Interactions between *Glossina pallidipes* salivary gland hypertrophy virus and tsetse endosymbionts in wild tsetse populations

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**Abstract**

**Background:** Tsetse control is considered an effective and sustainable tactic for the control of cyclically transmitted trypanosomosis in the absence of effective vaccines and inexpensive, effective drugs. The sterile insect technique (SIT) is currently used to eliminate tsetse fly populations in an area-wide integrated pest management (AW-IPM) context in Senegal. For SIT, tsetse mass rearing is a major milestone that associated microbes can influence. Tsetse flies can be infected with microorganisms, including the primary and obligate *Wigglesworthia glossinidia*, the commensal *Sodalis glossinidius*, and *Wolbachia pipientis*. In addition, tsetse populations often carry a pathogenic DNA virus, the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) that hinders tsetse fertility and fecundity. Interactions between symbionts and pathogens might affect the performance of the insect host.

**Methods:** In the present study, we assessed associations of GpSGHV and tsetse endosymbionts under field conditions to decipher the possible bidirectional interactions in different *Glossina* species. We determined the co-infection pattern of GpSGHV and *Wolbachia* in natural tsetse populations. We further analyzed the interaction of both *Wolbachia* and GpSGHV infections with *Sodalis* and *Wigglesworthia* density using qPCR.

**Results:** The results indicated that the co-infection of GpSGHV and *Wolbachia* was most prevalent in *Glossina austeni* and *Glossina morsitans morsitans*, with an explicit significant negative correlation between GpSGHV and *Wigglesworthia* density. GpSGHV infection levels > 10³ seem to be absent when *Wolbachia* infection is present at high density (> 10⁷), suggesting a potential protective role of *Wolbachia* against GpSGHV.

**Conclusion:** The result indicates that *Wolbachia* infection might interact (with an undefined mechanism) antagonistically with SGHV infection protecting tsetse fly against GpSGHV, and the interactions between the tsetse host and its associated microbes are dynamic and likely species specific; significant differences may exist between laboratory and field conditions.

**Keywords:** Hytrosaviridae, Tsetse microbiota, Virus transmission, *Wigglesworthia*, *Wolbachia*, *Sodalis*

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**Introduction**

Mutualistic bacteria are functionally essential to the physiological well-being of their animal hosts. They benefit their hosts by providing essential nutrients, aiding in digestion and maintaining intestinal equilibrium. Furthermore, mutualistic symbionts foster the development, differentiation, and proper function of their host’s
immune system [1–5]. Insects provide a useful model for studying host-microbe interactions because they are associated with bacterial communities that can be easily manipulated during their host’s development [6]. Tsetse flies (Glossina spp.) accommodate various types of bacteria, including two gut-associated bacterial symbionts, the obligate Wigglesworthia glossinidius and the commensal Sodalis glossinidius, the widespread symbiont Wolbachia pipiensis, and a recently discovered Spiroplasma endosymbiont [7–13]. In addition, tsetse flies can house different types of viral infection, including the salivary gland hypertrophy virus (GpSGHV), iflavirus, and negevirus, besides trypanosome parasites [14–17]. Symbiotic associations between insect disease vectors, gut and endosymbiotic bacteria have been particularly well studied to determine how these microbes influence their host’s ability to be infected [18–22]. For example, in tsetse flies, the obligate bacteria W. glossinidius are essential for maintaining female fecundity and the host immune system by providing important nutritional components (vitamin B6) and folate (vitamin B9) [22–24]. In addition, Sodalis may modulate tsetse susceptibility to infection with trypanosomes, and several studies using field-captured tsetse have noted that the prevalence of trypanosome infections positively correlates with increased Sodalis density in the fly’s gut [25–29]. In contrast, the exogenous bacterium Kosakonia cowanii inhibits trypanosome infection by creating an unfavorable environment for trypanosome establishment in the mid-gut [30].

Flies in the genus Glossina (tsetse flies) are unique to Africa and are of great medical and economic importance as they serve as a vector for the trypanosomes responsible for sleeping sickness in humans (human African trypanosomosis or HAT) and nagana in animals (African animal trypanosomosis or AAT) [31, 32]. The presence of tsetse and trypanosomes is considered one of the major challenges to sustainable development in Africa [33, 34]. The lack of adequate and affordable vaccines coupled with pathogen resistance to drug treatments severely limits AAT control, leaving vector control as the most feasible option for sustainable management of the disease [31, 32]. In addition to various pesticide- and trapping-based methods for tsetse control, the sterile insect technique (SIT) is considered an efficient, sustainable and environmentally friendly method when implemented in the frame of area-wide integrated pest management (AW-IPM) [35, 36]. However, the SIT requires the mass rearing of many males to be sterilized with ionizing radiation before release into the targeted area [33, 37].

