Diversity of fall armyworm, *Spodoptera frugiperda* and their gut bacterial community in Kenya

Joseph Gichuhi[1,2], Sevgan Subramanian[1], Fathiya M. Khamis[1], Johnnie Van den Berg[2], Hannalene du Plessis[2], Sunday Ekesi[1], and Jeremy K Herren[1,3]

[1] International Centre of Insect Physiology and Ecology (ICIPE), Kasarani, Nairobi, Kenya

[2] Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa,

[3]MRC-University of Glasgow Centre for Virus Research, Henry Wellcome Building, Glasgow, UK

For correspondence: jherren@icipe.org Tel: +254716660160

Abstract

The invasive fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a polyphagous pest that causes widespread damage particularly to maize and sorghum in Africa. The microbiome associated with *S. frugiperda* could play a role in their success and adaptability. However, these bacterial communities remain poorly studied, especially for *S. frugiperda* in Africa. We investigated the composition, abundance and diversity of microbiomes associated with larval and adult specimens of *S. frugiperda* collected from four maize growing regions in Kenya through high throughput sequencing of bacterial 16S rRNA gene. We identified Proteobacteria and Firmicutes as the most dominant phyla and lesser proportions of Bacteroidetes and Actinobacteria. We also observed differences in bacterial microbiome diversity between larvae and adults that are a likely indication that some prominent larval bacterial groups are lost during metamorphosis. Several bacterial groups were found in both adults and larvae suggesting that they are transmitted across developmental stages. Reads corresponding to several known entomopathogenic bacterial clades as well as the non-bacterial entomopathogen, *Metarhizium rileyi* (Farl.) Kepler, Rehner & Humber (2014), were observed. Mitochondrial DNA haplotyping of the *S. frugiperda* population in Kenya indicated the presence of both 'Rice' and 'Corn' strains, with a higher prevalence of the 'Rice' strain. Insights into the microbiota may ultimately provide alternative avenues for controlling of this pest.

Keywords: Fall armyworm, *Spodoptera frugiperda*, gut bacteria, 16S sequencing, corn strain, rice strain, mtDNA haplotype

Introduction

Invasions by exotic pests can have major detrimental effects on agricultural production and natural resources (Huber et al. 2002). The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a polyphagous pest that is native to tropical regions of the western hemisphere, where it is known for its ability to cause economic damage to several crop species. In 2016, it was first detected in West Africa (Goergen et al. 2016), and since then this pest has rapidly spread across the continent (Goergen et al. 2016; Tindo et al. 2016; Day et al. 2017; Nagoshi et al. 2017, 2018; Cock et al. 2017; Rwomushana et al. 2018; Uzayisenga et al. 2018; Jacobs et al. 2018). By 2018, *S. frugiperda* was reported

present in all countries in Sub-Saharan Africa except Djibouti and Lesotho (Rwomushana et al. 2018). Furthermore, *S. frugiperda* has now also reached the Asian continent (Deole and Paul 2018; Sisodiya et al. 2018). Maize and other economically important food crops in these regions are extensively damaged by *S. frugiperda* larvae (Day et al. 2017) causing extensive economic losses threatening food security.

There is a lack of information about *S. frugiperda* - host plant interactions and other factors that may be leading to the rapid spread of *S. frugiperda* in the geographic regions that have recently been invaded. Many of the control measures used in the western hemisphere (e.g. transgenic maize, chemical insecticides) might not be readily available and economically viable for subsistence farmers in Africa. Further, the use of highly hazardous pesticides is not considered a sustainable long term control measure for any pest (FAO 2018). In addition, *S. frugiperda* is known to readily develop resistance to most chemical insecticides (e.g. pyrethroids, organophosphates and carbamates) and to transgenic maize that are used in its control (Yu 1991; Jakka et al. 2016; Banerjee et al. 2017; Flagel et al. 2018; Botha et al. 2019). In light of this, there is a great need for alternative, cost-effective control strategies for *S. frugiperda* (FAO 2018). A recent survey in Ethiopia, Kenya and Tanzania indicated that *S. frugiperda* has established interactions with indigenous parasitoid species (Sisay et al. 2018) that could be harnessed for biological control. A study on *S. frugiperda* host plant interactions in East Africa has also suggested a climate adapted push-pull system (Midega et al. 2018) and maize-legume intercropping (Hailu et al., 2018) for management of pests including fall armyworm on maize farms. However, many factors related to *S. frugiperda* rapid spread, host plant interactions, bio-ecology and insect-microbiome interactions in the African region remain poorly understood.

Insect microbiomes can have important consequences for the outcome of insect pest-natural enemies- host plant interactions (Ferrari et al. 2011). Strategies that involve modifying insect microbiomes are currently being evaluated for control and management of pests and vectors of plant diseases (Crotti et al. 2012; Perilla-henao and Casteel 2016; Arora and Douglas 2017; Beck and Vannette 2017). Insect microbiomes play a key role in the adaptation of insect to their environment and are therefore a major and often poorly understood determinant of the host plant and geographic range of insect pests (Su et al. 2013). In general, a greater diversity of microbial symbionts exist within the insect's gut lumen, while few others exist inside cells of the host, or on the cuticle (Douglas 2016). Gut microbial symbionts are known to influence their host's nutrition, usually by promoting digestion and availability of nutrients (Douglas 2009). These symbionts can also modulate the immune response and accessibility of the host to invading organisms, and therefore have direct or indirect effects on host susceptibility to parasites and pathogens (Dillon et al. 2005; Dong et al. 2009; Garcia et al. 2010; Vorburger et al. 2010; Narasimhan et al. 2014; Mclean and Godfray 2015; Vorburger and Rouchet 2016; Ubeda et al. 2017). Previous studies have also identified important roles of bacterial symbionts in the interactions between phytophagous insects and host plants (Frago et al. 2012; Biere and Bennett 2013; Brady and White 2013). In addition, microbial symbionts can break down complex molecules such as insecticides and promote insecticide resistance (Kikuchi et al.

2012; Xia et al. 2018). It is also notable that pathogenic bacteria can reside in host guts, only initiating or facilitating pathogenesis under certain conditions (Wei et al. 2017). Studying the gut microbiome is not only important from the standpoint of understanding mutualistic relationships but also for the potential development of microbial biocontrol agents.

There are an increasing number of studies examining the microbial diversity of lepidopterans. While in some of the assessed species, consistent bacterial communities have been observed in both field and laboratory collected populations as well as in insects reared on different diets (Broderick et al. 2004; Xiang et al. 2006; Pinto-Tomás et al. 2011), other studies reported no host specific resident communities that occurred, regardless of the insect diet (Hammer et al. 2017). It is possible that lepidopterans are less prone to forming robust 'core' microbiomes due to several factors: 1) very high pH in the midgut, 2) low retention time of food, 3) lack of microbe housing structures in the intestinal tract, and 4) continual replacement of the peritrophic matrix (Hammer et al. 2017). Nevertheless, bacterial communities do continually associate with lepidopterans and influence a variety of important host processes (Broderick et al. 2006; Anand et al. 2010; Wang et al. 2017).

Few studies have assessed the *Spodoptera*-associated gut microbiome. In a recent study, the microbial diversity of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was examined by 16S rDNA sequence profiling (Gao et al. 2018). In *Spodoptera exigua,* the dominant bacterial clades are Proteobacteria and Firmicutes, with the predominant genus in larvae being *Enterococcus*. In *S. frugiperda*, previous studies have isolated several bacterial strains using culture-dependent methods (De Almeida et al. 2017; Acevedo et al. 2017). In this study, we used 16S rDNA sequence profiling to characterize the diversity of bacteria associated with populations of *S. frugiperda* in Kenya and assessed the prevalence of the corn and rice strains in these populations using Mitochondrial COI gene sequences.

Materials and methods

Insect collection

Spodoptera frugiperda larvae were collected from infested maize fields in Kenya between June and December 2017 at the following locations: Ngeria (N00.37024 E035.9862) and Burnt Forest (N00.22505 E035.42479) in Uasin Gishu County; Msamia, Kitale (N00.98009 E034.97170) in Trans Nzoia County; Shimba Hills (S04.33228 E039.34361) in Kwale County and Chala Irrigation Scheme (S03.27338 E037.13816) and Wundanyi (S03.337538 E038.33612) in Taita Taveta County. Part of the field collected insects from each sampled region in Kenya were reared on fresh maize leaves in ventilated cages to pupation and eclosion at 27 °C and 60% humidity, while the rest were stored in absolute ethanol at -20°C.

