tsetse flies. Field-caught tsetse flies of ten different taxa and from 15 countries were screened using PCR to detect the presence of *Sodalis* and *Trypanosoma* species and analyse their interaction. The results indicate that the prevalence of *Sodalis* and *Trypanosoma* varied with country and tsetse species. Trypanosome prevalence was higher in east, central and southern African countries than in west African countries. Tsetse fly infection rates with *Trypanosoma vivax* and *T. brucei* spp were higher in west African countries, whereas tsetse infection with *T. congolense* and *T. simiae* (tsavo) and *T. godfreyi* were higher in east, central and south African countries. *Sodalis* prevalence was high in *Glossina morsitans morsitans* and *G. pallidipes* but absent in *G. tachinoides*. Double and triple infections with *Trypanosoma* taxa and coinfection of *Sodalis* and *Trypanosoma* were rarely observed but it occurs in some taxa and locations. A significant Chi square value (< 0.05) seems to suggest that *Sodalis* and *Trypanosoma* infection correlate in *G. palpalis gambiensis*, *G. pallidipes* and *G. medicorum*. *Trypanosoma* infection seemed significantly associated with an increased density of *Sodalis* in wild *G. m. morsitans* and *G. pallidipes* flies, however, there was no significant impact of *Sodalis* infection on trypanosome density.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| SIT          | Sterile insect techniques |
| qPCR         | Quantitative polymerase chain reaction |
| BKF          | Burkina Faso |
| ETH          | Ethiopia |
| GHA          | Ghana |
| GUI          | Guinea |
| KEN          | Kenya |
| MAL          | Mali |
| MOZ          | Mozambique |
| SAF          | South Africa |
| SWA          | Swaziland |
| ZAI          | Democratic Republic of the Congo |
| ZAM          | Zambia |
| ZIM          | Zimbabwe |
| Ga           | Glossina austeni |
| Gb           | G. brevipalpis |
| Giff         | G. fusipes fusipes |
| Gmm          | G. morsitans morsitans |
| Gmsm         | G. m. submorsitans |
| Gpp          | G. palpalis gambiensis |
| Gpp          | G. p. palpalis |
| Tc           | Trypanosoma congolense |
| Tv           | Trypanosoma vivax |
| Tz           | T. brucei spp. |

Tsetse flies (Diptera: Glossinidae) are distributed in sub-Saharan Africa between 15° north and 26° south latitude. Glossina spp. are the cyclic vectors of unicellular protozoa of the genus Trypanosoma that cause African animal trypanosomosis (AAT) or nagana and human African trypanosomosis (HAT) or sleeping sickness. Nagana in cattle is mainly caused by T. congolense, T. vivax and T. brucei brucei and causes annual losses to agriculture estimated at $4.75 billion. In addition, around 35 million doses of trypanocidal drugs are administered to livestock per year for managing AAT. Human African trypanosomosis is fatal without treatment and is caused by two Trypanosoma subspecies, i.e. T. brucei rhodesiense responsible for the acute form of HAT in East Africa and T. b. gambiensis for the chronic form of HAT in western and central Africa. The lack of effective vaccines and the development of resistance to the available trypanocidal drugs makes the control of AAT in the vertebrate host unsustainable. Consequently, an effective tool to reduce Trypanosoma transmission would be the control of the tsetse vector. One effective method to manage populations of tsetse flies is the sterile insect technique (SIT) when used as part of an area-wide integrated pest management (AW-IPM) approach. The SIT method relies on the mass-production and sterilization of male flies by ionizing radiation. The sterile males are released in the target area for mating with wild females and the absence of offspring will gradually reduce the density of the targeted tsetse populations.

The biological transmission of the Trypanosoma species requires the parasite to undergo a series of proliferation and differentiation steps in the tsetse alimentary tract and finally mature into an infective form in the mouthparts (T. congolense) or salivary glands (T. brucei spp.). However, tsetse flies are refractory to Trypanosoma infection meaning that the probability that Trypanosoma ingested during a blood meal complete their developmental cycle in the fly to result in a mature infection is rather low. The endogenous bacterial microbiome seems important in providing tsetse flies the natural ability to mitigate Trypanosoma infections. Three major endosymbiotic bacteria have been identified in tsetse flies, i.e. Wigglesworthia glossinidius, Sodalis glossinidius (hereafter mentioned as Sodalis) and Wollbachia pipiens. Some studies suggested that the obligate mutualist Wigglesworthia must be present in the larval stage during the development of a mature tsetse fly to properly develop a well-functioning immune system contributing to a refractory phenotype against Trypanosoma.

Sodalis, the second mutualistic symbiont, can be found in the midgut, hemolymph, muscles, fat body, milk glands, and salivary glands of certain tsetse species and is inherited by the progeny through transovarial transmission. The biological role/importance of Sodalis for tsetse remain unclear and needs to be clarified. This symbiont might provide some benefits to the host as flies without Sodalis have a significantly shorter lifespan compared with flies with it, however the establishment of a Sodalis free colony was feasible. Sodalis also presents many ideal characteristics to be used for expressing molecular effectors in paratransgenic tsetse. In addition, previous work suggested that Sodalis may modulate the ability of Trypanosoma to establish an infection in the tsetse midgut as some studies reported that the elimination of this bacterial endosymbiont results in an increased tsetse fly refractoriness to Trypanosoma infection. Moreover, Geiger et al. suggested that specific genotypes of Sodalis presents in G. p. gambiensis from insectary colonies facilitate Trypanosoma infection. Soumana et al. revealed that a variation in the Sodalis population caused by a hosted prophage can influence the trypanosome infections. In contrast, a recent study demonstrated that the absence or presence of S. glossinidius in the tsetse fly does not affect the fly’s susceptibility toward Trypanosoma infection. In conclusion, from the above-described results, it is clear that our knowledge on the impact of Sodalis on Trypanosoma infection in tsetse remains limited and fragmented and is still under debate. Moreover, exploring on a large scale the occurrence and possible association between Sodalis and Trypanosoma infection in wild flies is highly required. The above described potential impact of Sodalis to facilitate Trypanosoma infection in tsetse, and the fact that Sodalis is
found in all laboratory-reared tsetse colonies and some wild populations indicates that mitigating action, such as feeding the flies 2–3 times on blood supplemented with trypanocidal drugs before release, is required in SIT programs to minimize the risk of disease transmission by the large number of released males that harbour Sodalis. Field studies in two HAT foci in Cameroon used PCR to detect Trypanosoma and Sodalis in G. palpalis palpalis and the results indicate that the presence of Sodalis favours Trypanosoma infections especially by T. brucei s.l. Furthermore, in the wildlife-livestock-human interface in the Maasai Mara National Reserve in Kenya, it was shown that G. pallidipes infected with Sodalis was associated with increased Trypanosoma infection rates. However, other studies have found no strong association between trypanosome and Sodalis in some tsetse species collected in four locations in Kenya. Channumsoin et al. suggested that the association between Trypanosoma infection and the presence of Sodalis will vary depending on tsetse and Trypanosoma species. Similarly, studies carried out in the Fontem focus in Cameroon did not find a relationship between the endosymbiont and the parasite in G. p. palpalis, and no significant Sodalis-Trypanosoma infection association was found in G. tachinoides in two sites of the Faro and Déo Division in Adamawa region of Cameroon. Likewise, no association between the presence of the parasite and Sodalis was found in G. brevipalpis, G. m. morsitans and G. pallidipes in the Luambe National Park of Zambia.

The overall objective of this study was to evaluate the prevalence of Sodalis and Trypanosoma in wild tsetse populations at a continental scale, i.e. Burkina Faso, Democratic Republic of Congo (DRC), Eswatini, Ethiopia, Ghana, Guinea, Kenya, Mali, Mozambique, Senegal, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe and analyse these data in the context of a possible association between the occurrence of Sodalis and Trypanosoma infection in tsetse. Such information might guide the decision maker for SIT programmes to take the appropriate action, if necessary, to minimize any potential risk of increased transmission.

**Results**

**Trypanosoma prevalence.** Adult tsetse flies (n = 6860) were screened for infection with T. brucei ssp (Tz) (T. b. brucei, T. b. gambienses, T. b. rhodesiense), Tc (T. congolense savannah; T. congolense kilifi; T. congolense forest), Tsg (T. simiae; T. simiae tsavo; T. godfreyi) and Tv (T. vivax). The results indicate that 1736 (25.3%) adults were infected with one or more Trypanosoma taxa (Tables 1, 2 and 3). The Trypanosoma prevalence varied significantly between tsetse taxa (X² = 750.18, df = 9, P << 0.001) and between countries (X² = 2038.1, df = 14, P << 0.001). The Permanova analysis indicated as well significant differences between countries (P = 0.009) and taxa (P = 0.041) (Table 4). As all taxa were not collected from all countries, the interaction between taxa and countries was only analyzed where a taxon was collected from several countries.