Tsetse fly biology is characterized by its viviparous reproduction rendering tsetse mass rearing a real challenge. Tsetse flies nourish their intrauterine larvae from glandular secretions and give birth to fully developed larvae (obligate adenotrophic viviparity) [38, 39]. They also live considerably longer than other vector insects, which somewhat compensates for their slow reproductive rate [40]. The ability to nourish larvae on the milk gland secretion, although limiting the number of larvae produced per female lifetime (8–12), facilitates the transmission of endosymbiotic bacteria and pathogens from females to larvae such as Wigglesworthia, Sodalis, Wolbachia, Spiroplasma, and GpSGHV [8, 10, 13, 41]. Moreover, as strictly hematophagous, tsetse rely on the associated endosymbionts to obtain essential nutrients for female reproduction. Therefore, tsetse well-being in mass rearing for SIT is affected by the status of its endosymbionts as well as infection with pathogenic viruses and the interactions between them. Although Wigglesworthia is an obligate endosymbiont and found in all tsetse species, Sodalis, Wolbachia, and Spiroplasma infection varied from one species to another [8, 10, 42–45]. In addition, infection with GpSGHV, although reported in different tsetse species, is mainly symptomatic in G. pallidipes [46–48]. As GpSGHV is horizontally transmitted via the feeding system under laboratory conditions, leading to high infection rates [49–51], and the virus has a negative effect on the reproductive system of the host causing reduced fecundity and fertility [52, 53], control of the virus infection is important in tsetse mass rearing for efficient production of irradiated males for SIT program implementation.

The variable responses of different tsetse species to the GpSGHV infection might indicate a possibility of the tsetse microbiota modulating the molecular dialogue among the virus, symbiont, and host, shaping the response of each species to the virus infection. It was necessary, therefore, to investigate the infection status of the major tsetse endosymbionts (Wigglesworthia, Sodalis, and Wolbachia) in different tsetse species and their potential interactions. We have recently investigated the interaction between GpSGHV and tsetse symbionts in six tsetse species after virus injection under laboratory conditions [54]. The results indicated that the interaction between the GpSGHV and tsetse symbionts is a complicated process that varies from one tsetse species to another. It is worth noting that the study of Demirbas-Uzel et al. [54] was conducted in tsetse flies maintained under controlled laboratory conditions (sustainable food availability, constant environmental conditions (temperature and humidity), and high density of the flies), which favors the increase of tsetse symbionts [45, 55, 55–57]. In addition, this study was done using adults artificially infected with GpSGHV by injection. Therefore, we investigated the associations of the GpSGHV and tsetse symbionts in field-collected samples by evaluating the
prevalence of co-infection of GpSGHV and Wolbachia and their potential association with Wigglesworthia and Sodalis infection in natural tsetse populations. The results are also discussed in the context of developing an effective and robust mass production system of high-quality sterile tsetse flies for implementing SIT programs.

Methods
Tsetse samples, extraction of total DNA, and PCR amplifications
The field collection of tsetse fly samples, DNA extraction, and the PCR-based prevalence of GpSGHV and Wolbachia infections were reported previously [7, 47, 58, 59]. Based on these publications, and using G. m. morsitans, G. pallidipes, G. medicorum, G. brevipalpis, and G. austeni samples collected from Burkina Faso, South Africa, Tanzania, Zambia, and Zimbabwe, four infection patterns (i.e. presence) were determined: (i) flies PCR positive for both GpSGHV and Wolbachia (W+/V+), (ii) flies PCR positive for Wolbachia alone (W+/V−), (iii) flies PCR positive for GpSGHV alone (W−/V+), and (iv) flies PCR negative for both GpSGHV and Wolbachia (W−/V−). It has to be noted that the prevalence of the symbionts was assessed using a conventional PCR assay while their densities (see below) were determined using a qPCR assay. Since these two assays were different in several aspects including the size of the amplicons and visualization process, this resulted in some discrepancies regarding the infections status of some virus samples initially considered virus free by conventional PCR that were found to be positive during the qPCR analysis.

Analysis of the associations among SGHV and Wolbachia, Sodalis, and Wigglesworthia infection in wild tsetse populations
The associations among GpSGHV and Wolbachia, Sodalis, and Wigglesworthia were assessed by qPCR analysis. Tsetse fly samples were selected for qPCR analysis only if a given population of each species was characterized by the presence of two or three of the infection patterns (W+/V+), (W+/V−), and (W−/V+). Based on this criterion, 203 individual flies (78, 103, and 22 flies with an effective and robust mass production system of high-quality sterile tsetse flies for implementing SIT programs.

Statistical analysis
The proportion of single and double infections (GpSGHV and Wolbachia) in wild flies was analyzed by location and species and for all samples together using the Chi-squared test. The Chi-squared tests for independence, Spearman correlation coefficient, and Cochran-Mantel-Haenszel test for repeated tests of independence were performed using Excel 2010. P-values were calculated from the data with the significance threshold selected as 0.05.