DNA extraction and 16S rDNA sequencing

106 Guts from 9 live stage 5-6 larvae and 9 one-day old emerging adults from the Kenya collected samples
107 were dissected separately in phosphate buffered saline (PBS) following surface sterilization and used for
108 DNA extraction. Insects were surface sterilized in 70% ethanol, in 5% v/v sodium hypochlorite solution
109 followed by 3 washes in PBS for 3 minutes in each solution. DNA was extracted using the ISOLATE II
110 Genomic DNA Kit (Bioline, London, UK) according to the manufacturer's instructions. DNA extracted from
111 gut samples was submitted for high throughput sequencing targeting the v4 region of the bacterial 16s
112 rRNA gene using the Illumina Miseq platform (Center for Integrated Genomics, University of Lausanne,
113 Switzerland). Sequence reads were checked for quality using FastQC v 0.11.28 (Andrews 2010) and pre-
114 processed to remove adapters and sequencing primers using Cutadapt v1.18 (Martin 2011). Forward and
115 reverse reads were imported into the QIIME2-2018.11 (Boylen et al. 2018). The deblur plugin (Amir et
116 al. 2017) was used to further filter the reads based on per base quality scores, merge the paired-end-
117 reads and cluster reads into operational taxonomic units (OTUs). A total of 457501 sequence reads were
118 retained after removal of spurious reads and all reads shorter than 220 nucleotides in length for further
119 analysis. These sequences clustered into 1796 OTUs. Of these, 197 OTUs survived low count and
120 interquartile range-based variance filtering to eliminate OTUs that could arise from sequencing errors and
121 contamination. Taxonomic assignment was done using the blast classifier against the Silva132 reference
122 database (Quast et al. 2013) at a 99% identity cut-off. OTUs initially characterized as "*Candidatus*
123 *Hamiltonella*" by comparison to the Silva132 reference database were re-analyzed by homology searches
124 against the NCBI nr nucleotide database through blast (Altschul et al. 1990) and found to be *Pseudomonas*,
125 highlighting a potential incorrect assignment in the reference database. OTU prevalence and variance
126 based filtering as well as alpha and beta diversity measures were applied to the data in the Microbial Analyst
127 Marker Data Profiling (Dhariwal et al. 2017). Shannon diversity indices were applied along with Mann-
128 Whitney and analysis of variance statistics in profiling alpha diversity between sets of samples. Beta
129 diversity was evaluated using Bray-Curtis and unweighted Unifrac distances. Significance testing was done
130 using analysis of group similarities (ANOSIM) and non-metric multidimensional scaling (NMDS) used for
131 ordination. The empirical analysis of digital gene expression data in R (edgeR) algorithm (Robinson et al.
132 2009) was used to evaluate differential abundance of bacterial genera reads between sample groups. All
133 sequence reads were archived in the Sequence Read Archive (SRA) under the BioProject: PRJNA521837.

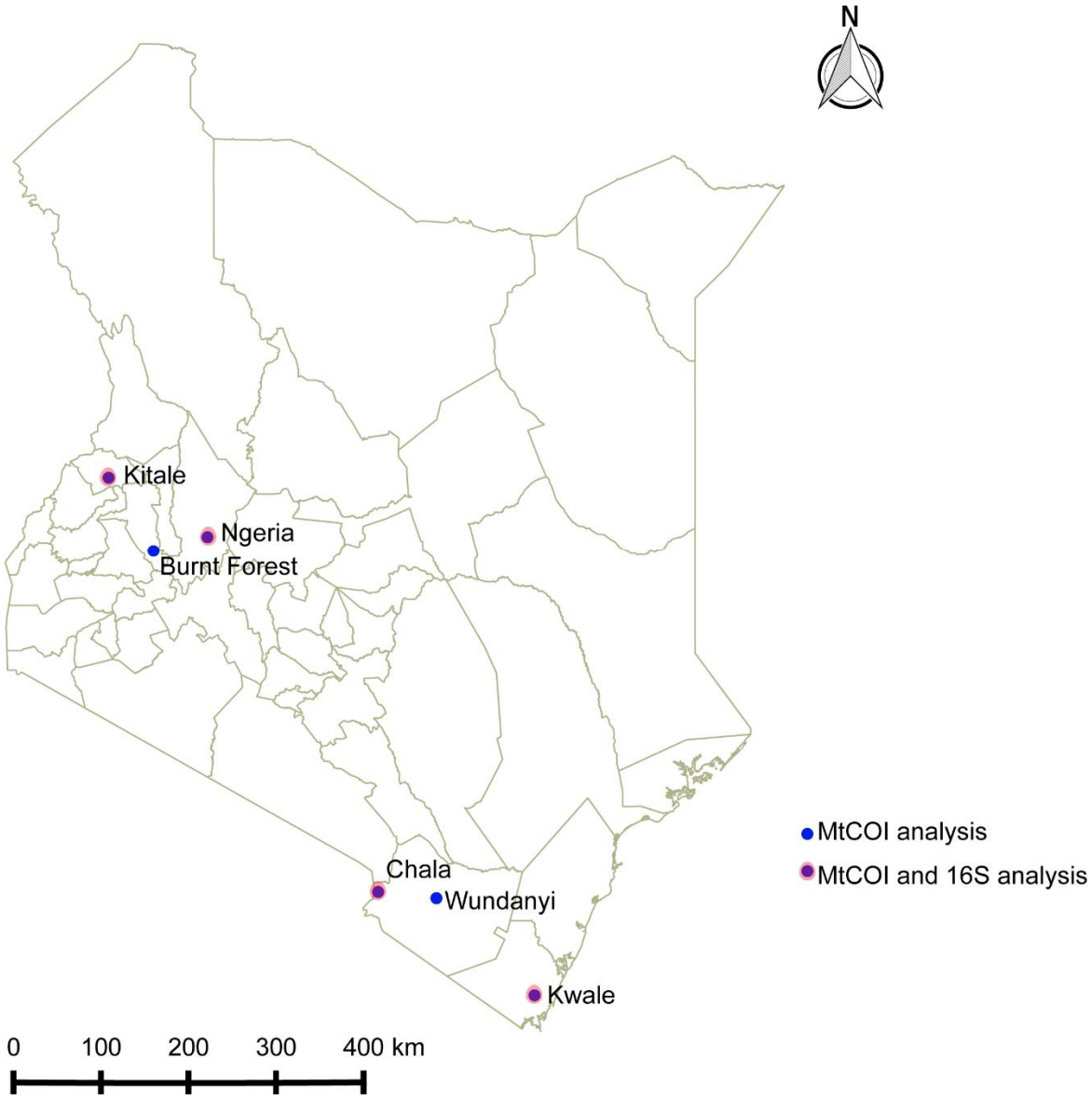
134 **mtDNA haplotyping**

135 DNA was extracted from surface-sterilized whole insects using the ISOLATE II Genomic DNA Kit (Bioline,
136 London, UK) according to the manufacturer's instructions. Mitochondrial COI gene sequences were
137 amplified from insect DNA by PCR using the primer LCO1490 and HCO2198 (Folmer et al. 1994).
138 Reactions were set up in total volumes of 10 µl each, containing 5× MyTaq reaction buffer (5 mM dNTPs,
139 15 mM MgCl2, stabilizers and enhancers) (Bioline, London, UK), 2 µM of each primer, 0.25 mM MgCl2
140 (Thermo Fischer Scientific, Massachusetts, USA), 0.125 µl MyTaq DNA polymerase (Bioline, London, UK),
141 and 7.5 ng/µl of DNA template. These reactions were set up in a Master cycler Nexus gradient thermo-

142 cycler (Thermo Fischer Scientific, Massachusetts, USA) using the following cycling conditions: initial
143 denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 45 s at 50.6 °C and 1 min at 72 °C,
144 then a final elongation step of 10 min at 72 °C. PCR products were run through 1% agarose gel
145 electrophoresis and visualized by ethidium bromide staining and UV trans-illumination. Direct sequencing
146 was done for all host mtCOI gene and the sequences deposited in the GenBank.

147 **Results**

148 We profiled the bacterial microbiome for 18 samples from 4 different locations in Kenya. In addition,
149 samples were collected from these 4 sites plus two additional sites for mtDNA haplotyping (Fig. 1).



150

151 **Fig. 1** Sites from which *Spodoptera frugiperda* larvae were collected in Kenya

The most abundant bacterial Phyla observed across the fall armyworm gut samples were Proteobacteria, Firmicutes, Bacteroidetes and a small proportion of Actinobacteria (Fig.10, Supplementary material). OTUs clustering in the orders Enterobacteriales and Pseudomonadales were predominant in the majority of the samples (Fig. 2).

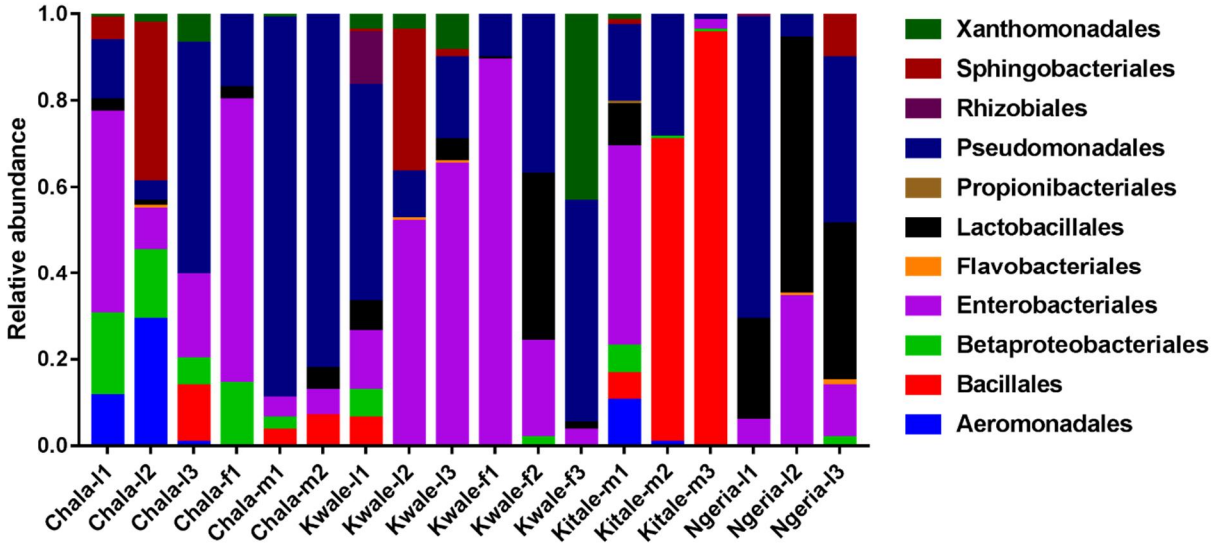


Fig. 2 Composition of bacterial OTUs at Order level in screened *Spodoptera frugiperda* samples

We note that despite the high genus-level diversity between samples (Fig. 3), there were some similarities based on developmental stage and location. For example, there was a very high proportion of: 1) *Pseudomonas* in the two adult male samples from Chala, 2) *Citrobacter* in two larval samples from Kwale, 3) *Lysinibacillus* in two male samples from Kitale and 4) *Enterococcus* in two larval samples from Ngeria. It was noted that *Stenotrophomonas*, *Sphingobacterium*, *Serratia*, *Pseudomonas*, *Morganella*, *Enterococcus* and *Delftia* were observed in both larvae and adult samples.