Regardless of tsetse taxon, in west African countries the average Trypanosoma prevalence was 20% (n = 3733), with the highest prevalence recorded in Ghana (61%) and the lowest recorded in Guinea (2.2%). The prevalence in Burkina Faso, Mali and Senegal was 21.9, 6.9 and 14.2% respectively (Fig. 1, and Table 1). In east, central and southern African countries, the Trypanosoma infection prevalence was a bit higher than in west African countries with an averaged infection of 31.5% (n = 3127), with the highest prevalence (53.6%) in Zimbabwe and lowest prevalence (2.9%) in DRC. No Trypanosoma infection was detected in Eswatini (Fig. 1 and Table 1). Regardless of the country, Trypanosoma prevalence varied from one taxon to another, and G. m. morsitans showed the highest Trypanosoma prevalence (41%) followed by G. pallidipes (38.5%) and the lowest prevalence was detected

| Region                        | Country          | Sodalis prevalence (%) | Trypanosome prevalence (%) |
|-------------------------------|------------------|-------------------------|-----------------------------|
| East, central and southern Africa | Ethiopia        | 94/459 (20.48)ªªª       | 92/459 (20.04)ªªª            |
|                               | Kenya            | 288/1008 (28.57)ªª      | 448/1008 (44.44)ªªª          |
|                               | Democratic R. of Congo | 4/35 (11.43)ªª        | 1/35 (2.86)ªª               |
|                               | Mozambique       | 7/100 (7.00)ªªªª        | 80/526 (15.21)ªª            |
|                               | South Africa     | 9/526 (1.71)ªªªª        | 0/50 (0.00)ªª               |
|                               | Eswatini         | 0/30 (0.00)ªªªª         | 8/100 (8.00)ªª              |
|                               | Tanzania         | 227/338 (67.16)ªªªª     | 128/338 (37.87)ªª           |
|                               | Uganda           | 91/210 (43.33)ªª        | 19/210 (9.05)ª              |
|                               | Zambia           | 11/210 (5.24)ªª        | 97/210 (46.19)ªª            |
|                               | Zimbabwe         | 39/211 (18.48)ªªªª     | 113/211 (53.55)ªª           |
| West Africa                   | Burkina Faso     | 11/2274 (0.48)ªª       | 498/2274 (21.90)ªª          |
|                               | Ghana            | 0/234 (0.00)ªªªª        | 143/234 (61.11)ªª           |
|                               | Guinea           | 90/314 (28.66)ªªªª     | 77/314 (22.22)ªª            |
|                               | Mali             | 0/364 (0.00)ªªªª        | 25/364 (6.86)ªª             |
|                               | Senegal          | 0/547 (0.00)ªªªª       | 78/547 (14.25)ªª            |
|                               | Subtotal         | 770/3127 (24.62)       | 986/3127 (31.53)            |
|                               | Burkina Faso     | 1/112 (0.09)ªª         | 84/112 (75.40)ª              |
|                               | Guinea           | 0/90 (0.00)ªªªª        | 7/90 (7.78)ªª               |
|                               | Mali             | 0/234 (0.00)ªªªª        | 14/234 (6.01)ªª              |
|                               | Senegal          | 0/111 (0.00)ªªªª       | 97/111 (87.36)ªª             |
|                               | Subtotal         | 101/3733 (2.70)        | 750/3733 (20.09)             |
|                               | Total (average)  | 87/6860 (12.69)        | 1736/6860 (25.30)            |

Table 1. Global prevalence of Sodalis and Trypanosomes in tsetse samples analyzed per country. *Values indicated by the same lower-case letter do not differ significantly at the 5% level.

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in *G. brevipalpis* (9.71%) in east, central and southern Africa. In west Africa, *G. medicorum* showed the highest *Trypanosoma* prevalence (39.5%) and the lowest prevalence was detected in *G. p. palpalis* (2.8%) (Table 2).

Some tsetse taxa were collected from several countries as presented in Fig. 2 and Table 3. The highest *Trypanosoma* prevalence was recorded in *G. tachinoides* in Ghana (61%). This was followed by high prevalence in *G. m. morsitans* collected from Zimbabwe (53.9%), Tanzania (53%) and Zambia (48.4%). *G. pallidipes* from Zimbabwe, Kenya, Zambia and Tanzania also showed high *Trypanosoma* prevalence of 52.7%, 50.9%, 45.2% and 37.3%, respectively. The lowest *Trypanosoma* prevalence was found in *G. p. gambiensis* from Guinea (2.2%). Based on the *Trypanosoma* prevalence presented in Fig. 2 and Table 3, the tested samples can be categorized as: (i) tsetse samples with high prevalence (> 35%) detected in *G. tachinoides* from Ghana; *G. medicorum* from Burkina Faso, *G. m. morsitans* from Kenya, Tanzania and Zambia; *G. p. palpalis* from Democratic Republic of Congo; and *G. tachinoides* from Ghana; (ii) tsetse samples with moderate prevalence (35% - 25%) detected in *G. austeni* from Mozambique, South Africa, Eswatini and Tanzania; *G. brevipalpis* from Mozambique and South Africa; *G. f. fuscipes* from Kenya, Uganda; *G. m. morsitans* from Kenya, Tanzania, Zambia and Zimbabwe; *G. m. submorsitans* from Burkina Faso and *G. pallidipes* from Ethiopia; (iii) tsetse samples with low prevalence (< 25%) detected in *G. austeni* from South Africa, Eswatini; *G. brevipalpis* from South Africa; *G. f. fuscipes* from Uganda; *G. medicorum* from Burkina Faso; *G. m. morsitans* from Zambia; *G. m. submorsitans* from Burkina Faso; *G. pallidipes* from Kenya, Uganda; *G. p. palpalis* from Democratic Republic of Congo; (iv) tsetse samples with low prevalence (< 10%) detected in *G. austeni* from Mozambique, Eswatini; *G. brevipalpis* from South Africa, Mozambique; *G. f. fuscipes* from Uganda; *G. m. morsitans* from Tanzania; *G. m. submorsitans* from Burkina Faso; *G. pallidipes* from Ethiopia; *G. p. palpalis* from Democratic Republic of Congo; (v) tsetse samples with very low prevalence (< 5%) detected in *G. austeni* from Mozambique, South Africa, Eswatini; *G. brevipalpis* from South Africa; *G. f. fuscipes* from Uganda; *G. m. morsitans* from Kenya, Tanzania, Zambia; *G. m. submorsitans* from Burkina Faso; *G. pallidipes* from Ethiopia; *G. p. palpalis* from Democratic Republic of Congo; (vi) tsetse samples with low prevalence (< 1%) detected in *G. austeni* from Mozambique, South Africa, Eswatini; *G. brevipalpis* from South Africa; *G. f. fuscipes* from Uganda; *G. m. morsitans* from Kenya, Tanzania, Zambia; *G. m. submorsitans* from Burkina Faso; *G. pallidipes* from Ethiopia; *G. p. palpalis* from Democratic Republic of Congo; (vii) tsetse samples with very low prevalence (< 0.1%) detected in *G. austeni* from Mozambique, South Africa, Eswatini; *G. brevipalpis* from South Africa; *G. f. fuscipes* from Uganda; *G. m. morsitans* from Kenya, Tanzania, Zambia; *G. m. submorsitans* from Burkina Faso; *G. pallidipes* from Ethiopia; *G. p. palpalis* from Democratic Republic of Congo.