The difference in Wigglesworthia, Sodalis, Wolbachia, and GpSGHV density between different locations and tsetse species and the correlation between densities as well as preparing figures were executed in R v 4.0.5 [61] using RStudio v 1.4.1106 [62, 63] with packages ggplot2 v3.3.2.1 [64], lattice v0.20-41 [65], car (version 3.1-0) [66], gghithms (version 4.2.4) [67], and MASS v7.3-51.6 [68]. All regression analyses of symbionts and GpSGHV densities were conducted using the generalized linear model (glm) for different tsetse species and different countries with analysis of deviance table (type II tests). Pearson correlation coefficient between the density of Wolbachia and Wigglesworthia and the log transformed density of GpSGHV and Sodalis was conducted in R. The analysis details are presented in Additional file 1. Overall similarities in Wolbachia, Wigglesworthia, Sodalis, and GpSGHV density levels between tsetse species, countries, and infection pattern were shown using the matrix display and metric multidimensional scaling (mMDS) plot with bootstrap averages in PRIMER version 7 + and were displayed with a Bray and Curtis matrix based on the square root transformation [69]. The tests were based on the
Table 1  SGHV and Wolbachia infection status of tsetse flies in natural populations of different Glossina species

| Glossina taxon | Country (area, collection date) | N  | W+/V+ | W+/V- | W-/V+ | W-/V- | χ²  | P   |
|----------------|---------------------------------|----|-------|-------|-------|-------|-----|-----|
| G. austeni     | Tanzania (Jozani, 1997)a         | 42 | 0     | 22    | 2     | 18    |     |     |
| G. austeni     | Tanzania (Zanzibar, 1995)ac      | 78 | 3     | 72    | 0     | 3     |     |     |
| G. austeni     | South Africa (Zululand, 1999)bc | 83 | 51    | 28    | 1     | 3     |     |     |
| G. austeni     | Coastal Tanzania (Muhoro, NA)    | 2  | 0     | 2     | 0     | 0     |     |     |
| G. austeni     | All locations                    | 205| 54    | 124   | 3     | 24    | 4.32| 0.04|
| G. brevipalpis | South Africa (Zululand, 1995)a  | 50 | 0     | 1     | 0     | 49    |     |     |
| G. brevipalpis | Coastal Tanzania (Muhoro, NA)    | 1  | 0     | 1     | 0     | 0     |     |     |
| G. brevipalpis | Coastal Tanzania (Muyuyu, NA)    | 1  | 0     | 1     | 0     | 0     |     |     |
| G. brevipalpis | All locations                    | 52 | 0     | 3     | 0     | 49    |     |     |
| G. f. fuscipes | Uganda (Buvuma Island, 1994)ab  | 53 | 0     | 6     | 47    |       |     |     |
| G. medicorum   | Burkina Faso (Comoe, 2008)f      | 94 | 2     | 18    | 7     | 67    | 0.01| 0.94|
| G. m. submorsitans | Burkina Faso (Nazinga, 2009)     | 3  | 0     | 0     | 0     | 3     |     |     |
| G. m. submorsitans | Burkina Faso (Comoe Folonzo, 2007) | 30 | 0     | 2     | 3     | 25    |     |     |
| G. m. submorsitans | Burkina Faso (Comoe, 2008)g     | 109| 0     | 4     | 9     | 96    |     |     |
| G. m. submorsitans | All locations                    | 142| 0     | 6     | 12    | 124   | 0.58| 0.45|
| G. p. palpalis | Democratic Republic of Congo (Zaire, 1995)h | 48 | 0     | 0     | 1     | 47    |     |     |
| G. tachinoides | Burkina Faso (Nazinga, 2009)     | 15 | 0     | 0     | 0     | 15    |     |     |
| G. tachinoides | Burkina Faso (Comoe Folonzo, 2007) | 112| 3     | 2     | 26    | 81    |     |     |
| G. tachinoides | Burkina Faso (Comoe, 2008)       | 72 | 0     | 0     | 8     | 64    |     |     |
| G. tachinoides | Ghana (Pong Tamale, Walewaile, 2008) | 46 | 0     | 5     | 0     | 41    |     |     |
| G. tachinoides | Ghana (Walewaile, 2008)          | 149| 0     | 27    | 6     | 116   |     |     |
| G. tachinoides | Ghana (Fumbissi, 2008)           | 39 | 0     | 0     | 0     | 39    |     |     |
| G. tachinoides | All locations                    | 433| 3     | 34    | 40    | 356   | 0.15| 0.70|
| G. m. morsitans | Coastal Tanzania (Utethe, NA)    | 3  | 0     | 2     | 0     | 1     |     |     |
| G. m. morsitans | Zambia (MFWE, Eastern Zambia, 2007)abc | 122| 26    | 96    | 0     | 0     |     |     |
| G. m. morsitans | Tanzania (Ruma, 2005)h           | 100| 29    | 71    | 0     | 0     |     |     |
| G. m. morsitans | Zimbabwe (Gokwe, 2006)h         | 74 | 0     | 7     | 8     | 59    |     |     |
| G. m. morsitans | Zimbabwe (Kemukura, 2006)h      | 26 | 0     | 26    | 0     | 0     |     |     |
| G. m. morsitans | Zimbabwe (M.Chiy, 1994)hbc      | 36 | 5     | 28    | 0     | 3     |     |     |
| G. m. morsitans | Zimbabwe (Makuti, 2006)hbc      | 99 | 11    | 84    | 1     | 3     |     |     |
| G. m. morsitans | Zimbabwe (Mukond, 1994)h         | 36 | 0     | 35    | 0     | 1     |     |     |
| G. m. morsitans | Zimbabwe (Mushumb, 2006)h       | 8  | 0     | 3     | 0     | 5     |     |     |
| G. m. morsitans | Zimbabwe (Rukomeshi, 2006)hbc   | 100| 8     | 90    | 0     | 2     |     |     |
| G. m. morsitans | All locations                    | 604| 79    | 442   | 9     | 74    | 1.07| 0.30|
| G. pallidipes  | Zambia (MFWE, Eastern Zambia, 2007)abc | 203| 1     | 4     | 97    | 101   |     |     |
| G. pallidipes  | Kenya (Mewa, Katotoi, Meru national park, 2007)h | 470| 0     | 0     | 10    | 460   |     |     |
| G. pallidipes  | Ethiopia (Arba Minch, 2007)h     | 454| 0     | 2     | 87    | 365   |     |     |
| G. pallidipes  | Tanzania (Ruma, 2005)hbc        | 83 | 2     | 1     | 42    | 38    |     |     |
| G. pallidipes  | Tanzania (Mlambuli and Tunguli, 2009)h | 94 | 0     | 0     | 0     | 94    |     |     |
| G. pallidipes  | Zimbabwe (Mushumb, 2006)h       | 50 | 0     | 0     | 1     | 49    |     |     |
| G. pallidipes  | Zimbabwe (Gokwe, 2006)h         | 150| 0     | 0     | 19    | 131   |     |     |
| G. pallidipes  | Zimbabwe (Rukomeshi, 2006)h     | 59 | 0     | 5     | 0     | 54    |     |     |
| G. pallidipes  | Zimbabwe (Makuti, 2006)hbc      | 96 | 1     | 3     | 5     | 87    |     |     |
| G. pallidipes  | Mainland Tanzania (Death Valley, NA) | 6  | 0     | 4     | 0     | 2     |     |     |
| G. pallidipes  | Coastal Tanzania (Muhoro, NA)    | 4  | 0     | 3     | 0     | 1     |     |     |
| G. pallidipes  | Coastal Tanzania (Muyuyu, NA)    | 3  | 0     | 3     | 0     | 0     |     |     |
| G. pallidipes  | All locations                    | 1672| 4     | 25    | 261   | 1382  | 0.09| 0.76|
| G. p. gambiensis | Senegal (Diacksao Peul and Pout, 2009)h | 188| 0     | 1     | 31    | 156   |     |     |
| G. p. gambiensis | Guinea (Kansaba, Mini Pontda, Kindooya, Ghada Oundou, 2009)h | 180| 0     | 0     | 13    | 167   |     |     |
multivariate null hypothesis via the non-parametric statistical method PERMANOVA [70]. The PERMANOVA test was conducted on the average of the qPCR density data based on the country-species sample.