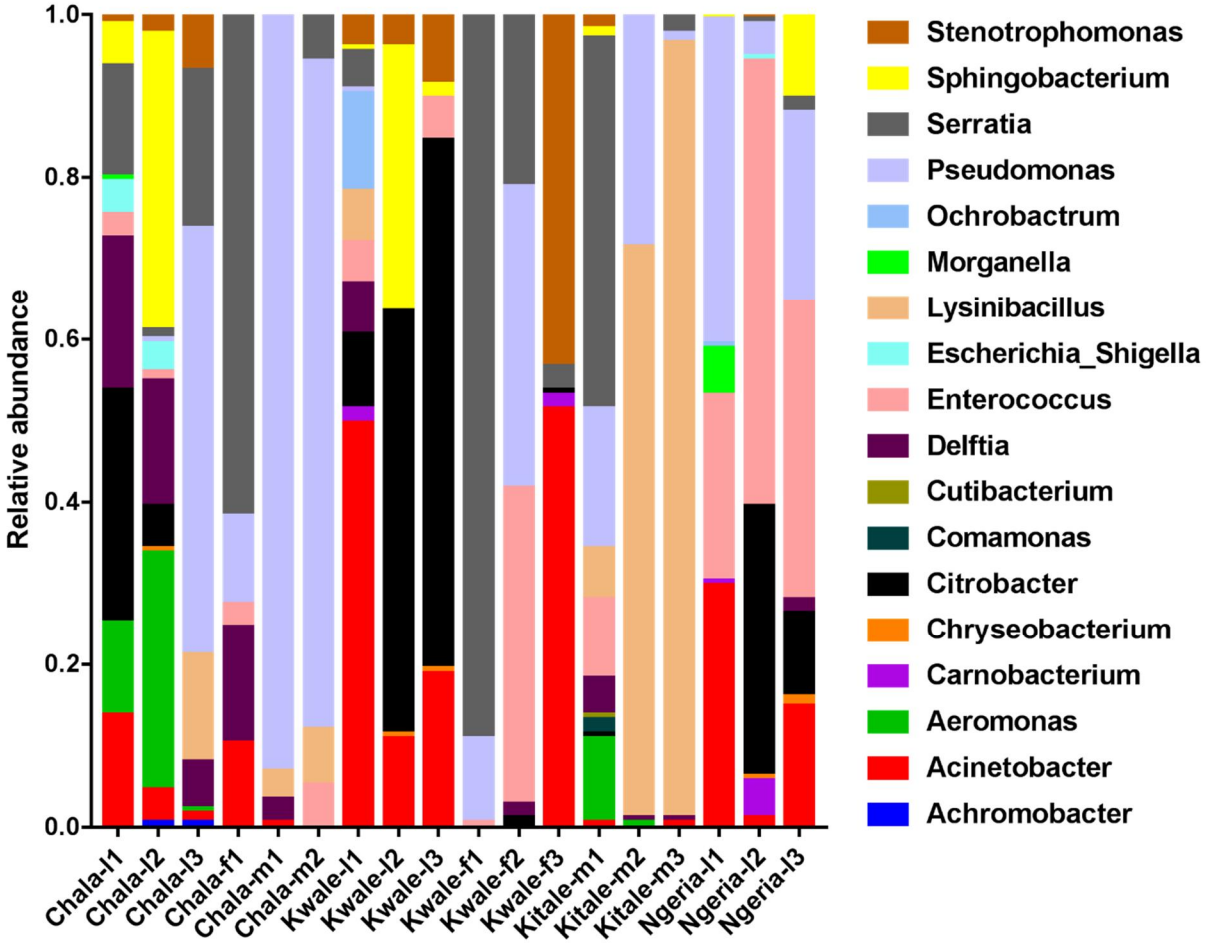


Fig. 3 Genus level composition of (% of OTUs) in the different samples of *S. frugiperda*. The relative abundances of the 19 most abundant genera are represented here

In one of the larval samples from the Ngeria site (Ngeria-l2), we observed an excessive number of non-bacterial reads. Upon closer inspection, these were found to be closely related to *Metarhizium rileyi* (Farl.) Kepler, Rehner & Humber (2014) (formerly *Nomuraea rileyi*), an entomopathogenic fungi that is known to infect *S. frugiperda* (Fig. 4).

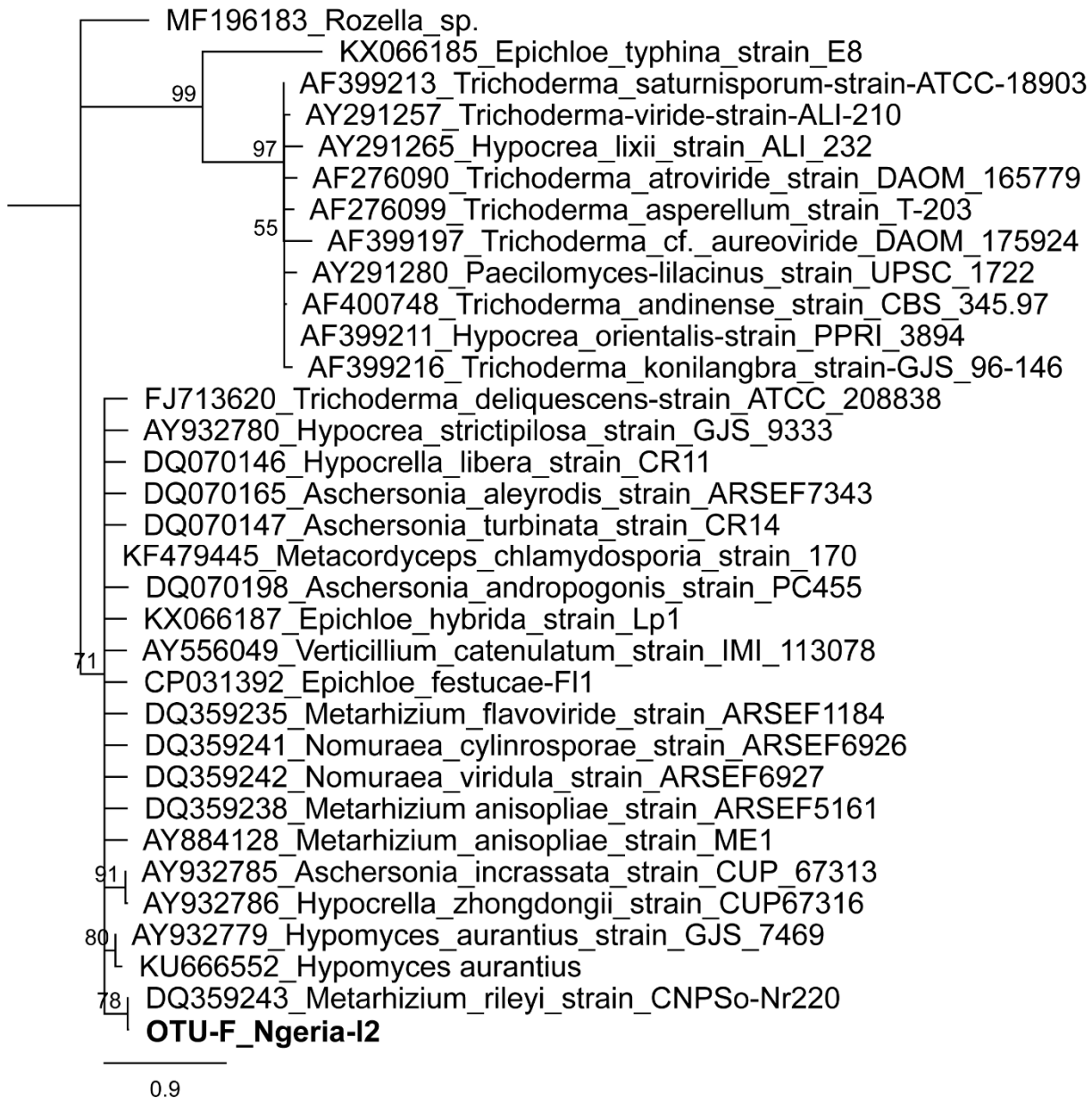


Fig. 4 Neighbor-joining tree of fungus OTU detected in *S. frugiperda* sample (Ngeria-I2; in bold) and GenBank accessions of small subunit ribosomal RNA gene sequences from related fungi. Sequences are labelled by their GeneBank Accessions followed by genus, species and strain where available. Bootstrap values are indicated above the branches. Branches with a bootstrap value less than 50 are collapsed. A sequence from a species in the genus *Rozella* is included as an out-group

The bacterial OTU richness appeared to be higher in *S. frugiperda* larvae than adults, however this difference was not statistically significant (p-value: 0.062526; [Mann-Whitney] statistic: 19) using Shannon diversity metrics (Fig. 5a). In addition, no significant variation in OTU richness and abundance was observed between larvae from different sampling sites (p-value: 0.32834; [ANOVA] F-value: 1.3486) (Fig. 5b).