### Table 2. Global prevalence of *Sodalis* and *Trypanosoma* in tsetse samples analyzed per tsetse species. *Values indicated by the same lower-case letter do not differ significantly at the 5% level.*

| Species       | Sodalis prevalence (%) | Trypanosome prevalence (%) |
|---------------|------------------------|----------------------------|
| *G. austeni*  | 5/346 (1.44)           | 58/346 (16.76)             |
| *G. brevipalpis* | 14/350 (4)          | 34/350 (9.71)              |
| *G. f. fuscipes* | 24/183 (13.11)       | 31/183 (16.93)             |
| *G. medicorum* | 8/154 (5.2)          | 61/154 (39.6)              |
| *G. m. morsitans* | 156/369 (42.27)     | 152/369 (41.19)            |
| *G. m. submorsitans* | 1/343 (0.29)      | 62/343 (18.07)             |
| *G. pallidipes* | 567/1844 (30.74)     | 711/1844 (38.55)           |
| *G. p. gambiensis* | 92/2168 (4.24)    | 343/2168 (15.82)           |
| *G. p. palpalis* | 4/35 (11.4)          | 1/35 (2.8)                 |
| *G. tachinoides* | 0/1068 (0.0)        | 283/1068 (26.49)           |
| Total (average) | 871/6860 (12.6)     | 1736/6860 (25.3)           |

### Table 3. Global prevalence of *Sodalis* and trypanosomes in tsetse samples analyzed per country and tsetse species. *Values indicated by the same lower-case letter do not differ significantly at the 5% level.*

| Species       | Country          | Sodalis prevalence (%) | Trypanosome prevalence (%) |
|---------------|------------------|------------------------|----------------------------|
| *G. austeni*  | Mozambique       | 0/50 (0.00)            | 5/50 (10.00)               |
|               | South Africa     | 2/226 (0.88)           | 49/226 (21.68)             |
|               | Eswatini         | 0/30 (0.00)            | 0/30 (0.00)                |
|               | Tanzania         | 3/40 (7.50)            | 4/40 (10.00)               |
| *G. brevipalpis* | Mozambique    | 7/50 (14.00)           | 3/50 (6.00)                |
|               | South Africa     | 7/300 (2.33)           | 31/300 (10.33)             |
| *G. f. fuscipes* | Kenya          | 20/89 (22.47)          | 21/89 (23.60)              |
|               | Uganda           | 4/94 (4.25)            | 10/94 (10.63)              |
| *G. medicorum* | Burkina Faso    | 8/154 (5.20)           | 61/154 (39.61)             |
| *G. m. morsitans* | Kenya        | 54/85 (63.52)          | 2/85 (2.35)                |
|               | Tanzania         | 62/81 (76.54)          | 43/81 (53.08)              |
|               | Zambia           | 8/64 (12.50)           | 31/64 (48.43)              |
|               | Zimbabwe         | 32/139 (23.02)         | 75/139 (53.95)             |
| *G. m. submorsitans* | Burkina     | 1/343 (0.30)           | 62/343 (18.07)             |
| *G. pallidipes* | Ethiopia        | 94/459 (20.48)         | 92/459 (20.04)             |
|               | Kenya            | 214/834 (25.65)        | 425/834 (50.95)            |
|               | Tanzania         | 162/217 (74.65)        | 81/217 (37.32)             |
|               | Uganda           | 87/116 (75.00)         | 9/116 (7.75)               |
|               | Zimbabwe         | 7/72 (9.72)            | 38/72 (52.77)              |
|               | Zambia           | 3/146 (2.05)           | 66/146 (45.20)             |
| *G. p. palpalis* | Democratic R. of Congo | 4/35 (11.42) | 1/35 (2.86)              |
| *G. p. gambiensis* | Burkina Faso   | 2/943 (0.21)          | 235/943 (24.92)            |
|               | Guinea           | 96/314 (28.66)         | 7/314 (2.22)               |
|               | Mali             | 0/364 (0.00)           | 25/364 (6.87)              |
|               | Senegal          | 0/547 (0.00)           | 78/547 (14.25)             |
| *G. tachinoides* | Burkina Faso  | 0/834 (0.00)           | 140/834 (16.79)            |
|               | Ghana            | 0/234 (0.00)           | 143/234 (61.11)            |
| Total (average) | 871/6860 (12.6) | 1736/6860 (25.30)      |
Table 4. Permanova analysis for Countries and tsetse species for *Sodalis* and trypanosome (single and multiple) infection prevalence. Within the table, statistically significant differences (*P* < 0.05) can be seen in bold values in countries and tsetse species. Perm(s) = permutations.

| Source      | df | SS    | MS    | Pseudo-F | P (perm) | Unique perms |
|-------------|----|-------|-------|----------|----------|--------------|
| Countries   | 11 | 13,040| 1185.4| 2.6004   | 0.009    | 998          |
| Species     | 7  | 7899.8| 1128.5| 2.4756   | 0.041    | 999          |
| Residuals   | 5  | 2279.3| 455.87|          |          |              |
| Total       | 25 | 34,074|       |          |          |              |

Figure 1. The geographical locations of tsetse samples in Africa. Circles indicate the total prevalence of *Sodalis* and *Trypanosoma* per country. Black dots indicate samples collection site(s) per country.

*G. pallidipes* from Kenya, Zambia, and Zimbabwe, *G. m. morsitans* from Tanzania, Zambia, and Zimbabwe; (ii) tsetse samples with medium prevalence (10–35%) detected in *G. austeni* from South Africa, *G. f. fuscipes* from Kenya and Uganda, *G. m. submorsitans* from Burkina Faso, *G. p. gambiensis* from Burkina Faso and Senegal and *G. tachinoides* from Burkina Faso; (iii) tsetse samples with low prevalence (< 10%) detected in the rest of the samples listed in Table 3 except the *G. austeni* collected from Eswatini. Despite the difference in *Trypanosoma*
Figure 2. Prevalence of the *Sodalis* and *Trypanosoma* (single and multiple) infections per country (A) and tsetse species (B). Prevalence data were square root transformed and averaged based on country-species and the matrix display was conducted in PRIMER version 7+ software. Tree on the left of the matrix is the similarity dendrogram based on the similarity index of the square root of the prevalence values. The colour index is the square root of the prevalence values ranged 0–9 which is the square root of 0–81% prevalence. Country abbreviations follow the UNDP list of country codes https://web.archive.org/web/20060713221355/http://refgat.undp.org/genericlist.cfm?entid=82&pagenumber=1&requesttimeout=360 as follows: BKF: Burkina Faso; ETH: Ethiopia; GHA: Ghana; GUI: Guinea; KEN: Kenya; MLI: Mali; MOZ: Mozambique; SAF: South Africa; SWA: Eswatini; ZAI: Democratic Republic of the Congo; ZAM: Zambia; ZIM: Zimbabwe. Tsetse, *Sodalis* and *Trypanosoma* taxa were abbreviated as following: Ga: Glossina austeni; Gb: *G. brevipalpis*; Gff: *G. fuscipes fuscipes*, Gm: *G. morsitans morsitans*; Gmsm: *G. m. submorsitans*; Gpg: *G. palpalis gambiensis*; Gpp: *G. palpalis palpalis*. Sod: *Sodalis*, Tc: *Trypanosoma. conglobense savannah*; T. conglobense kilifi*; T. conglobense forest, Tsg: T. simiae; T. simiae Tsavo; T. godfreyi, Tv: T. vivax, Tz: T. brucei brucei, T. b. gambiens, T. b. rhodesiense.
prevalence for each tsetse species, the differences were significant only in G. p. gambiensis ($X^2 = 26.71, df = 4, P < 0.001$) and G. tachinoides, ($X^2 = 9.38, df = 1, 2, P = 0.002$). In contrast, no significant difference was detected between countries for G. austeni ($X^2 = 1.47, df = 4, P = 0.688$), G. brevipalpis ($X^2 = 0.34, df = 2, P = 0.559$), G. f. fusipes ($X^2 = 0.15, df = 2, P = 0.702$) and G. m. morsitans ($X^2 = 1.04, df = 3, P = 0.593$) and G. pallidipes ($X^2 = 4.983, df = 1,6, P = 0.418$) (Table 3). No Trypanosoma infection was recorded in G. austeni from Eswatini. The best glm model (lowest AICc) selected for the overall Trypanosoma prevalence retained the countries as variables that fitted the data well (AICc = 1521.35) (Supplementary File 1).

**Prevalence of different Trypanosoma taxa and mixed infections.** The above-mentioned prevalence of Trypanosoma infection was comprised of several different Trypanosoma species and sub-species. Based on the size of the amplified fragment by PCR, the Trypanosoma infection was categorized into four groups: (i) the Tc group including the different forms of T. congolense; (ii) Tv group including T. vivax infections; (iii) T. brucei ssp (Tz) group including T. b. brucei, T. b. gambiensis, T. b. rhodesiense, and infections; and (iv) Tsg group including the infections with T. simiae, T. simiae tsavo and T. godfreyi. The screening results revealed that tsetse flies could be infected with single or multiple (double or triple) taxa of Trypanosoma, and the proportion of the infections with the different Trypanosoma taxa and the mixed infection varied with country ($X^2 = 63.56, df = 14, P < 0.001$) and species ($X^2 = 21.86, df = 9, P < 0.001$) (Supplementary File 1).