### Results

#### Prevalence of co-infection with GpSGHV and Wolbachia in wild tsetse flies

Analysis of the Wolbachia and GpSGHV infection status for each individual tsetse adult in the previously reported data [7, 47, 58, 59] indicated that the single infection rate was 10.21% (n = 459) and 15.12% (n = 680) for GpSGHV and Wolbachia, respectively, over all taxa and locations combined (Additional file 4: Fig. S1A). No Wolbachia infection was found in two taxa, G. f. fuscipes and G. p. palpalis, and these were excluded from further examination (Table 1). A Cochran-Mantel-Haenszel test for repeated tests of independence showed that infection with GpSGHV and Wolbachia did not deviate from independence across all taxa ($\chi^2_{MH} = 0.848$, $df = 1$, n.s.), and individual Chi-squared tests for independence for each taxon did not show any significant deviation from independence at the Bonferroni corrected $\alpha = 0.00714$ (Additional file 3: Table S2). The prevalence of co-infection of GpSGHV and Wolbachia ($W^+/V^+$) in wild tsetse populations varied based on the taxon and the location (Table 1 and Additional file 4: Fig. S1B). No co-infection was found in G. brevipalpis, G. m. submorsitans, and G. p. gambiensis, and co-infection was absent in many locations in the remaining taxa. However, a low prevalence of co-infection was found in G. medicorum (2%), G. tachinoides (0.7%), and G. pallidipes (0.2%). A relatively high prevalence of co-infection was only observed in G. auseteni (26%) and G. m. morsitans (13%) (Additional file 4: Fig. S1B).