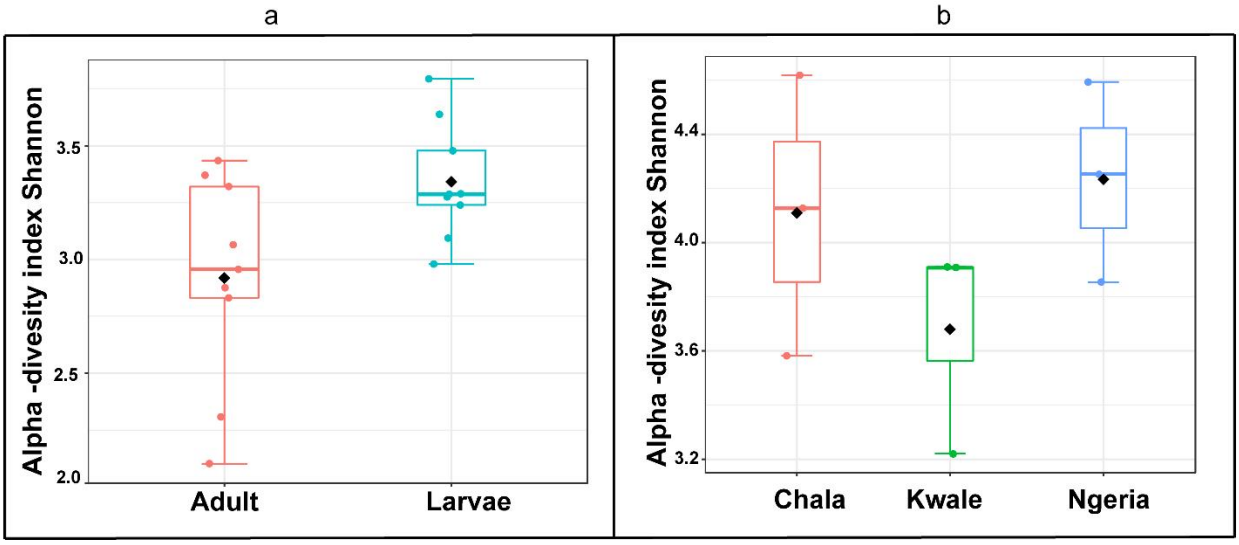


Fig. 5 A comparison of the Shannon diversity indices for: **(a)** adult and larval samples from all sites and **(b)** larvae collected from different sites. The Shannon diversity index (H') was calculated based on the OTU-level of classification. The boxplots show the distribution of H' values across all samples

The composition of bacterial OTUs between larvae and adult *S. frugiperda* was observed to overlap, with no significant dissimilarity ([ANOSIM] R=0.17365; p-value < 0.081 stress=0.14876) (Fig. 6a). However, OTU composition was observed to vary significantly among larval samples from different sites ([ANOSIM] R: 0.45679; p-value < 0.017 stress=0.05711) (Fig. 6b).

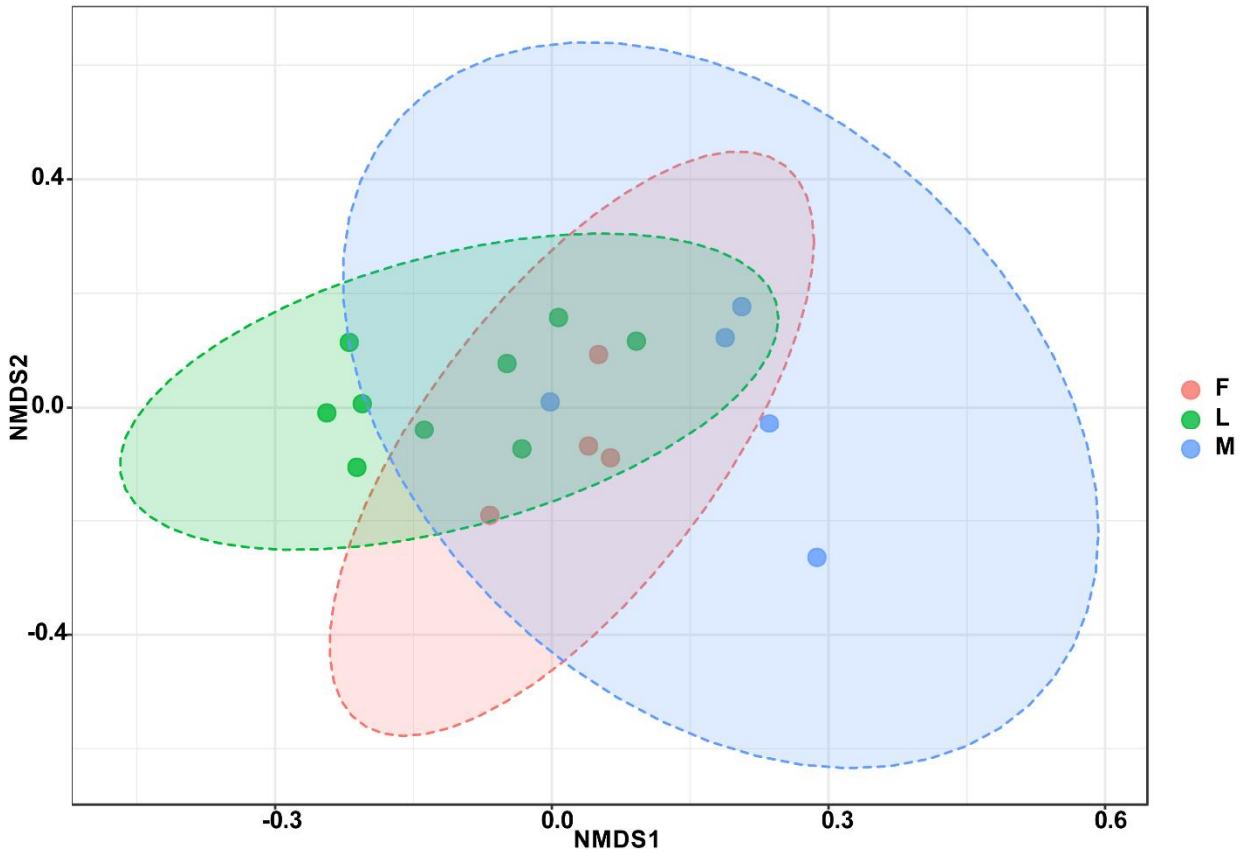


Fig. 6a Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities in bacterial communities detected in the *S. frugiperda* samples. Samples are colored according to their developmental stage and sex as indicated on the legend where F=female, L=larvae and M=male

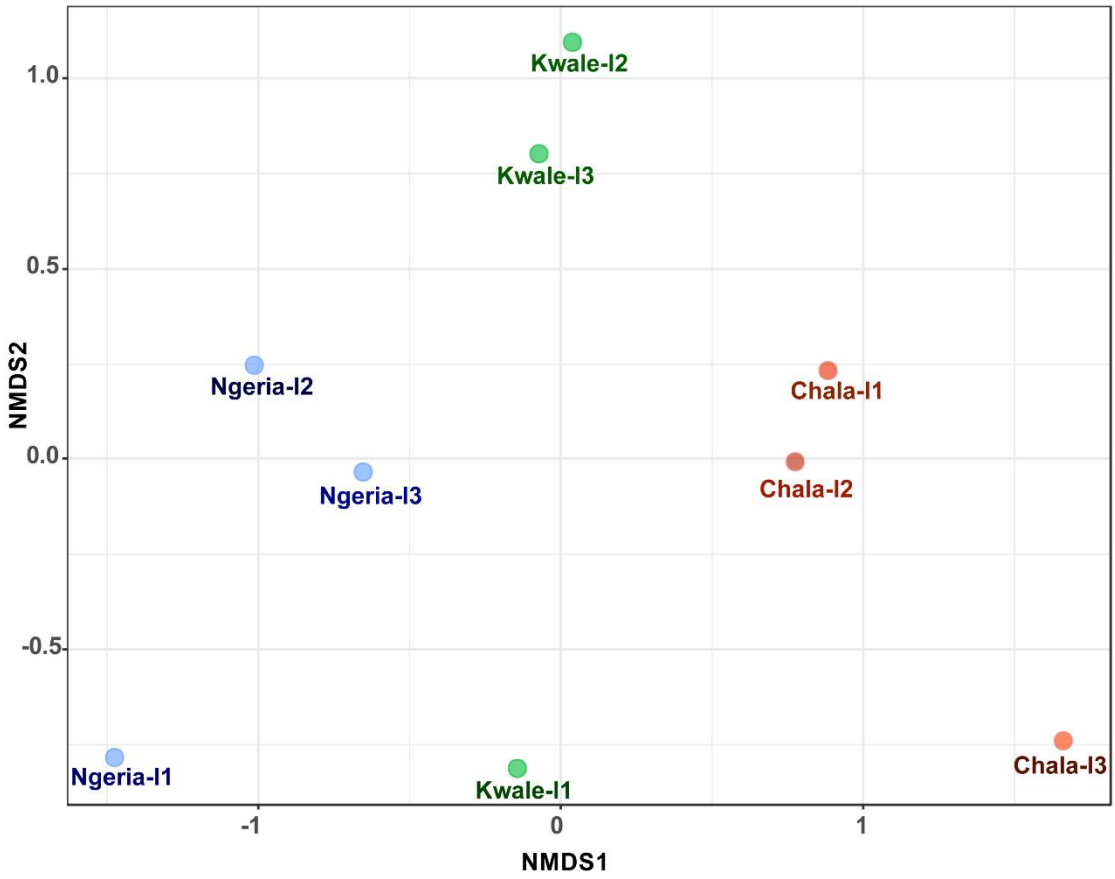


Fig. 6b Non-metric multidimensional scaling (NMDS) ordination based on Weighted Unifrac distance dissimilarities in bacterial OTU composition between larval sample pairs from different sites

A significant differential abundance was observed for 3 bacterial genera between larvae and adult *S. frugiperda* samples using the EdgeR algorithm at an adjusted p-value of 0.05. Two of these: *Citrobacter* (log2FC=4.4178, p value=3.6E-6, FDR=7.218E-5) and *Sphingobacterium* (log2FC=3.625, p value=1.01E-4, FDR=0.0010118) were more abundant in larvae whereas the third: *Lysinibacillus* (log2FC=-3.2247, p value= 4.4E-3, FDR=0.029375) was more abundant in adults (Fig. 7).