The prevalence of the different Trypanosoma species with respect to the above-mentioned groups, indicate that infections with the Tsg group was the highest regardless of countries or tsetse species with an average of 7.06%. The infection rate was higher (14.13%) in east, central and southern African countries than in west Africa (1.13%). Tv infection averaged at 6.75% but with higher prevalence in west African countries (10.37%) than in east, central and southern Africa (2.43%). The prevalence of Tc infection was lower than Tv and Tsg group with an average of 4.78% with higher prevalence in central and southern Africa (8.38%) than in west Africa (1.77%). The Tz group had the lowest prevalence with an average of 2.29%. Like Tv infection, the Tz prevalence was higher in west Africa (3.16%) than central and southern Africa (1.25%). The prevalence of infection by a single Trypanosoma group varied significantly from one country to another and from one tsetse species to another. For Tc, Tv, Tz and Tsg the infection prevalence varied significantly with country ($X^2 = 47.74, df = 14, P = 0.001, X^2 = 27.40, df = 14, P = 0.01705, X^2 = 106.11, df = 14, P = 0.001$ and, $X^2 = 44.74, df = 14, P = 0.001$ respectively). Regardless of tsetse species, the highest infection rate for Tc, Tv, Tz and Tsg was found in Tanzania (14.20%), Ghana (14.10%), Ghana (19.66%) and Zimbabwe (39.81%), respectively (Supplementary Table 1). Similarly, the prevalence of Tc, Tz and Tsg varied significantly with tsetse species ($X^2 = 40.36, df = 1,9, P << 0.001, X^2 = 58.253, df = 1,9, P << 0.001 and, X^2 = 34.871, df = 1,9, P << 0.001$, respectively), however no significant difference was found in Tv prevalence between tsetse species ($X^2 = 5.475, df = 1,9, P = 0.07868$). Regardless of the country, the highest infection rate of Tc, Tv, Tz and Tsg was found in G. pallidipes (10.68%), G. tachinoides (12.92%), G. medicorum (13.64%) and G. m. morsitans (22.76%), respectively (Supplementary Table 2). No Tc infection was found in samples of G. austeni collected from Eswatini and Tanzania, G. brevipalpis from Mozambique, G. p. palpalis from DRC and G. p. gambiensis from Guinea. In addition, no Tv infection was detected in G. austeni collected from Eswatini and Mozambique, G. m. morsitans from Kenya and Zambia, G. pallidipes from Uganda and Zimbabwe. For Tz, G. austeni collected from Eswatini and Mozambique, G. brevipalpis from Mozambique, G. f. fusipes from Kenya, G. m. morsitans from Kenya and Zambia, G. p. palpalis from DRC and G. p. gambiensis from Guinea did not show any infection (Fig. 2 and Supplementary Table 3).

Mixed infections of Trypanosoma groups (double or triple) are rare events with an average prevalence between 0.09 and 1.71% regardless of country or tsetse species. However, double infections seem to be more frequent in some countries than others ($X^2 = 35.01, df = 14, P = 0.001$) for Tv–Tz and in some tsetse species than others ($X^2 = 21.20, df = 9, P = 0.012$) for Tg–Tc (Supplementary File 1). The highest prevalence of the mixed infections Tv–Tz and Tc–Tz were observed in Ghana with 12.39% and 10.68%, respectively, regardless of tsetse species. Although the average Tc–Tsg prevalence was higher than that of Tv–Tz and Tc–Tz, the highest mixed infection with it was found in Zambia with 9.05%. Regardless of the country, the highest mixed infection of Tc–Tsg detected per tsetse species was ~ 5% in G. m. morsitans and G. pallidipes. The mixed infection of Tsg with either Tv or Tz or both was lower than 2% regardless of the country or tsetse species. Taking into account both the country and tsetse species, the highest mixed infection of Tc–Tsg (12.5%) was detected in G. m. morsitans in Zambia. However, the highest prevalence of Tc–Tz (10.68%) and Tv–Tz (12.39%) was detected in G. tachinoides from Ghana. Although the average prevalence of Tv–Tsg was low (0.54%), a relative high infection rate of 6.17% was found in G. m. morsitans from Tanzania.

A triple infection of Trypanosoma groups (Tc–Tv–Tz) was only detected in G. medicorum from Burkina Faso (1.30%) and G. tachinoides from Ghana (1.71%) (Fig. 2 and Supplementary Table 3, Supplementary File 1).

**Prevalence of Sodalis infection.** The prevalence of Sodalis infection based on the PCR results varied significantly with country ($X^2 = 108.02, df = 1, 14, P < 0.001$) and tsetse species ($X^2 = 69.60, df = 9, P < 0.001$). The best glm model (lowest AICc) selected for the overall Sodalis prevalence retained the countries, the species and their interaction (where possible) as variables that fitted the data well (AICc = 1296.12). Similar to the prevalence of Trypanosoma, the average Sodalis prevalence in east, central and southern Africa (24.6%) was higher than in west Africa (27.0%). Regardless of tsetse species, the highest prevalence of Sodalis infection was found in Tanzania (67.1%) followed by Uganda (43.3%), Kenya (28.5%) and Ethiopia (20.4%) (Table 1). The highest prevalence of Sodalis infection in west Africa was found in Guinea (28.6%). No Sodalis infection was found in Ghana, Mali, Senegal or Eswatini. Regardless of the country, the highest Sodalis prevalence per tsetse species was detected in G. m. morsitans (42.27%) followed by G. pallidipes (30.74%). No Sodalis infection was detected in G. tachinoides. The prevalence of Sodalis infection changed when both the countries and tsetse species are taken into
consideration (Table 4). Based on the Sodalis prevalence the tsetse samples can be categorized into four groups: (i) samples with high prevalence (> 50%) (ii) samples with medium prevalence (between < 10% and > 50%) (iii) samples with low prevalence (between > 0% and 10%) and (iv) samples with no Sodalis infection as shown in Fig. 2 and Table 4. The samples showing high Sodalis prevalence includes G. m. morsitans from Kenya (63.5%) and Tanzania (76.5%) and G. pallidipes from Tanzania (74.6%) and Uganda (75%), however the samples with no Sodalis infection includes G. austeni from Eswatini, G. p. gambiensis from Mali and Senegal and G. tachinoides from Burkina Faso and Ghana indicating that there is 95% confidence that the infection rate is less than or equal to 10%, 0.82%, 0.55%, 1.28% and 0.36%, respectively.

**Interactions between Sodalis and Trypanosoma infections.** Prevalence of co-infections of Sodalis with Trypanosoma. The screening results indicated that the single infection rate was 9.3% (n = 638) and 21.9% (n = 1503) for Sodalis and Trypanosoma, respectively, over all taxa and countries (Fig. 3A). No Sodalis infection was found in G. tachinoides, and therefore was excluded from the analysis. A Cochran–Mantel–Haenszel test for repeated tests of independence showed that infection with Sodalis and Trypanosoma did deviate from independence across all taxa ($X^2_{MH} = 41.73$, df = 1, $P < 0.001$) and individual Chi squared tests for independence for each
taxon showed significant deviation from independence at the Bonferroni corrected α = 0.00833 in G. pallidipes (P < 0.001) and G. p. gambiensis (P < 0.001) (Supplementary Table 4). The prevalence of coinfection of Sodalis and Trypanosoma in wild tsetse populations varied with tsetse taxon and location. No coinfection was found in many taxa and many locations. The co-infection was found only in G. f. fusiceps (2.73%), G. m. morsitans (15.72%) and G. pallidipes (9.22%) in east, central and southern Africa (Fig. 3B, Table 5 and Supplementary Table 4).

Impact of co-infection on Trypanosoma and Sodalis density. Attempts were to assess the density of Trypanosoma and Sodalis under single (S−/T+) and (S+/T−) or double infection (S+/T+) conducted using qPCR with primers mentioned in Supplementary Table 5. The results show that Sodalis infections did not have a significant impact on Trypanosoma density (X² = 0.648, df = 2, P = 0.723), however the median value of (S+/T+) files were slightly lower than (S−/T−) and (S+/T+) files and the number of outliter samples with higher trypanosome density (S−/T+) flies (Fig. 4A). Trypanosoma infections significantly reduced the density of Sodalis as indicated by comparing (S+/T−) flies with (S+/T+) files (P = 0.014) although the median values in (S+/T+) flies is higher than the other samples indicating that the increased of Sodalis density in (S+/T+) might be affected with the outliter flies with high Sodalis density (Fig. 4B). No significant different was found in the Trypanosoma density determined by qPCR in the flies tested negative (S−/T−) or positive (S+/T−) and (S+/T+) with the standard PCR, however, Sodalis density showed significant difference between flies with different infection type (X² = 14.54, df = 2, P < 0.001) (Fig. 4B). The results showed no correlation between Sodalis and Trypanosoma density (r = 0.007, t = 0.055, df = 69, P = 0.9561) Supplementary Fig. 2, Supplementary File 1).