#### Impact of co-infection ($W^+/V^+$) on GpSGHV, Wolbachia, Sodalis, and Wigglesworthia density

**GpSGHV density**

The GpSGHV qPCR data showed overall no statistically significant difference between flies with different infection patterns ($W^+/V^+$), ($W^+ / V^+$), and ($W^+ / V^-$) ($X^2 = 1.4625$, $df = 2$, $P = 0.481$) regardless of tsetse taxon (Additional file 5: Fig. S2A). Moreover, no significant difference in GpSGHV copy number was observed between tsetse taxa ($X^2 = 0.752$, $df = 3$, $P = 0.861$) (Additional file 4: Fig. S1A). However, a significant difference in the virus copy number was observed between different countries ($X^2 = 16.234$, $df = 4$, $P = 0.0027$) where the virus copy number in the flies collected from Zambia

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Table 1 (continued)

| Glossina taxon | Country (area, collection date) | N  | $W^+/V^+$ | $W^+ / V^+$ | $W^+ / V^-$ | $X^2$ | $P$    |
|----------------|---------------------------------|----|-----------|-------------|-------------|------|-------|
| G. p. gambiensis | Guinea (Alahine, 2009)$^a$ | 29 | 0         | 3           | 26          |      |       |
| G. p. gambiensis | Guinea (Boureya Kolonko, 2009)$^b$ | 36 | 0         | 1           | 35          |      |       |
| G. p. gambiensis | Guinea (Fefe, 2009)$^b$ | 29 | 0         | 1           | 28          |      |       |
| G. p. gambiensis | Guinea (Kansaba, 2009)$^b$ | 19 | 0         | 4           | 15          |      |       |
| G. p. gambiensis | Guinea (Kindoya, 2009)$^b$ | 12 | 0         | 1           | 11          |      |       |
| G. p. gambiensis | Guinea (Lemonako, 2009)$^a$ | 30 | 0         | 4           | 26          |      |       |
| G. p. gambiensis | Guinea (Togoue, 2009)$^a$ | 32 | 0         | 1           | 31          |      |       |
| G. p. gambiensis | Guinea (Conakry, 2010) | 138 | 0         | 5           | 133         |      |       |
| G. p. gambiensis | Burkina Faso (Comoe, 2008) | 12 | 0         | 7           | 5           |      |       |
| G. p. gambiensis | Burkina Faso (Comoe Folonzo, 2007) | 53 | 0         | 14          | 38          |      |       |
| G. p. gambiensis | Burkina Faso (Kenedougou, 2007) | 37 | 0         | 1           | 36          |      |       |
| G. p. gambiensis | Burkina Faso (Houet Bama, 2007) | 69 | 0         | 1           | 41          | 27   |       |
| G. p. gambiensis | Guinea (Fefe, Togoue, Alahine, Boureya Kolonko, 2009–2010) | 94 | 0         | 5           | 89          |      |       |
| G. p. gambiensis | Guinea (Boureya Kolonko, Kansaba, Kindoya, Ghada Oundou, 2009–2010) | 94 | 0         | 3           | 91          |      |       |
| G. p. gambiensis | Mali (Fijira, 2009) | 14 | 0         | 0           | 14          |      |       |
| G. p. gambiensis | Senegal (Diaka Madia, 2009) | 42 | 0         | 0           | 42          |      |       |
| G. p. gambiensis | Senegal (Tambacounda, 2008) | 38 | 0         | 3           | 35          |      |       |
| G. p. gambiensis | Senegal (Simenti, 2008) | 33 | 0         | 6           | 27          |      |       |
| G. p. gambiensis | Senegal (Kédougou, 2008) | 15 | 0         | 1           | 14          |      |       |
| G. p. gambiensis | All locations | 1194 | 0         | 28          | 120         | 1046 | 3.20 | 0.07 |

$^a$ In these samples, the presence of Wolbachia was tested in Doudoumis et al. [7]

$^b$ The individuals of G. f. fuscipes were considered negative for Wolbachia based on the results of the initial PCR amplification. The results from the reamplification method were not considered so that the conditions were consistent for all species

$^c$ Samples used for qPCR analysis to determine the density of Wigglesworthia, Sodalis, Wolbachia, and GpSGHV
was significantly lower than those collected from South Africa, Tanzania, and Zimbabwe (Additional file 1 and 6: Fig. S3A).

**Wolbachia density**
The copy number of *Wolbachia* infection was significantly different between tsetse taxa ($X^2 = 6.568$, $df = 2$, $P = 0.037$) (Additional file 4: Fig. S1B), between the infection statuses ($X^2 = 23.723$, $df = 2$, $P < 0.001$) (Additional file 5: Fig. S2B), and between the countries ($X^2 = 73.507$, $df = 3$, $P < 0.001$) (Additional file 6: Fig. S3B). Wolbachia density was significantly higher in *G. m. morsitans* than in *G. austeni* ($t = 2.029$, $df = 1$, $P = 0.0478$) (Additional file 1 and 4: Fig. S1B).

Overall, a significant difference in Wolbachia density was observed in the flies with different infection patterns previously determined by conventional PCR, where flies with a $(W^+/V^-)$ infection pattern showed significantly higher Wolbachia density than flies with a $(W^-/V^+)$ infection pattern regardless of the tsetse species ($X^2 = 23.723$, $df = 2$, $P < 0.001$). This trend was observed in *G. m. morsitans* ($t = 3.184$, $P = 0.0022$) (Additional file 1). The Wolbachia density was highest in the flies collected from Zambia (Additional file 6: Fig. S3B). Analyzing only the flies with co-infection $(W^+/V^+)$ indicated that the Wolbachia density was statistically significantly higher in *G. m. morsitans* than in *G. austeni* ($t = -2.353$, $df = 1$, $P = 0.024$) (Additional file 1 and 7: Fig. S4B).