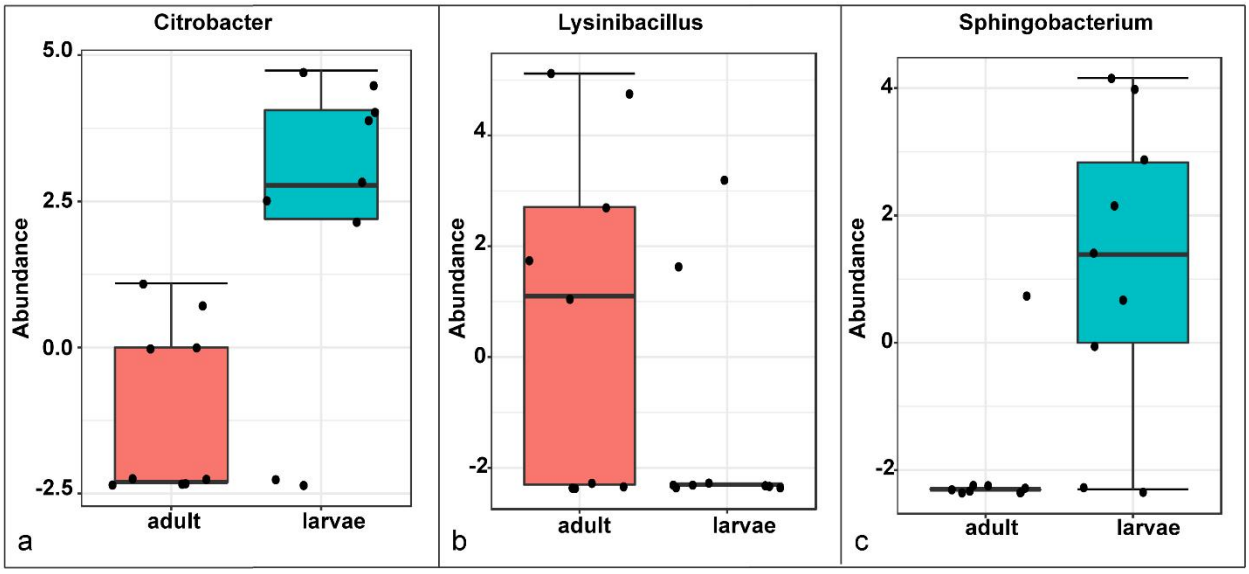
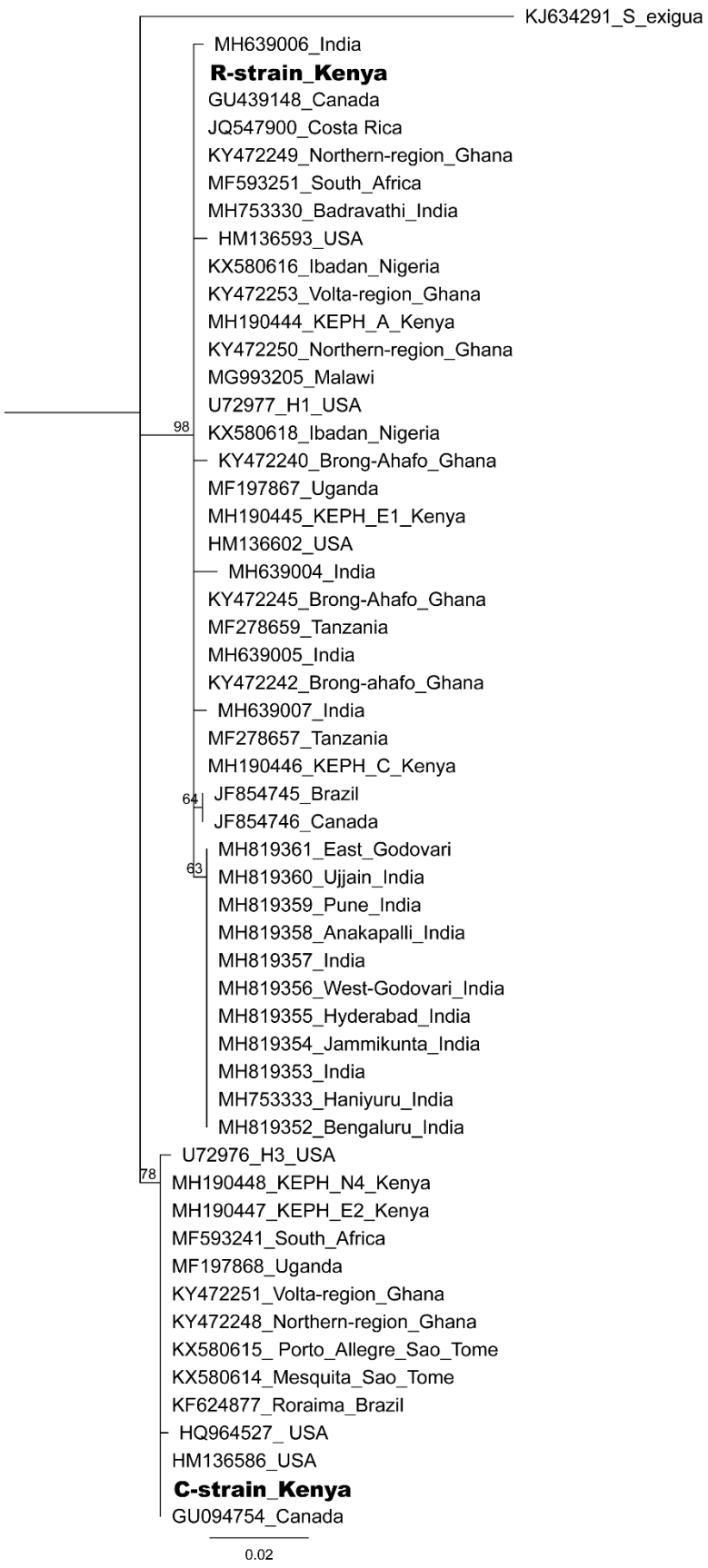


Fig. 7 Comparative abundance of A) *Citrobacter,* B) *Lysinibacillus* and C) *Sphingobacterium* between adults and larvae of *S. frugiperda.* Abundance is shown on a log transformed scale of original counts

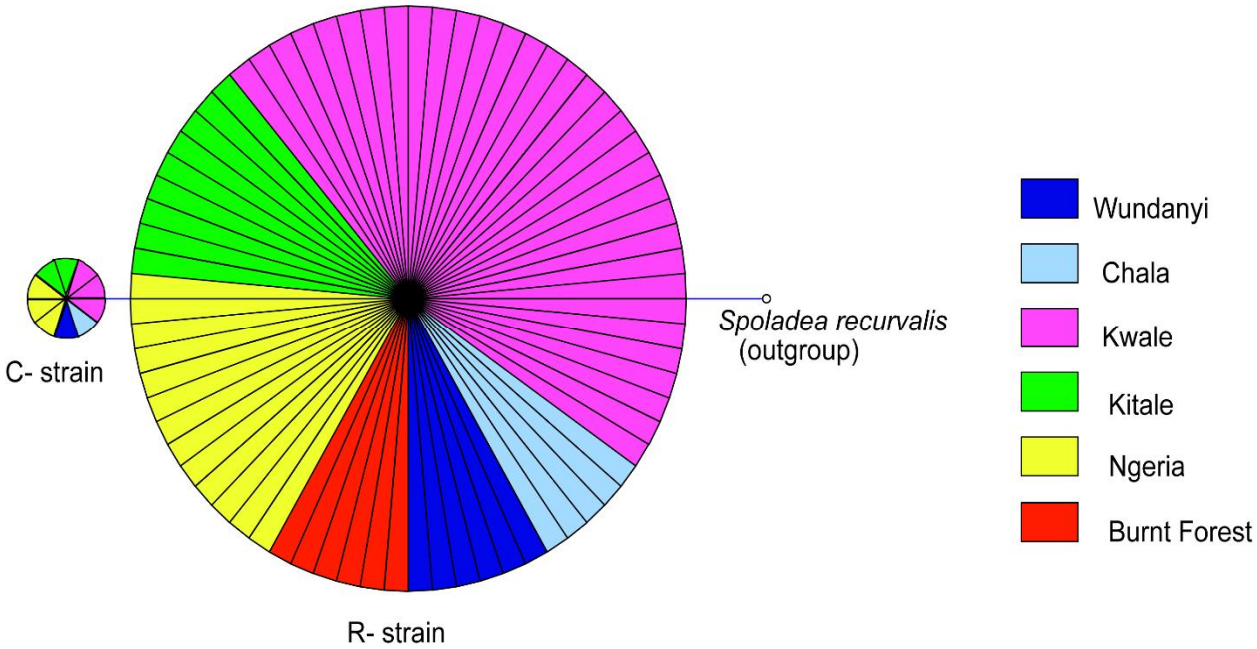
Based on mtDNA sequences, the *S. frugiperda* strains detected in this study were identical to strains from Canada, USA and Brazil, as well as strains that were recently reported in Kenya and other parts of Africa and India (Fig. 8).