Discussion

The implementation of the SIT in the context of an AW-IPM strategy to eradicate tsetse flies relies on the release of sterile males in the targeted area. This was successful in eradicating a population of G. austeni from Unguja Island of Zanzibar22 and significant progress has been made in the eradication programme implemented against G. p. gambiensis in the Niayes area of Senegal35. However, as both male and female tsetse flies are vectors of Trypanosoma species, the release of large numbers of sterile male flies bears a potential risk of temporally increasing disease transmission during the initial release phase of an SIT programme38. Therefore, mitigating measures are required to reduce or eliminate this potential risk, especially in areas where sleeping sickness (HAT) is endemic. To date, to mitigate such risks, sterile males are offered two or three blood meals mixed with the trypanocidal drug isometamidium chloride, before being released which reduces the risk of Trypanosoma transmission significantly but does not eliminate it40,41. In addition, other approaches were proposed to minimize such risks such as paratransgenesis42,43 and combining paratransgenesis with SIT44.

The vector competence of tsetse flies for different trypanosome species is highly variable and is suggested to be affected by various factors, amongst which bacterial endosymbionts. Here, the interaction of Sodalis glossinidius with tsetse trypanosome infection is still under debate. Several studies reported a potential positive correlation between Sodalis and Trypanosoma infections28,30,32,36,45–48, leading to the hypothesis that Sodalis might facilitate the establishment of Trypanosoma infections in the tsetse midgut23,26,27. However, other studies indicated the lack of correlation between Sodalis and Trypanosoma infection34–36. The presence of Sodalis infections in tsetse rearing colonies has been well studied and previous studies indicated that Sodalis is more frequently present in colonized tsetse flies than in wild tsetse populations46,49 with a prevalence of 80 and 100% in colonized G. m. morsitans and G. p. gambiensis, respectively45,50, which is higher than the symbiont prevalence in wild populations of these tsetse species. This seems to indicate that the rearing process of tsetse flies favours the transmission and spread of Sodalis infections within the colonized population. Recently, colonies of G. pallidipes, G. p. gambiensis, G. f. fusiceps, G. m. morsitans, G. m. centralis and G. m. submorsitans maintained at the FAO/IAEA Insect Pest Control Laboratory were screened for Sodalis infections and showed a 100% prevalence of Sodalis; only the G. brevipalpis colony had a lower prevalence of 95% (data not shown). Taken into consideration that mass-rearing conditions enhances Sodalis infections and that Sodalis infections might facilitate the establishment of a Trypanosoma infection in the midgut, sterile male tsetse flies that are derived from colonies might be effective vectors for different Trypanosoma species and, therefore, might increase the trypanosome transmission risk. It is therefore important that the managers and planners of SIT programmes are aware which tsetse species show a positive correlation between Sodalis and Trypanosoma infections to be able to take the necessary mitigating actions.

Various studies have examined the prevalence of Sodalis and Trypanosoma species in wild tsetse populations30,32,36,45,51, but our study presents for the first time the prevalence of Sodalis and Trypanosoma species on a continent-wide scale. In addition, the DNA extraction and PCR methods we have used were standardized and were all carried out in one laboratory to avoid discrepancies in the results due to different handling of tsetse samples or to different methods to discriminate trypanosome species in tsetse tissues. Our results indicate that Sodalis and Trypanosoma prevalence varied with tsetse species and geographical location (with an overall trypanosome prevalence of 23.5%), which agrees with many previous studies52. A high Trypanosoma prevalence (> 30%) was found in G. m. morsitans and G. pallidipes from central and east Africa. This finding is in agreement with previous reports on G. m. morsitans and G. m. centralis from Zambia53,54 and G. m. morsitans sampled in Malawi50. Moreover, a high prevalence of Trypanosoma in G. pallidipes was also previously reported in Tanzania30 and Kenya31. However, another study in northern Tanzania indicated a lower prevalence of Trypanosoma infection (< 10%) both in G. m. morsitans and G. pallidipes35.

Our study showed that the prevalence of different Trypanosoma species and or subspecies can be different in different tsetse taxa. In G. tachinoides in Ghana, the Trypanosoma vivax (Tv) infection was high (> 10%) as well as the infections of the T. brucei ssp (Tv) and the T. simiae/T. godfreyi (Tsg) group and the mixed infections of Tv–Tsg. In contrast, the prevalence of T. congolense was very low. These results are in agreement with the
| Glossina taxon | Country (Area, Collection Date) | N  | S+/T+ | S+/T− | S−/T+ | S−/T− | χ² | P    |
|----------------|---------------------------------|----|-------|-------|-------|-------|-----|------|
| G. austeni     | Tanzania (Jozani, 1997)         | 4  | 0     | 0     | 1     | 3     |     |      |
| G. austeni     | Tanzania (Zanzibar, 1995)       | 6  | 0     | 1     | 0     | 5     |     |      |
| G. austeni     | Tanzania (Ugwa Island, 1995)    | 30 | 0     | 2     | 3     | 25    |     |      |
| G. austeni     | South Africa (North eastern KwaZulu Natal, 1999) | 39 | 0    | 2     | 2     | 35   |     |      |
| G. austeni     | South Africa (Lower Mkhuzi, 2018) | 53 | 0   | 0     | 23    | 30    |     |      |
| G. austeni     | South Africa (Saint Lucia, 2018) | 57 | 0   | 0     | 22    | 35    |     |      |
| G. austeni     | South Africa (False Bay Park, 2018) | 77 | 0   | 0     | 2     | 75    |     |      |
| G. austeni     | Mozambique (Reserva Especial de Maputo, 2019) | 50 | 0   | 0     | 5     | 45    |     |      |
| G. austeni     | Eswatini (Mlawula Nature Reserve, 2019) | 30 | 0 | 0     | 0     | 30    |     |      |
| G. austeni     | All locations                   | 346| 0   | 5     | 58    | 283   | 1.02| 0.31|
| G. brevipalpis | South Africa (North eastern KwaZulu Natal, 1995) | 50 | 0   | 2     | 48    |       |     |      |
| G. brevipalpis | South Africa (Phinda, 2018)     | 170| 0   | 7     | 163   |       |     |      |
| G. brevipalpis | South Africa (Saint Lucia, 2018) | 30 | 0   | 0     | 13    | 17    |     |      |
| G. brevipalpis | South Africa (Hluhluwe, 2018)   | 50 | 0   | 0     | 16    | 34    |     |      |
| G. brevipalpis | Mozambique (Reserva Especial de Maputo, 2019) | 50 | 0   | 7     | 3     | 40    |     |      |
| G. brevipalpis | All locations                   | 350| 0   | 14    | 34    | 302   | 1.57| 0.21|
| G. f. fuscipes | Uganda (Buvuma island, 1994)    | 94 | 0   | 4     | 10    | 80    |     |      |
| G. f. fuscipes | Kenya (Ikapolo, 2007)           | 51 | 5   | 15    | 14    | 17    |     |      |
| G. f. fuscipes | Kenya (Obekai, 2007)            | 38 | 0   | 0     | 2     | 36    |     |      |
| G. f. fuscipes | All locations                   | 183| 5   | 19    | 26    | 133   | 0.3 | 0.59|
| G. medicorum   | Burkina Faso (Comoe, 2008)      | 94 | 0   | 8     | 32    | 54    |     |      |
| G. medicorum   | Burkina Faso (Folonzo, 2008)    | 60 | 0   | 0     | 29    | 31    |     |      |
| G. medicorum   | All locations                   | 154| 0   | 8     | 61    | 85    | 5.53| 0.02|
| G. m. submoritans | Burkina Faso (Comoe, 2007) | 206| 0   | 0     | 20    | 186   |     |      |
| G. m. submoritans | Burkina Faso (Folonzo, 2008) | 154| 0   | 1     | 42    | 91    |     |      |
| G. m. submoritans | Burkina Faso (Sissili, 2008) | 3  | 0   | 0     | 0     | 3     |     |      |
| G. m. submoritans | All locations                  | 343| 0   | 1     | 62    | 280   | 0.22| 0.64|
| G. p. palpalis  | Democratic Republic of Congo (Zaire, 1995) | 35 | 0 | 4     | 1     | 30    |     |      |
| G. m. moritans  | Tanzania (Kwekivu 2, 2005)      | 81 | 35  | 27    | 9     | 10    |     |      |
| G. m. moritans  | Zambia (Miwuwe, Eastern Zambia, 2007) | 64 | 1   | 7     | 30    | 26    |     |      |
| G. m. moritans  | Zimbabwe (Mukondore, 2007)      | 13 | 1   | 2     | 0     | 10    |     |      |
| G. m. moritans  | Zimbabwe (M. chiuyi, 2007)      | 9  | 0   | 1     | 0     | 8     |     |      |
| G. m. moritans  | Zimbabwe (Rukomeshi, 2006)      | 15 | 0   | 3     | 0     | 12    |     |      |
| G. m. moritans  | Zimbabwe (Kemukura, NA)         | 18 | 0   | 4     | 1     | 13    |     |      |
| G. m. moritans  | Zimbabwe (Mushumbi, 2006)       | 6  | 0   | 0     | 2     | 4     |     |      |
| G. m. moritans  | Zimbabwe (Makuti, 2006)         | 78 | 19  | 2     | 52    | 5     |     |      |
| G. m. moritans  | Kenya (Kari, 2006)              | 85 | 2   | 52    | 0     | 31    |     |      |
| G. m. moritans  | All locations                   | 369| 58  | 98    | 94    | 119   | 1.8 | 0.18|
| G. pallidipes   | Zambia (Mfuwe, Eastern Zambia, 2007) | 146| 2   | 1     | 64    | 79    |     |      |
| G. pallidipes   | Kenya (Mwea, Katoloi, Emusos, Kari, Koibus, Meru and Ruma national park, 2007) | 834| 88  | 126   | 337   | 283   |     |      |
| G. pallidipes   | Ethiopia (Arba Minch, 2007)     | 459| 15  | 79    | 77    | 288   |     |      |
| G. pallidipes   | Tanzania (Kwekivu 1, 2005)      | 217| 54  | 108   | 27    | 28    |     |      |
| G. pallidipes   | Zimbabwe (Mushumbi 2006)        | 26 | 1   | 0     | 4     | 21    |     |      |
| G. pallidipes   | Zimbabwe (Gokwe, 2006)          | 4  | 0   | 0     | 4     |       |     |      |
| G. pallidipes   | Zimbabwe (Rukomeshi, 2006)      | 4  | 0   | 0     | 4     |       |     |      |
| G. pallidipes   | Zimbabwe (Makuti, 2006)         | 38 | 6   | 0     | 27    | 5     |     |      |
| G. pallidipes   | Uganda (Lira, Omogo, Budaka, Moyu, NA) | 116| 4   | 83    | 5     | 24    |     |      |
| G. pallidipes   | All locations                   | 1844| 170| 397   | 541   | 736   | 25.4| 0    |
also agree with former studies\textsuperscript{56,57}, in our study. Our results of trypanosome infection rates in \textit{G. tachinoides} averaged 15.5%, but in certain locations the prevalence exceeded 40%\textsuperscript{60}. In Nigeria, \textit{Sodalis} infection in \textit{G. m. morsitans} found in the proboscis of (major vector). It is worth noting that all previous studies of \textit{Sodalis} was associated with its status as a poor vector, whereas it is not in both midgut and proboscis of \textit{G. pallidipes} under laboratory conditions\textsuperscript{28,29,64}. The correlation between prevalence of \textit{T. brucei} and its interaction with \textit{Trypanosoma} infection on \textit{G. austeni} in Eswatini for \textit{Sodalis} co-infection of and \textit{G. medicorum}, which is in agreement with the high prevalence reported in our study for both species. In another study in Kenya, Wamwiri et al.\textsuperscript{32} reported a high prevalence for \textit{T. brucei} (64.4%) but much lower for all other \textit{Trypanosoma} infections (< 10%)\textsuperscript{30}. The mixed infection of \textit{Trypanosoma} species/subspecies is in agreement with previous reports\textsuperscript{29,30,32,33,37,39}.