**Interaction between GpSGHV infection and Wolbachia, Wigglesworthia, and Sodalis infection**
The qPCR results of both *Wigglesworthia* and *Sodalis* in tsetse adults with different infection patterns $(W^+/V^-)$, $(W^-/V^+)$, and $(W^+/V^+)$ indicated that *Wigglesworthia* density varies significantly between different infection patterns ($X^2 = 10.706$, $df = 2$, $P = 0.0047$) and its density in flies with co-infection $(W^+/V^+)$ was significantly lower than in those with Wolbachia infection only $(W^+/V^-)$ ($t = 3.137$, $df = 2$, $P = 0.0024$) but did not differ significantly from flies with virus infection only $(W^-/V^+)$ ($t = 1.656$, $P = 0.102$) (Additional file 5: Fig. S2C). *Wigglesworthia* density varies also between tsetse taxa ($X^2 = 33.479$, $df = 4$, $P < 0.001$) with higher density in *G. m. morsitans* and *G. pallidipes* than in *G. austeni* (Additional file 4: Fig. S1C) as well as between countries ($X^2 = 10.706$, $df = 2$, $P = 0.0047$) (Additional file 1 and 6: Fig. S3C). *Sodalis* density also varies between tsetse taxa ($X^2 = 21.612$, $df = 3$, $P < 0.001$) (Fig. 1D) and between countries ($X^2 = 21.179$, $df = 4$, $P < 0.001$) (Additional file 6: Fig. S3D) but there was no significant difference between tsetse flies with different infection patterns ($X^2 = 0.63888$, $df = 2$, $P = 0.727$) (Additional file 1 and 5: Fig. S2D).

Analyzing the pairwise correlation between the GpSGHV and each of the tsetse endosymbionts in *G. austeni* and *G. m. morsitans* (species with the highest number of flies with co-infection) indicated different types of correlation based on the insect taxa. In *G. m. morsitans*, the GpSGHV density has a significant negative correlation with *Wolbachia* density ($r = -0.558$, $t = -4.150$, $df = 38$, $P < 0.001$). No flies were observed with high virus density ($> 10^{3.3}$ copy number) when *Wolbachia* density was high ($> 10^{5.3}$ copy number), although this observation should be considered with caution as it is based on a small sample size. Contrary to *Wolbachia*, GpSGHV has a significant positive correlation with *Wigglesworthia* ($r = 0.531$, $t = 3.868$, $df = 38$, $P < 0.001$) but no correlation with *Sodalis* density ($r = 0.203$, $t = 1.276$, $df = 38$, $P = 0.209$). *Wolbachia* density also showed significant negative correlation with *Wigglesworthia* density ($r = -0.637$, $t = -5.095$, $df = 38$, $P < 0.001$). No flies with high *Wigglesworthia* density ($> 10^6$ copy number) were detected when *Wolbachia* density was high ($> 10^{7.3}$ copy number). In contrast, *Sodalis* density did not show significant correlation with either *Wolbachia* ($r = 0.193$, $t = 1.214$, $df = 38$, $P = 0.232$) or *Wigglesworthia* densities ($r = 0.072$, $t = 0.443$, $df = 38$, $P = 0.66$) (Fig. 2, Additional file 1). In *G. austeni*, the only significant correlation was found to be positive between *Sodalis* and *Wigglesworthia* density ($r = 0.602$, $t = 2.386$, $df = 10$, $P = 0.038$) (Fig. 2, Additional file 1).

The qPCR results showed that Wolbachia-infected flies had relatively high Wolbachia density (median $10^{7.3}$ copies/fly) compared to the GpSGHV and other tsetse symbionts (*Wigglesworthia* and *Sodalis*) regardless of the species, country, or infection pattern (Fig. 3). The heat map analysis of the qPCR data of *G. austeni* and *G. m. morsitans* clearly indicates the contrast between Wolbachia copy number and *Wigglesworthia* copy number considering the infection pattern, tsetse taxa, or countries. In addition, it clearly shows the low copy number of GpSGHV in the samples showing a high Wolbachia copy number (Fig. 3, Additional file 8: Fig. S5). The bootstrap averages of the metric multidimensional scaling (mMDS) produced clusters based on the species, country, and infection pattern (Fig. 4). The PERMANOVA analysis of the density of GpSGH, Wolbachia, Wigglesworthia, and *Sodalis* based on the country, tsetse species, and infection pattern indicated that the clusters observed between infection pattern ($P = 0.026$) and country ($P = 0.001$) were statistically significant. The interaction between country and infection pattern was not statistically significant ($P = 0.123$) (Table 2).
**Discussion**

The prevalence of GpSGHV and *Wolbachia* in natural tsetse populations clearly indicated that the two infections were independent (not correlated) in most of the tested tsetse species with only *G. m. morsitans* and *G. austeni* presenting a high proportion of co-infections. However, the number of co-infections originally determined by conventional PCR may have been...
underestimated with conventional PCR as the qPCR analysis carried out in the frame of the present study clearly indicated that a number of initially considered virus-free samples were found to be positive, albeit at low density. It should also be noted that the *Wolbachia* strains infecting *G. m. morsitans* and *G. austeni* are closely related but different, as has been shown by both MLST analysis and, more recently, genome sequencing [7, 40, 71].