KJ634291_S_exigua
MH639006_India
R-strain_Kenya
GU439148_Canada
JQ547900_Costa Rica
KY472249_Northern-region_Ghana
MF593251_South_Africa
MH753330_Badravathi_India
HM136593_USA
KX580616_Ibadan_Nigeria
KY472253_Volta-region_Ghana
MH190444_KEPH_A_Kenya
KY472250_Northern-region_Ghana
MG993205_Malawi
U72977_H1_USA
98
KX580618_Ibadan_Nigeria
KY472240_Brong-Ahafo_Ghana
MF197867_Uganda
MH190445_KEPH_E1_Kenya
HM136602_USA
MH639004_India
KY472245_Brong-Ahafo_Ghana
MF278659_Tanzania
MH639005_India
KY472242_Brong-ahafo_Ghana
MH639007_India
MF278657_Tanzania
MH190446_KEPH_C_Kenya
64
JF854745_Brazil
JF854746_Canada
MH819361_East_Godovari
63
MH819360_Ujjain_India
MH819359_Pune_India
MH819358_Anakapalli_India
MH819357_India
MH819356_West-Godovari_India
MH819355_Hyderabad_India
MH819354_Jammikunta_India
MH819353_India
MH753333_Haniyuru_India
MH819352_Bengaluru_India
U72976_H3_USA
78
MH190448_KEPH_N4_Kenya
MH190447_KEPH_E2_Kenya
MF593241_South_Africa
MF197868_Uganda
KY472251_Volta-region_Ghana
KY472248_Northern-region_Ghana
KX580615_ Porto_Allegre_Sao_Tome
KX580614_Mesquita_Sao_Tome
KF624877_Roraima_Brazil
HQ964527_ USA
HM136586_USA
C-strain_Kenya
GU094754_Canada
0.02

208

209 **Fig 8** Neighbor-joining tree based on mtCOI sequences of *S. frugiperda* from the GenBank and
210 representative haplotypes from this study (in bold). Bootstrap values are indicated above branches.
211 Branches with bootstrap values less than 50 are collapsed. A sequence from *Spodoptera exigua is* included
212 as an out-group. Sequences are labelled with their GenBank accession numbers, collection site where
213 available and country of collection

214 All the samples clustered in two major clades widely referred to as either the 'Rice' or the 'Corn' strain
215 (hereafter referred to as R- strain and C- strain). We investigated the frequency of mtDNA haplotypes of *S.*
216 *frugiperda* samples collected at several sites in Kenya. Overall, 90% of the samples (n=85) clustered as R-
217 strain, whereas 10% (n=9) clustered as C-strain. Proportions of the R-strain in populations at the different
218 sites were 100% (n=6) for Burnt Forest, 83% (n=6) for Chala, 86% (n=7) for Wundanyi, 82% (n=11) for
219 Kitale, 91% (n=35) for Kwale and 82% (n=17) for Ngeria (Fig. 9).



220

221 **Fig. 9** Mitochondrial COI haplotype map of the *S. frugiperda* samples collected at various sites in Kenya.
222 Node size is proportional to number of samples and individual samples are represented as fractions of the
223 nodes. A sequence of *Spoladea recurvalis* is included as an out-group. Sequences for all samples are
224 accessible from GenBank using the accessions: MK492929-MK493010

225

226 **Discussion**

227 We found that the gut bacterial communities of most *S. frugiperda* samples were dominated by
228 Proteobacteria. This observation is similar to proportions reported in other phytophagous insects, in
229 particular lepidopterans (Belda et al. 2011; Xia et al. 2013, 2017; Landry et al. 2015; Ramya et al. 2016;
230 Snyman et al. 2016; Strano et al. 2017; Chen et al. 2018). Only three samples, two adult males from Kitale
231 (Kitale-m2 and Kitale-m3) and one larvae from Ngeria (Ngeria-l2) were dominated by Firmicutes. Four
232 genera of bacteria, *Pseudomonas*, *Delftia*, *Enterococcus* and *Serratia* that were recorded in this study have

233 previously been isolated from *S. frugiperda* (De Almeida et al. 2017; Acevedo et al. 2017). Surprisingly,
234 *Staphylococcus*, *Microbacterium*, *Arthrobacter* and *Leclercia* that were previously isolated from *S.*
235 *frugiperda* in Brazil (De Almeida et al. 2017) were not found in any of the samples we profiled in Kenya.
236 Similarly, *Pantoea*, *Enterobacter*, *Raoultella* and *Klebsiella* previously identified in oral secretions of *S.*
237 *frugiperda* in Pennsylvania, USA (Acevedo et al. 2017) were not found in the profiled Kenyan samples.

238 We observed significant differences in OTU composition between larvae from different sites. This was most
239 likely caused by complex biological and environmental factors in the diverse agro-ecological zones that
240 were sampled. Diet is known to strongly influence the microbiome of lepidopterans (Strano et al. 2017;
241 Sittenfeld et al. 2002; Priya et al. 2012; Montagna et al. 2016), however in this study all samples were
242 collected from maize plants. Hence, the observed compositional differences are not likely to be caused
243 solely by diet. We observed differences in bacterial OTU composition between larvae and adults, however
244 with a relatively low number of samples these differences were not statistically significant. It is interesting
245 that many of the detected bacterial genera such as *Stenotrophomonas*, *Sphingobacterium*, *Serratia*,
246 *Pseudomonas*, *Morganella*, *Enterococcus* and *Delftia* are found in both life stages, which suggests that gut
247 bacterial community members are transmitted across developmental stages. Bacteria that are continually
248 transmitted across developmental stages (and across generations) may evolve a closer, mutualistic
249 relationship with their hosts (Moran 2006). Future studies should investigate the effects of these microbes
250 on host fitness and investigate the extent to which they are vertically transmitted from parents to offspring.
251 In contrast, *Citrobacter* and *Sphingobacterium* were observed to be differentially abundant in larvae than in
252 adults, a likely indicator that these two genera may be part of the fraction of bacterial communities that are
253 lost during transition of *S. frugiperda* into the adult stage. *Lysinibacillus*, on the other hand, was more
254 abundant in adults than in larvae and therefore could have an adult-specific function.

255 Notably, we identified *Serratia*, *Lysinibacillus* (formerly *Bacillus*) and *Pseudomonas*, species of which have
256 been reported to have entomopathogenic properties (Castagnola and Stock 2014). In addition, one sample
257 had a high number of reads attributed to a relative of a non-bacterial entomopathogen, *Metarhizium rileyi,*
258 which has been previously isolated and tested for efficiency against *S. frugiperda* (Maniania and Fargues
259 1985; Mallapur et al. 2018). It will be worthwhile to explore the pathogenicity of these microbes for *S.*
260 *frugiperda* and to determine if they could be incorporated into biological pest management strategies (Ruiu
261 et al. 2015).

262 Based on the mtCOI gene sequence, we observed two mtDNA haplotypes in Kenya (C- and R- strains).
263 These findings confirm that both haplotypes are present in Kenya, as has been demonstrated for other
264 countries in Africa (Rwomushana et al. 2018). The majority of the *S. frugiperda* samples collected were
265 characterized as R-strain suggesting that this strain is dominant in *S. frugiperda* populations in Kenya.
266 These observations are in agreement with a previous study (Goergen et al. 2016) that observed C- and R-
267 strains appear to have an East-West axis alignment in the African region with the Eastern Africa having

268 progressively lower frequencies of the mtCOI C-strain (Goergen et al. 2016). We observed that some
269 variants of the rice strain have been reported in other places such as Ghana and India but those were not
270 detected in this study. It is interesting to note that in addition to a similar rice strain as the one detected in
271 Kenya, a variant differing by a single nucleotide polymorphism has been recorded from various locations in
272 India (Fig. 8). This variant has however not been reported in Africa. It is therefore possible that the invasion
273 into India may not have come directly from the African continent, or invasion could have included strains
274 from Africa and elsewhere.

275 Symbiotic bacteria play a key role in the biology of insects. We characterized the gut bacterial communities
276 in *S. frugiperda* larvae and adult samples collected from several locations in Kenya, finding some important
277 differences and similarities across samples and in relation to other studies on this species (Acevedo et al.
278 2017; De Almeida et al. 2017). Understanding the gut microbial symbionts of this pest species may facilitate
279 the development of novel, cost-effective control strategies.

280 **Acknowledgements**

281 The authors gratefully acknowledge the financial support for this research by the following organizations
282 and agencies: European Union Funded project "Integrated pest management" strategy to counter the
283 threat of invasive fall armyworm to food security in Eastern Africa (FAW-IPM) (FOOD/2018/402-634); UK
284 Aid from the UK Government; Swedish International Development Cooperation Agency (Sida); the Swiss
285 Agency for Development and Cooperation (SDC); and the Kenyan Government. Financial assistance for
286 sequencing at the Center for Integrated Genomics, Lausanne, was generously provided by the Global
287 Health Institute of the École Polytechnique Fédérale de Lausanne. The views expressed herein do not
288 necessarily reflect the official opinion of the donors. Authors also acknowledge the technical assistance of
289 Mr. Peter Malusi during sample collection.