Likewise, the prevalence of \textit{Sodalis} infection varied significantly with tsetse taxon and location and the highest prevalence was found in \textit{G. m. morsitans} and \textit{G. pallidipes}. Our results agree with the high prevalence of \textit{Sodalis} reported in \textit{G. pallidipes} (~50%) in one location in Kenya regardless of the fly age\textsuperscript{31}; however, the same study reported low \textit{Sodalis} prevalence in another location. In another study in Kenya, Wamwiri et al.\textsuperscript{32} reported moderate \textit{Sodalis} prevalence in \textit{G. pallidipes} (16%) and low prevalence in \textit{G. austeni} (3.7%), which is in agreement with our results. On the other hand, our results are different from the low prevalence (<8%) found in \textit{G. m. morsitans} and \textit{G. pallidipes} in Zambia\textsuperscript{66}. In another study in Zambia, \textit{Sodalis} prevalence in \textit{G. m. centralis}, was reported to be 15.9% with no significant difference between inter-site prevalence\textsuperscript{32}. The prevalence of \textit{Sodalis} in \textit{G. brevipalpis} in our study was found to be low (<2.3%) which contradicts with the high prevalence (93.7%) reported in this species in Zambia\textsuperscript{66}. In the DRC, the global prevalence of \textit{Sodalis} in \textit{G. fuscipes quanzensis} midgut averaged 15.5%, but in certain locations the prevalence exceeded 40%\textsuperscript{66}. In Nigeria, \textit{Sodalis} prevalence in \textit{G. pallidipes} and \textit{G. tachinoides} was 35.7\%\textsuperscript{67} which is higher that the prevalence reported in our study for both species.

Our data indicate that the \textit{Trypanosoma} and \textit{Sodalis} infections were very low or absent in some tsetse taxa from certain locations such as \textit{G. austeni} in Eswatini for \textit{Trypanosoma} and \textit{Sodalis} infections and several species in west Africa for \textit{Sodalis}. The lack of \textit{Sodalis} and/or \textit{Trypanosoma} infection in these samples might be due to (i) low number of tested samples (ii) the use of the DNA extracted from the whole body of tsetse adults (iii) the possibility of the collected samples being infected with different strains/genotypes that might not be detected with the primers used and (iv) the infection of \textit{Sodalis} and \textit{Trypanosoma} are under the detection limit of the used PCR. It is important to note that due to the high number of samples tested in our study, the more sensitive nested PCR to detect low infection level was excluded due to logistic reasons.

Our results indicate significant deviation from independence (correlation) of \textit{Sodalis} and \textit{Trypanosoma} infections in \textit{G. medicorum}, \textit{G. p. gambiensis} and \textit{G. pallidipes}. However, the lack of detection of any tsetse adult with co-infection of \textit{Sodalis} and \textit{Trypanosoma} in \textit{G. medicorum}, and \textit{G. p. gambiensis} might indicate a negative correlation. Such negative trend might be supported by the lower density of \textit{Sodalis} in the flies with co-infection (S+/T+) compared to these with \textit{Sodalis} infection only (S+/T−). On other hand the lack of impact of \textit{Sodalis} infection on \textit{Trypanosoma} density does not support the negative trend and agreed with the results of Treppe- niers et al.\textsuperscript{24} reported on colonized flies. This results also agreed with previous results reporting the absence of direct correlation between the presence of \textit{Sodalis} and the acquisition of a \textit{Trypanosoma} infection\textsuperscript{68}. However, an inverse correlation was reported between \textit{Sodalis} and the vector competence where the presence of \textit{Sodalis} in both midgut and proboscis of \textit{G. p. gambiensis} was associated with its status as a poor vector, whereas it is not found in the proboscis of \textit{G. m. morsitans} (major vector). It is worth noting that all previous studies of \textit{Sodalis} infection in \textit{G. p. gambiensis} and its interaction with \textit{Trypanosoma} infection was carried out with flies reared under laboratory conditions\textsuperscript{28,29,64}. The correlation between \textit{Sodalis} and \textit{Trypanosoma} infection in \textit{G. pallidipes} is

### Table 5. Distribution of the association between the presence of \textit{Trypanosoma} spp and the presence of \textit{Sodalis} according to the tsetse species and the country.

| Glossina taxon     | Country (Area, Collection Date) | N  | S+/T+ | S+/T− | S−/T+ | S−/T− | χ²  | P   |
|--------------------|---------------------------------|----|-------|-------|-------|-------|-----|-----|
| \textit{G. p. gambiensis} | Burkina Faso (Comoe)            | 123 | 0     | 0     | 3     | 120   |
| \textit{G. p. gambiensis} | Burkina Faso (Folonzo)          | 212 | 0     | 2     | 25    | 185   |
| \textit{G. p. gambiensis} | Burkina Faso (Kartasso)         | 136 | 0     | 0     | 0     | 136   |
| \textit{G. p. gambiensis} | Burkina Faso (Kenedougou)       | 41  | 0     | 0     | 0     | 41    |
| \textit{G. p. gambiensis} | Burkina Faso (Moussodougou)     | 142 | 0     | 0     | 49    | 93    |
| \textit{G. p. gambiensis} | Guinea (Bafing)                 | 33  | 0     | 0     | 1     | 32    |
| \textit{G. p. gambiensis} | Guinea (Dekomkore)              | 16  | 0     | 0     | 1     | 15    |
| \textit{G. p. gambiensis} | Guinea (Kangoliya)              | 126 | 0     | 90    | 0     | 36    |
| \textit{G. p. gambiensis} | Guinea (Kerfala)                | 13  | 0     | 0     | 1     | 12    |
| \textit{G. p. gambiensis} | Guinea (Kifala)                 | 30  | 0     | 0     | 0     | 30    |
| \textit{G. p. gambiensis} | Guinea (Lemonako)               | 20  | 0     | 0     | 0     | 20    |
| \textit{G. p. gambiensis} | Guinea (Mimi)                   | 45  | 0     | 0     | 1     | 44    |
| \textit{G. p. gambiensis} | Guinea (Tinkisso)               | 31  | 0     | 0     | 2     | 29    |
| \textit{G. p. gambiensis} | Mali                            | 364 | 0     | 0     | 25    | 339   |
| \textit{G. p. gambiensis} | Senegal                         | 547 | 0     | 0     | 79    | 469   |
| \textit{G. p. gambiensis} | All locations                   | 2168| 0     | 92    | 343   | 1733  | 18.06| 0   |
| \textit{G. tachinoides} | Burkina Faso                    | 834 | 0     | 0     | 140   | 694   |
| \textit{G. tachinoides} | Ghana                           | 234 | 0     | 0     | 143   | 91    |
| \textit{G. tachinoides} | All locations                   | 1068| 0     | 0     | 283   | 785   |