Analysis of *G. morsitans* and *G. austeni* co-infected samples suggested that low density of GpSGHV is associated with high density of *Wolbachia*. Due to the low number of individuals showing this correlation, further analysis is required. Moreover, the screen of wild tsetse populations for GpSGHV and *Wolbachia* infection indicated that not all *Glossina* species harbor *Wolbachia* or GpSGHV. Furthermore, *Wolbachia* and GpSGHV prevalence was found to differ not only between different

**Fig. 2** Interaction between the GpSGHV and tsetse endosymbionts *Wigglesworthia*, *Wolbachia*, and *Sodalis* in natural populations of *G. austeni* and *G. m. morsitans*. The density of tsetse symbionts was analyzed by qPCR, and the data of each two organisms were plotted in R. The density of GpSGHV was plotted versus the density of *Wolbachia* (A), *Sodalis* (B), and *Wigglesworthia* (C). The density of *Wigglesworthia* was plotted versus *Wolbachia* (D), and the density of *Sodalis* was plotted versus *Wolbachia* (E) and *Wigglesworthia* (F). Vertical bar A and D indicates the *Wolbachia* density at $10^8$ and $10^{8.2}$ copy number, respectively.
Fig. 3  Relative density of GpSGHV, Wigglesworthia, Sodalis, and Wolbachia in G. austeni and G. m. morsitans field-collected tsetse flies. The density of GpSGHV and tsetse symbionts was analyzed by qPCR. Data were transformed to square root and averaged based on country (A), tsetse species (B), and infection status (Sample) (C). The top and the left of the graph indicate the group averaged Bray-Curtis similarity.
Fig. 4 Metric multidimensional scaling (mMDS) of GpSGHV, Wigglesworthia, Sodalis, and Wolbachia relative density in field-collected tsetse flies. The mMDS of GpSGHV, Wigglesworthia, Sodalis, and Wolbachia relative density was performed in respect to infection status (Sample) (A), tsetse species (B), or country (C). av average.
tsetse host species but also between different populations within the same tsetse species [7, 11, 47, 58, 59, 72].

The potential negative impact (antagonistic effect) of Wolbachia density on the GpSGHV density in natural tsetse populations is in agreement with the recent report on the interaction of Wolbachia and GpSGHV infection in colonized tsetse populations [54]. However, the number of tested flies was not equally distributed between the tsetse taxa and locations, which might explain the lack of detected co-infections in some taxa and, therefore, the low number of taxa (G. austeni and G. m. morsitans) used for investigating the interactions between the GpSGHV and tsetse symbionts. The negative correlation between Wolbachia and GpSGHV infections was also reported in wild-caught G. f. fuscipes collected from Uganda [72]. This conflicts with our findings as no G. f. fuscipes flies with GpSGHV were reported, which might be due to the low number of tested flies used in our study (n = 53).

Several reports have discussed and well documented the negative effect of Wolbachia on RNA viruses in different insect models such as mosquitoes and Drosophila [73–75], although there have also been reports about Wolbachia enhancement of both RNA and DNA viruses [76–78]. It is worth mentioning that the negative correlation of Wolbachia with GpSGHV was observed only when Wolbachia density was high as the results show the absence of high density (>10^7.5) GpSGHV infection with high-density Wolbachia infection (>10^5). However, at low Wolbachia density co-infection occurs with a prevalence of > 10%. Although our study indicated a correlation between high-density Wolbachia and low-density GpSGHV, previous reports suggested that the negative impact of Wolbachia on insect viruses is density dependent [76, 79].

The assessment of the infection density (copy number per fly) of all four microbes (GpSGHV, Wolbachia, Wigglesworthia, and Sodalis) in the same tsetse flies indicated that Wolbachia infection at high density has a significant negative correlation with Wigglesworthia infection in G. m. morsitans but not in G. austeni. However, the latter might be due to the low number of analyzed G. austeni flies (n = 21) compared to G. m. morsitans (n = 91). On the other hand, Wolbachia density levels do not correlate with Sodalis. The nature of the negative interaction between Wolbachia and Wigglesworthia is unclear. Whether this negative correlation between Wolbachia and Wigglesworthia is present in other tsetse species beyond G. m. morsitans remains to be seen.