290 **Author contributions**

291 SE, SS, JH and FMK conceived and designed the research, JG conducted experiments and analyzed
292 data, JG and JH wrote the manuscript, JVB and HP contributed materials. All authors read and approved
293 the manuscript

294 **Conflict of Interest**

295 The authors declare that they have no conflict of interest.

296

297

298

References

Acevedo FE, Peiffer M, Tan C-W, Stanley BA, Stanley A, Wang J, Jones AG, Hoover K, Rosa C, Luthe D, Felton G (2017) Fall armyworm-associated gut bacteria modulate plant defense responses. Mol Plant-Microbe Interact 30:127–137. doi: 10.1094/MPMI-11-16-0240-R

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Amir A, McDonald D, Navas-Molina, Jose A. Kopylova E, Morton JT, Xu ZZ, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Rob K (2017) Deblur rapidly resolves single-nucleotide community sequence patterns. mSystems 2:1–7

Anand AAP, Vennison SJ, Sankar SG, Prabhu DIG, Vasan PT, Raghuraman T, Geoffrey CJ, Vendan SE (2010) Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. J Insect Sci 10:1–20. doi: 10.1673/031.010.10701

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Arora AK, Douglas AE (2017) Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. J Insect Physiol 103:10–17. doi: 10.1016/j.jinsphys.2017.09.011

Banerjee R, Hasler J, Meagher R, Nagoshi R, Hietala L, Huang F, Narva K, Jurat-Fuentes JL (2017) Mechanism and DNA-based detection of field-evolved resistance to transgenic Bt corn in fall armyworm (*Spodoptera frugiperda*). Sci Rep 7:1–10. doi: 10.1038/s41598-017-09866-y

Beck JJ, Vannette RL (2017) Harnessing insect-microbe chemical communications to control insect pests of agricultural systems. J Agric Food Chem 65:23–28. doi: 10.1021/acs.jafc.6b04298

Belda E, Pedrola L, Peretó J, Martínez-Blanch JF, Montagud A, Navarro E, Urchueguía J, Ramón D, Moya A, Porcar M (2011) Microbial diversity in the midguts of field and lab-reared populations of the European corn borer *Ostrinia nubilalis*. PLoS One 6:e21751. doi: 10.1371/journal.pone.0021751

Biere A, Bennett AE (2013) Three-way interactions between plants, microbes and insects. Funct Ecol 27:567–573. doi: 10.1111/1365-2435.12100

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E., Da Silva, R., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, II, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Knight, R., Caporaso, J.G.: (2018) QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. PeerJ. doi: 10.7287/peerj.preprints.27295

Brady CM, White JA (2013) Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. Ecol Entomol 1–5. doi: 10.1111/een.12020

Broderick AN, Raffa KF, Handelsman J (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. PNAS 103:15196–15199. doi: 10.1371/journal.pone.0170933

Broderick N, Raffa K, Goodman R, Handelsman J (2004) Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. Appl Environ Microbiol 70:293–300. doi: 10.1128/AEM.70.1.293

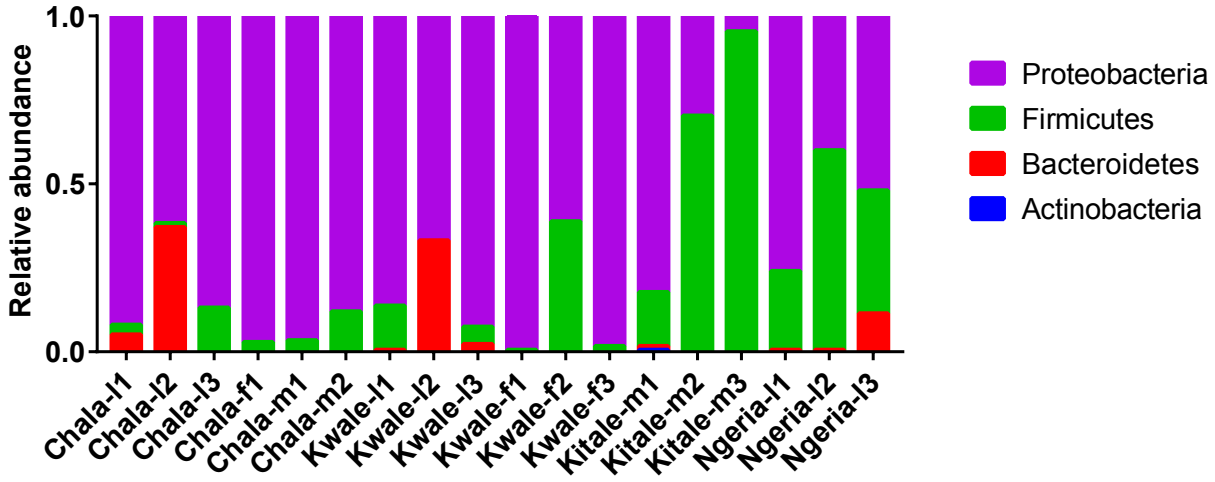
Castagnola A, Stock SP (2014) Common virulence factors and tissue targets of entomopathogenic bacteria for biological control of Lepidopteran pests. Insects 5:139–166. doi:

10.3390/insects5010139
Chen B, Du K, Sun C, Vimalanathan A, Liang X, Li Y, Wang B, Lu X, Li L, Shao Y (2018) Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. ISME J 12:2252–2262. doi: 10.1038/s41396-018-0174-1
Cock MJW, Beseh PK, Buddie AG, Cafá G, Crozier J (2017) Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. Sci Rep 7:1–10. doi: 10.1038/s41598-017-04238-y
Crotti E, Balloi A, Hamdi C, Sansonno L, Marzorati M, Gonella E, Favia G, Cherif A, Bandi C, Alma A, Daffonchio D (2012) Review microbial symbionts: a resource for the management of insect-related problems. Microb Biotechnol 5:307–317. doi: 10.1111/j.1751-7915.2011.00312.x
Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, Colmenarez Y, Corniani N, Early R, Godwin J, Gomez J, Moreno PG, Murphy ST, Birgitta O-M, Phiri N, Pratt C, Silvestri S, Witt A (2017) Fall armyworm: impacts and implications for Africa. Outlooks Pest Manag 2016:196–201. doi: 10.1564/v28
De Almeida LG, De Moraes LAB, Trigo JR, Omoto C, Cônsoli FL (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: a potential source for biotechnological exploitation. PLoS One 12:1–19. doi: 10.1371/journal.pone.0174754
Deole S, Paul N (2018) First report of fall army worm, *Spodoptera frugiperda* (J. E. Smith), their nature of damage and biology on maize crop at Raipur, Chhattisgarh. J Entomol Zool Stud 6:219–221
Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J (2017) MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45:W180–W188. doi: 10.1093/nar/gkx295
Dillon RJ, Vennard CT, Buckling A, Charnley AK (2005) Diversity of locust gut bacteria protects against pathogen invasion. Ecol Lett 8:1291–1298. doi: 10.1111/j.1461-0248.2005.00828.x
Dong Y, Manfredini F, Dimopoulos G (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLOS Pathog 5:e1000423. doi: 10.1371/journal.ppat.1000423
Douglas AE (2009) The microbial dimension in insect nutritional ecology. Funct Ecol 23:38–47. doi: 10.1111/j.1365-2435.2008.01442.x
Douglas AE (2016) Multiorganismal insects: diversity and function of resident microorganisms. Annu Rev Entomol 60:17–34. doi: 10.1146/annurev-ento-010814-020822
FAO (2018) Sustainable management of the fall armyworm in Africa: a framework for partnership. Rome, Italy. http://www.fao.org/3/I9160EN/i9160en.pdf
Ferrari J, Vavre F, Lyon D (2011) Bacterial symbionts in insects or the story of communities affecting communities. Philos Trans R Soc Boyal Soc B 366:1389–1400. doi: 10.1098/rstb.2010.0226
Flagel L, Lee YW, Wanjugi H, Swarup S, Brown A, Wang J, Kraft E, Greenplate J, Simmons J, Adams N, Wang Y, Martinelli S, Haas JA, Gowda A, Head G (2018) Mutational disruption of the ABCC2 gene in fall armyworm, *Spodoptera frugiperda*, confers resistance to the Cry1Fa and Cry1A.105 insecticidal proteins. Sci Rep 8:1–11. doi: 10.1038/s41598-018-25491-9
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299. doi: 10.1371/journal.pone.0013102
Frago E, Dicke M, Godfray HCJ (2012) Insect symbionts as hidden players in insect-plant interactions. Trends Ecol Evol 27:705–711. doi: 10.1016/j.tree.2012.08.013
Gao X, Li W, Luo J, Zhang L, Ji J, Zhu X, Wang L, Cui J (2018) Biodiversity of the microbiota in *Spodoptera exigua* (Lepidoptera: Noctuidae). J Appl Microbiol 126:1199–1208. doi: https://doi.org/10.1111/jam.14190
Garcia ES, Castro DP, Figueiredo MB, Azambuja P (2010) Immune homeostasis to microorganisms in the guts of triatomines (Reduviidae) - a review. Mem Inst Oswaldo Cruz 105:605–610
Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11:1–9. doi: 10.1371/journal.pone.0165632
Hammer TJ, H. Janzen D, Hallwachs W, Jaffe SP, Fierera N (2017) Caterpillars lack a resident gut microbiome. PNAS 114:9641–9646. doi: 10.1073/pnas.1707186114
Huber DM, Hugh-Jones ME, Rust MK, Sheffield SR, Simberloff D, Taylor CR (2002) Invasive pest species: impacts on agricultural production, natural resources and the environment. Counc Agric Sci Technol 20:1–18