prevalence of \textit{T. brucei} s.l (11%) and \textit{T. congolense} forest type (2.6%) reported in the same tsetse species in Cameroon. The same study reported a prevalence of 13.7% of \textit{T. congolense} savannah type\textsuperscript{35}, which was not observed in our study. Our results of trypanosome infection rates in \textit{G. tachinoides} also agree with former studies\textsuperscript{36,37}, except for \textit{Tc} for which a high fly infection rate (31.8%) was previously shown\textsuperscript{32}. The \textit{Tc} infection rates in our study were high in \textit{G. pallidipes} and \textit{G. m. morsitans}; for the latter tsetse fly species, a study in Malawi reported a high prevalence for \textit{T. brucei} (64.4%) but much lower for all other \textit{Trypanosoma} infections (< 10%)\textsuperscript{30}. The mixed infection of \textit{Trypanosoma} species/subspecies is in agreement with previous reports\textsuperscript{29,30,32,33,37,39}.
positive, evidenced with the relative high number (n = 170) of tsetse with co-infection. This positive correlation was also found in G. pallidipes from Kenya although with too few flies with co-infection to enable us to draw a definite conclusion. Although co-infections were found in G. m. morsitans and G. f. fuscipes in some locations, the global correlation was missing. This is in agreement with the positive correlation found between Sodalis and Trypanosoma infection in G. m. centralis in Zambia, in which there was a 6.2 fold increase in the likelihood of a fly being infected with Trypanosoma if Sodalis was present. More studies are needed to enhance the potential control interventions mediated by endosymbionts to reduce parasitic infections.

The results of this study clearly indicate that the interaction between Sodalis and Trypanosoma infection is complex, species-specific and requires further investigation. The prevalence results indicate that Sodalis and Trypanosoma infections are not independent in some species, such as G. p. gambiensis and G. medicorum in west Africa and G. pallidipes in central and east Africa. In case of a positive correlation between Sodalis and Trypanosoma infection in these species, additional measures could be suggested when implementing the SIT to reduce the Sodalis density in the sterile males released in the targeted area to maximize the safe implementation of the SIT. These measures might include the mixing of Sodalis phage(s) with the blood meals to feed the mass-reared flies to reduce the Sodalis density in these flies. In addition, the blood meal offered to the males before release can be supplemented with one or more of the following antimicrobial products to reduce Sodalis density, i.e. streptozotocin, indolicidin and OaBAC 5 mini. The use of the Sodalis phage as well as these antimicrobial agents requires further studies to (1) develop methods to isolate the phage, (2) determine the conditions (e.g. suitable concentration) for its use, and (3) determine the impact on Sodalis density, tsetse productivity and...

**Figure 4.** Impact of co-infection with Trypanosoma and Sodalis on Trypanosoma (A) and Sodalis (B) density in Glossina pallidipes and G. m. morsitans. Bars marked with the same lower-case letter do not differ significantly at the 0.05 level.
success. For *G. m. morsitans* and *G. pallidipes*, our results suggest that *Sodalis* infection does not have an impact on *Trypanosoma* infection so here no additional measures need to be taken during the implementation of SIT against these species.

**Conclusion**

*Sodalis* and *Trypanosoma* infection varied with tsetse taxon and location. There is a significant positive correlation between *Sodalis* and *Trypanosoma* infection in *G. medoruma*, *G. p. gambiensis* and *G. pallidipes*; however, no significant correlation was found in other tsetse taxa and locations. The results of this study will enable the decision makers of SIT projects to better plan and take the necessary measures to fine-tune and optimize SIT efficiency and safety.

**Methods**

**Tsetse collection and DNA extractions.** Tsetse flies were collected in 1995 and between 2005 and 2018 from 95 different geographical locations in fifteen countries in east, central, southern, and western Africa (Table 6, Supplementary Table 6). The tsetse flies were collected with species-specific traps which included the biconical trap[^97^], the monoconal trap[^98^], the Vavoua trap[^99^], the Ngui trap[^100^],[^71^], the odour-baited Epsilon trap[^72^], the NZI trap[^73^], and the odour baited H trap[^74^]. A total of 6860 tsetse flies, belonging to ten tsetse species, were collected for this study (Table 6). The majority of the samples were collected in Burkina Faso (2274), Kenya (1008), Senegal (547) and South Africa (526). As the distribution of most tsetse species is allopatric (only few species are sympatric), not all tsetse species were collected from each country. Following collection, fly samples were preserved in 95% ethanol or propylene glycol and shipped to the FAO/IAEA Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria and stored at −20 °C until analysis. Total DNA was extracted from individual whole bodies using the DNeasy tissue kit (QIAGEN Inc., Valencia, CA) following the supplier's instructions. The DNA quality and concentration were measured by spectrophotometry (Synergy H1 Multi-Mode Reader, BioTek, Instruments, Inc., USA) and subsequently kept at 4 °C until screened for *Sodalis* and *Trypanosoma* infections. To verify the quality of the extracted DNA, an S set of specific primers amplifying the Glossina spp. microsatellite GpCAG133 sequence (Supplementary Table 5) and only the successful samples were included in the analysis[^21^,^75^].

**Trypanosoma prevalence and genotyping.** Polymerase chain reaction (PCR), following the method of Njiru et al.[^76^] that used the primers ITS1-CF and ITS1-BR (Supplementary Table 5) previously designed to amplify the internal transcribed spacer (ITS1) of the ribosomal DNA, was used to detect *Trypanosoma* infection and *Trypanosoma* species in the fly samples. The PCR was carried out in 25 µl reaction mixtures containing 22.5 µl of 1.1 × Pre-Aliquoted PCR Master Mix (0.625 units Thermoprime Plus DNA Polymerase, 75 mM Tris−HCl (pH 8.8 at 25 °C), 20 mM (NH4)2SO4, 2.0 mM MgCl2, 0.01% (v/v) Tween-20 and 0.2 mM each of the dNTPs (ABgene, UK), 1 µl primers (at 200 nM final concentration of forward and reverse primer) and 1.5 µl of template DNA. PCR cycles were: 94 °C for 15 min, followed by 40 cycles of 94 °C for 30 s, 72 °C for 30 s and final extension 72 °C for 5 min. Interpretation of the results after the analysis of the amplification products in a 2% agarose gel (Fisher Biotech) stained with SafeGreen or ethidium bromide was based on the characteristic band size of *Trypanosoma* taxa: all members of the subgenus *T. brucei* spp (T. b. brucei, T. b. gambiensi, T. b. rhodesiensi: 480 bp); *T. congolense* savannah (700 bp); *T. congolense* forest (710 bp); *T. evansi* (300 bp); *G. pallidipes* (300 bp); *T. vivax* (250 bp). The positive control DNA was from *T. congolense* savannah, *T. congolense* forest, *T. b. brucei, T. b. gambiensi, T. b. rhodesiensi, T. evansi, and *T. vivax*. DNA samples validated with GpCAG133 primer amplification were screened for *Trypanosoma* infection. A tsetse sample was recorded as positive if one or more of the indicated band sizes was detected. *Trypanosoma* infection status and species were recorded for each fly.