The positive correlation between GpSGHV infection and Wigglesworthia infection observed in G. m. morsitans conflicts with the negative correlation observed in the same species of colonized flies [54]. This result might reflect a specific adaptation between a specific strain of Wigglesworthia, which reacts in a specific way to increase its density in the presence of GpSGHV as a manner to restore and enhance the host immune system against the virus infection [80]. The difference in the interaction between the GpSGHV and Wigglesworthia between the results of this study and the results of Demirbas-Uzel et al. [54] might be due to: (i) difference in the host strain/genotype as the G. m. morsitans individuals were collected from several countries in east Africa (Tanzania, Zambia, and Zimbabwe) while the colonized flies originated from Zimbabwe and have been maintained in the colony since 1997; (ii) different strain(s) of Wigglesworthia circulating in the field samples compared to the ones present in colonized flies [60]; (iii) different strain(s) of the GpSGHV in the field samples [58]; (iv) difference between field and laboratory conditions where the stress from handling the large number of flies in high density in the laboratory might negatively affect Wigglesworthia density levels and/or performance. The same reasons may also explain the difference observed between field and

| Source                  | df  | SS     | MS   | Pseudo-F | P(perm) | Uniqueperms |
|-------------------------|-----|--------|------|----------|---------|-------------|
| Country                 | 2   | 657.53 | 328.77 | 13.003   | 0.001   | 999         |
| Species                 | 0   | 0      | No test | No test | No test |
| Infection status        | 2   | 188.97 | 94.487 | 3.7369   | 0.026   | 998         |
| Country x species       | 0   | 0      | No test | No test | No test |
| Country x infection status | 2  | 100.59 | 50.296 | 1.9892   | 0.123   | 999         |
| Species x infection status | 0  | 0      | No test | No test | No test |
| Country x species x infection status | 0  | 0      | No test | No test | No test |
| Res                     | 104 | 2629.6 | 25.285 |
| Total                   | 113 | 4681.3 |       |

Within the table, statistically significant differences (P < 0.05) are shown in bold

Perm(s) permutations
laboratory samples regarding the interactions between GpSGHV and Sodalis.

Conclusions

The present study, despite its limitations regarding the size of samples and the lack of knowledge about the age, nutritional and trypanosome infection status, and environmental conditions at the time of collection of field specimens, shows a snapshot image of the density levels of tsetse symbionts and SGHV under field conditions and clearly indicates that the interactions/association between the tsetse host and its associated microbes are dynamic and likely species specific, and significant differences may exist between laboratory and field conditions. Further studies are needed to clarify the interaction between tsetse symbionts and GpSGHV under field conditions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13371-022-05336-9.

Additional file 1: Interactions between tsetse endosymbionts and Glossina pallidipes salivary gland hypertrophy virus in wild tsetse populations.

Additional file 2: Table S1. List of primers used for quantitative PCR (qPCR) analyses in Glossina species.

Additional file 3: Table S2. Cochran-Mantel-Haenszel test for repeated tests of independence with continuity correction on the coingestion of GpSGHV and Wolbachia in wild tsetse species.

Additional file 4: Figure S1. Prevalence of GpSGHV and Wolbachia coinfection in natural tsetse populations. A: In all tsetse species; B: in each tsetse species. GpSGHV and Wolbachia prevalence was determined by PCR as described previously [7,47].

Additional file 5: Figure S2. Density levels of GpSGHV (A), Wolbachia (B), Wigglesworthia (C), and Sodalis (D) determined by qPCR in tsetse flies with different GpSGHV and Wolbachia infection statuses. The copy number was determined by qPCR. Values indicated by a different small letter differ significantly at the 5% level. W+/V−: flies infected only with Wolbachia; W−/V+: flies infected only with Wigglesworthia; W−/V+: flies infected only with GpSGHV. GpSGHV and Wolbachia infection status was determined by conventional PCR as described previously [7,47].

Additional file 6: Figure S3. Density levels of GpSGHV (A), Wolbachia (B), Wigglesworthia (C), and Sodalis (D) in tsetse flies collected from different countries. The copy number was determined by qPCR. Values indicated by a different small letter differ significantly at the 5% level.

Additional file 7: Figure S4. Impact of GpSGHV and Wolbachia co-infection (W+/V+) on the density levels of GpSGHV (A), Wolbachia (B), Wigglesworthia (C), and Sodalis (D) in different tsetse species. The copy number was determined by qPCR. Values indicated by the same lower case letter do not differ significantly at the 5% level.

Additional file 8: Figure S5. Relative density of GpSGHV, Wigglesworthia, Sodalis, and Wolbachia in G. austeni and G. m. morsitans field-collected tsetse flies. The density of GpSGHV and tsetse symbionts was analyzed by qPCR. Data were transformed to square root and averaged based on countries and species (A), countries and infection status (sample) (B), and countries, species, and infection status (sample) (C). The top and the left of the graph indicate the group averaged Bray-Curtis similarity.

Acknowledgements

The authors thank Ms. Carmen Marín, Mr. Adun Henry, and Mr. Abdul Hasim Mohammed for their technical support.

Author contributions

MMD and DUG performed the experiments, analyzed the data, and drafted the manuscript. AAA and VD performed the experiments, and critically revised the manuscript. AGP analyzed the data and critically revised the manuscript. GT critically revised the manuscript. KB conceived the study, designed the experiments, interpreted the data, contributed to the drafting, and critically revised the manuscript. AMMA conceived the study, designed the experiments, interpreted the data, and drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Funding

This study was supported by the Joint FAO/IAEA Insect Pest Control Subprogramme.

Availability of data and materials

Materials described in the paper, including all relevant raw data, are available in this link. https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/K15POF.

Declarations

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential competing interests.

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Received: 27 June 2022 Accepted: 7 October 2022 Published online: 29 November 2022

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