409 Jacobs A, van Vuuren A, Rong IH (2018) Characterisation of the fall armyworm (*Spodoptera frugiperda*
410 J.E. Smith) (Lepidoptera: Noctuidae) from South Africa. African Entomol 26:45–49. doi:
411 10.4001/003.026.0045
412 Jakka SRK, Gong L, Hasler J, Banerjee R, Sheets JJ, Narva K, Blanco CA, Jurat-Fuentes JL (2016)
413 Field-evolved mode 1 resistance of the fall armyworm to transgenic Cry1Fa-expressing corn
414 associated with reduced Cry1Fa toxin binding andmidgut alkaline phosphatase expression. Appl
415 Environ Microbiol 82:1023–1034. doi: 10.1128/aem.02871-15
416 Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K (2012) Symbiont-mediated insecticide
417 resistance. Proc Natl Acad Sci 109:8618–8622. doi: 10.1073/pnas.1200231109/-
418 /DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1200231109
419 Landry M, Comeau AM, Derome N, Cusson M, Levesque RC (2015) Composition of the spruce budworm
420 (*Choristoneura fumiferana*) midgut microbiota as affected by rearing conditions. PLoS One 10:1–11.
421 doi: 10.1371/journal.pone.0144077
422 Mallapur CP, Naik AK, Hagari S, Praveen T, Patil RK (2018) Potentiality of *Nomuraea rileyi* (Farlow)
423 Samson against the fall armyworm, *Spodoptera frugiperda* (J E Smith) infesting maize. J Entomol
424 Zool Stud 6:1062–1067
425 Maniania NK, Fargues J (1985) Susceptibility of the fall armyworm, *Spodoptera frugiperda*, to the fungal
426 pathogens *Paecilomyces fumosoroseus* and *Nomuraea rileyi*. Florida Entomol 68:178–183
427 Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads.
428 EMBnet.journal 17:10. doi: 10.14806/ej.17.1.200
429 Mclean AHC, Godfray HCJ (2015) Evidence for specificity in symbiont-conferred protection against
430 parasitoids. Proc R Soc B Biol Sci 282:1–8
431 Midega CAO, Pittchar JO, Pickett JA, Hailu GW, Khan ZR (2018) A climate-adapted push-pull system
432 effectively controls fall armyworm, *Spodoptera frugiperda* (J E Smith), in maize in East Africa. Crop
433 Prot 105:10–15. doi: 10.1016/j.cropro.2017.11.003
434 Montagna M, Mereghetti V, Gargari G, Guglielmetti S, Faoro F, Locatelli D, Limonta L (2016) Evidence of
435 a bacterial core in the stored products pest *Plodia interpunctella*: the influence of different diets.
436 Environ Microbiol 18:4961–4973
437 Moran NA (2006) Symbiosis. Curr Biol 16:R866-871
438 Nagoshi RN, Goergen G, Tounou KA, Agboka K, Koffi D, Meagher RL (2018) Analysis of strain
439 distribution, migratory potential, and invasion history of fall armyworm populations in northern Sub-
440 Saharan Africa. Sci Rep 8:3710. doi: 10.1038/s41598-018-21954-1
441 Nagoshi RN, Koffi D, Agboka K, Tounou KA, Banerjee R, Jurat-Fuentes JL, Meagher RL (2017)
442 Comparative molecular analyses of invasive fall armyworm in Togo reveal strong similarities to
443 populations from the eastern United States and the Greater Antilles. PLoS One 12:1–15. doi:
444 10.1371/journal.pone.0181982
445 Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, Eppler-epstein R, Deponte K, Fish D, Fikrig
446 E (2014) Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the lyme
447 disease spirochete. Cell Host Microbe 15:58–71. doi: 10.1016/j.chom.2013.12.001
448 Perilla-henao LM, Casteel CL (2016) Vector-borne bacterial plant pathogens: interactions with
449 Hemipteran insects and plants. Front Plant Sci 7:1–15. doi: 10.3389/fpls.2016.01163
450 Pinto-Tomás AA, Sittenfeld A, Uribe-Lorío L, Chavarría F, Mora M, Janzen DH, Goodman RM, Simon HM
451 (2011) Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed
452 on different diets. Environ Entomol 40:1111–1122. doi: 10.1016/S0140-6736(02)87727-3
453 Priya NG, Ojha A, Kajla MK, Raj A, Rajagopal R (2012) Host plant induced variation in gut bacteria of
454 *Helicoverpa armigera*. PLoS One 7:1–10. doi: 10.1371/journal.pone.0030768
455 Quast C, Prusse EP, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA
456 ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic
457 Acids Res 41:D590–D596. doi: 10.1093/nar/gks1219
458 Ramya SL, Venkatesan T, Srinivasa Murthy KS, Jalali SK, Verghese A (2016) Detection of
459 carboxylesterase and esterase activity in culturable gut bacterial flora isolated from diamondback
460 moth, *Plutella xylostella* (Linnaeus), from India and its possible role in indoxacarb degradation.
461 Brazilian J Microbiol 47:327–336. doi: 10.1016/j.bjm.2016.01.012
462 Robinson MD, McCarthy DJ, Smyth GK (2009) edgeR: A Bioconductor package for differential expression
463 analysis of digital gene expression data. Bioinformatics 26:139–140. doi:
464 10.1093/bioinformatics/btp616

465 Ruiu L, Agraria D, Vegetale P (2015) Insect pathogenic bacteria in integrated pest management. Insects
466 6:352–367. doi: 10.3390/insects6020352
467 Rwomushana I, Bateman M, Beale T, Beseh P, Cameron K, Chiluba M, Clottey V, Davis T, Day R, Early
468 R, Godwin J, Gonzalez-Moreno P, Kansiime M, Kenis M, Makale F, Mugambi I, Murphy S, Nunda
469 W, Phiri N, Pratt C, Tambo J (2018) Fall armyworm: impacts and implications for Africa evidence note
470 update, October 2018
471 Sisay B, Simiyu J, Malusi P, Likhayo P, Mendesil E, Elibariki N, Wakgari M, Ayalew G, Tefera T (2018)
472 First report of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera : Noctuidae), natural enemies
473 from Africa. J Appl Entomol 142:800–804. doi: 10.1111/jen.12534
474 Sisodiya DB, Raghunandan BL, Bhatt NA, Verma HS, Shewale CP, Timbadiya BG, Borad PK (2018) The
475 fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae); first report of new
476 invasive pest in maize fields of Gujarat, India. J Entomol Zool Stud 6:2089–2091
477 Sittenfeld A, Uribe-lorío L, Mora M, Nielsen V, Arrieta G, Janzen DH (2002) Does a polyphagous
478 caterpillar have the same gut microbiota when feeding on different species of food plants? Rev Biol
479 Trop 50:547–560
480 Strano CP, Malacrinò A, Campolo O, Palmeri V (2017) Influence of host plant on *Thaumetopoea*
481 *pityocampa* gut bacterial community. Microb Ecol 75:487–494. doi: 10.1007/s00248-017-1019-6
482 Su Q, Zhou X, Zhang Y (2013) Symbiont-mediated functions in insect hosts. Commun Integr Biol
483 6:e23804 1-7. doi: https://doi.org/10.4161/cib.23804
484 Tindo M, Tagne A, Tigui A, Kengni F, Atanga J, Bila S, Abega R (2016) First report of the fall army worm,
485 *Spodoptera frugiperda* (Lepidoptera, Noctuidae) in Cameroon. Cameroon J Biol Biochem Sci 25:30–
486 32
487 Ubeda C, Djukovic A, Isaac S (2017) Roles of the intestinal microbiota in pathogen protection. Clin Transl
488 Immunol 6:1–10. doi: 10.1038/cti.2017.2
489 Uzayisenga B, Waweru B, Kajuga J, Karangwa P, Uwumukiza B, Edgington S, Thompson E, Offord L,
490 Cafá G, Buddie A (2018) First record of the fall armyworm, *Spodoptera frugiperda* (JE Smith,
491 1797)(Lepidoptera: Noctuidae), in Rwanda. African Entomol 26:244–246
492 Vorburger C, Gehrer L, Rodriguez P (2010) A strain of the bacterial symbiont *Regiella insecticola* protects
493 aphids against parasitoids. Biol Lett 6:109–111
494 Vorburger C, Rouchet R (2016) Are aphid parasitoids locally adapted to the prevalence of defensive
495 symbionts in their hosts? BMC Evol Biol 16:1–11. doi: 10.1186/s12862-016-0811-0
496 Wang J, Peiffer M, Hoover K, Rosa C, Zeng R, Felton GW (2017) *Helicoverpa zea* gut-associated
497 bacteria indirectly induce defenses in tomato by triggering a salivary elicitor(s). New Phytol
498 214:1294–1306. doi: 10.1111/nph.14429
499 Wei G, Lai Y, Wang G, Chen H, Li F, Wang S (2017) Insect pathogenic fungus interacts with the gut
500 microbiota to accelerate mosquito mortality. PNAS 114:5994–5999. doi: 10.1073/pnas.1703546114
501 Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H, Qin B, Lin J, Wang Y, Song F, Li Y, Lin H, You M (2017)
502 Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations
503 for herbivory. Front Microbiol 8:1–12. doi: 10.3389/fmicb.2017.00663
504 Xia X, Sun B, Gurr GM, Vasseur L, Xue M, You M (2018) Gut microbiota mediate insecticide resistance in
505 the diamondback moth, *Plutella xylostella* (L.). Front Microbiol 9:1–10. doi:
506 10.3389/fmicb.2018.00025
507 Xia X, Zheng D, Zhong H, Qin B, Gurr GM, Vasseur L, Lin H, Bai J, He W, You M (2013) DNA
508 sequencing reveals the midgut microbiota of diamondback moth, *Plutella xylostella* (L.) and a
509 possible relationship with insecticide resistance. PLoS One 8:1–8. doi:
510 10.1371/journal.pone.0068852
511 Xiang H, Wei G-F, Jia S, Huang J, Miao X-X, Zhou Z, Zhao L-P, Huang Y-P (2006) Microbial communities
512 in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*).
513 Can J Microbiol 52:1085–1092. doi: 10.1139/W06-064
514 Yu SJ (1991) Insecticide resistance in the fall armyworm, *Spodoptera frugiperda*. Pestic Biochem Physiol
515 39:84–91
516
517
518
519
520

521 **Supplementary material**



522
523 Fig. 10 Relative abundance of bacterial phyla detected in *S. frugiperda* samples