**Prevalence of *Sodalis* infection.** The detection of *Sodalis* in natural tsetse samples was based on the *Sodalis fliC* (flagellin) gene which results in an amplicon length of about 508 base pairs with the specific primers Sod-fliC-F and Sod-fliC-R (Supplementary Table 5). These primers were used in single pairs or in multiplex PCR with GpCAG133 primers. For all PCR reactions, 22.5 µl of 1.1 × Pre-Aliquoted PCR Master Mix (ABgene, UK) was used. In a final volume of 25 µl, 1.5 µl of template DNA plus forward and reverse primers were added to a final concentration of 0.2 mM per primer in a volume of 1 µl. Samples were considered *Sodalis*-infected if the expected symbiont PCR product amplicon was detected. Data were accepted only if the control gene GpCAG133 sequence was amplified. The PCR cycling conditions were: 95 °C for 5 min followed by 34 cycles of 95 °C for 30 s, 52.5 °C for 30 s, 72 °C for 30 s and lastly at 72 °C for 10 min; PCR products were separated by agarose (2%) gel electrophoresis and SafeGreen or ethidium bromide staining.

**Analysis of the *Trypanosoma* and *Sodalis* infection in wild tsetse populations.** Co-infection of tsetse adults with *Sodalis* and *Trypanosoma* infection was evaluated based on the PCR prevalence. The infection status was divided into four categories *Sodalis* positive and *Trypanosoma* positive (S+/T+), *Sodalis* positive and *Trypanosoma* negative (S+/T−), *Sodalis* negative and *Trypanosoma* positive (S−/T+), and *Sodalis* negative and *Trypanosoma* negative (S−/T−).

**Analysis of the *Trypanosoma* and *Sodalis* density.** Samples showing *Trypanosoma* infection (not *T. vivax*) with *Sodalis* (S+/T−) and samples not infected with *Trypanosoma* but infected with *Sodalis* (S−/T+) were evaluated with quantitative PCR (qPCR) to assess the impact of *Trypanosoma* infection (regardless the *Trypanosoma* type) on *Sodalis* density. The qPCR was performed using a CFX96 Real Time PCR Detection System (Bio-Rad). The fliC sequence was amplified. The PCR cycling conditions were: 95 °C for 5 min followed by 34 cycles of 95 °C for 30 s, 52.5 °C for 30 s, 72 °C for 30 s and lastly at 72 °C for 10 min; PCR products were separated by agarose (2%) gel electrophoresis and SafeGreen or ethidium bromide staining.

**Analysis of the *Trypanosoma* and *Sodalis* density.** Samples showing *Trypanosoma* infection (not *T. vivax*) with *Sodalis* (S+/T−) and samples not infected with *Trypanosoma* but infected with *Sodalis* (S−/T+) were evaluated with quantitative PCR (qPCR) to assess the impact of *Trypanosoma* infection (regardless the *Trypanosoma* type) on *Sodalis* density. The qPCR was performed using a CFX96 Real Time PCR Detection System (Bio-Rad). The fliC sequence was amplified. The PCR cycling conditions were: 95 °C for 5 min followed by 34 cycles of 95 °C for 30 s, 52.5 °C for 30 s, 72 °C for 30 s and lastly at 72 °C for 10 min; PCR products were separated by agarose (2%) gel electrophoresis and SafeGreen or ethidium bromide staining.
gene was amplified with the following primers: sodqPCR-FliCF and sodqPCR-FliCR78 (Supplementary Table 5) to assess the density of the symbiont present within *Trypanosoma* infected and noninfected, additional criteria for the selection of the samples was the presence of the two groups (S’/T’) and (S’/T’). In a given population. Based on the preceding criteria 96 individual flies (52 and 44 flies with infection status of (S’/T’) and (S’/T’)), respectively, were selected from the *G. pallidipes* and *G. m. morsitans* collected in Kenya, Tanzania and Zimbabwe. In addition, samples with (S’/T’) and (S’/T’) were used to assess the impact of *Sodalis* infection on *Trypanosoma* density. *Trypanosomatidae*18 specific primers (18S_Typ_F and18S_Typ_R) (Supplementary Table 5) were used to assess the *Trypanosoma* density in the tested samples. The DNA from all selected samples was diluted to a final concentration of 4 ng/μl and 5 μl of the diluted DNA was used for qPCR to determine *Sodalis* and *Trypanosoma* DNA density normalized to the housekeeping β-tubulin gene. The amplification mixture contained 5 μl of DNA template, 200 nM of each primer, and 7.5 μl iQ™ SYBER Green Supermix (Bio-Rad). qPCR cycling conditions for *Sodalis* were as follows: initial denaturation at 95 °C for 2 min; 39 cycles of 95 °C for 5 s, 55 °C for 30 s, one step at 95 °C for 5 s and a melting curve constructed from 65 °C to 95 °C in increments of 0.5 °C for 5 s. The same conditions were used for *Trypanosoma* except the annealing temperature was at 60 °C. The analysis of the *Sodalis, Trypanosoma* and Tubulin densities was based only on qPCR data with the expected melting curve at 81.5 °C, 85.5 °C and 86 °C, respectively.

**Data analysis.** The prevalence data were recorded and analyzed with the general linear model (GLM)79. The prevalence of *Sodalis, Trypanosoma* species and each *Trypanosoma* species and co-infection were tested for differences between the tsetse taxon and between taxa. For each country, the prevalence was assessed again for differences between the localities where the flies were collected and between the tsetse species present in each country. In the absence of PCR detected *Sodalis or Trypanosoma* infection, the upper 95% confidence interval for the true rate of infection was calculated following the method of Couey and Chew62. *Trypanosoma* prevalence between taxa was compared between species by a pairwise comparison of proportions with a Bonferroni correction and Benjamini–Hochberg correction. The analyses were executed in R v 4.0.579 using RStudio V 1.4.110681 with the packages ggplot2 v3.3.2.18, lattice v0.20-418, car84, gghstats85 and MASS v7.3-51.686 except for the Chi squared tests for independence, Spearman correlation coefficient and Cochran–Mantel–Haenszel test for repeated tests of independence, which were performed using Excel 2013. The R Markdown file is available in Supplementary File 1.

To analyse the qPCR data, normalized density of *Trypanosoma* and *Sodalis* against the house keeping gene (tubulin) was extracted from the CFX Maestro software. Samples giving a valid density (not N/A) for both *Trypanosoma* and *Sodalis* were retained for further statistical analysis in R. Similarities in the structure of *Sodalis* and *Trypanosoma* (single and multiple) infection and the role of different factors such as countries and tsetse taxa, were assessed using the matrix display and metric multidimensional scaling (mMDS) plot with bootstrap averages in PRIMER version 7+. The bootstrap averages plots were displayed with a Bray and Curtis matrix based on the square-root transformation of the *Sodalis* and *Trypanosoma* (single and multiple) infection abundance data87. The tests were based on the multivariate null hypothesis via the use of the non-parametric statistical method PERMANOVA88. The Permanova test was conducted on the average of the abundance data based on the country-species after excluding the data of Eswatini (low number of tested samples).

| Country       | No. of locations | No. of collection flies with valid DNA | Collection year |
|---------------|------------------|--------------------------------------|-----------------|
| Ethiopia      | 1                | 459                                   | 2007            |
| Kenya         | 11               | 1008                                 | 2007, 2008, 2009|
| Uganda        | 5                | 210                                   | 2007            |
| Tanzania      | 5                | 338                                   | 2005, 2009      |
| Democratic R. of Congo | 1   | 5                                     | 1995            |
| Zambia        | 1                | 210                                   | 2007            |
| Zimbabwe      | 7                | 211                                   | 2008            |
| South Africa  | 7                | 526                                   | 1995, 2018, 2019|
| Mozambique    | 1                | 100                                   | 2019            |
| Eswatini      | 1                | 30                                    | 2018, 2019      |
| Burkina Faso  | 14               | 2274                                 | 2008, 2010, 2013, 2015, 2018, 2019 |
| Ghanaa        | 11               | 234                                   | 2008            |
| Guinea        | 8                | 314                                   | 2008, 2009      |
| Mali          | 10               | 364                                   | 2008, 2010, 2011, 2012, 2013 |
| Senegal       | 12               | 547                                   | 2008, 2009      |
| Total         | 95               | 6860                                  |                 |

Table 6. List of collections of tsetse adults with valid DNA screened for *Sodalis* and *Trypanosoma* infection in wild tsetse population in east, central, southern and west Africa. *Part of the trypanosome infection in west Africa was screened by Ouedraogo et al. 2018.*
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Author contributions

A.M.M.A., R.L.M. and M.J.B.V. Conceived and designed Research; M.M.D., M.K.D., P.M., G.M.S.O., G.D.-U., E.G., F.C.M., L.N., S.M., J.-P.R., A.M.G.B., S.P. and C.J.B., collected data and conducted research; A.M.M.A., M.M.D., M.K.D., J.V.D.A. and A.G.P. analyzed and interpreted data; A.M.M.A., M.M.D., M.K.D. wrote the initial paper; A.M.M.A., A.G.P., J.V.D.A., R.L.M., and M.J.B.V. revised the paper; A.M.M.A had primary responsibility for final content. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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