Host-Adapted Strains of *Spodoptera frugiperda* Hold and Share a Core Microbial Community Across the Western Hemisphere

[1]Nathalia C. Oliveira, [1]Pedro A.P. Rodrigues, [1]Fernando L. Cônsoli

[1]Insect Interactions Laboratory, Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil

Nathalia Cavichiolli de Oliveira – email: nathaliaoliveira@usp.br ; ORCID iD: https://orcid.org/0000-0003-1256-1510

Pedro Augusto da Pos Rodrigues – email: rodrigues.pap@gmail.com; ORCID iD: https://orcid.org/0000-0002-3280-7576

Fernando Luís Cônsoli – email: fconsoli@usp.br; ORCID iD: https://orcid.org/0000-0002-2287-0782

Correspondence

Fernando L. Cônsoli, Insect Interactions Laboratory, Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil.

E-mail: fconsoli@usp.br

Abstract

The fall armyworm *Spodoptera frugiperda* is an important polyphagous agricultural pest in the Western Hemisphere and currently invasive to countries of the Eastern Hemisphere. This species has two host-adapted strains named "rice" and "corn" strains. Our goal was to identify the occurrence of core members in the gut bacterial community of Fall armyworm larvae from distinct geographical distribution and/or host strain. We used next-generation sequencing to identify the microbial communities of *S. frugiperda* from corn fields in Brazil, Colombia, Mexico, Panama, Paraguay, and Peru, and rice fields from Panama. The larval gut microbiota of *S. frugiperda* larvae did not differ between the host strains neither was it affected by the geographical distribution of the populations investigated. Our findings provide additional support for *Enterococcus* and *Pseudomonas* as core members of the bacterial community associated with the larval gut of *S. frugiperda,* regardless of the site of collection or strain, suggesting that these bacteria may maintain true symbiotic relationships with the fall armyworm. Further investigations are required for a deeper understanding of the nature of this relationship.

Keywords: Microbial ecology, dysbiosis, symbiosis, host adaptation

Declarations

Funding

This research was financed by the São Paulo Research Foundation (FAPESP) (process 2011/50877-0) and the Ministry of Science, Technology and Innovation (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq: process 462140-2014/8).

Conflicts of interest/Competing interests

The authors declare no competing interests.

Availability of data and material

Upon paper acceptance, the data will be archived and the data regarding the deposited database and information such as access numbers will be provided for all manuscript data.

Code availability

Not applicable

Contributions

N.C.O. and F.L.C. conceived the study and designed the experiment. N.C.O. processed the samples. N.C.O. and P.A.P.R. conducted the bioinformatics. FLC secured funds for the project. N.C.O. wrote the first draft of the manuscript. P.A.P.R. and F.L.C. revised and edited the initial draft. All authors approved the final version for publication.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable

Ethics approval

Not applicable

Consent to participate

All authors agree with the participation in this manuscript.

Consent for publication

All authors agree with the manuscript submission to Microbial Ecology Journal.

Introduction

The complexity and wide variety of host-microbe interactions are increasingly evident through new molecular techniques and the improvement of bioinformatic analysis tools. The advancement of understanding of this topic has brought support to some hypotheses, and challenged others. An example is the discussion on whether the gut microbiota is relevant for all animals [1]. The gut is a rich environment for holding a variety of host – microorganism associations, and the gut microbiota has been shown to play crucial roles in a wide range of aspects of host physiology, morphology and ecology. The insect gut microbiota can influence intra and interspecific interactions, such as sexual behavior [2, 3] and the relationship between host plants and natural enemies [4]. It also plays a key role in insect adaptation to their environment by providing essential nutrients [5, 6] and/or boosting the host immune response to parasites and pathogens [7, 8]. In addition, microbial symbionts can contribute to hosts by detoxifying xenobiotics as insecticides [9-12].

Such range of beneficial contributions has led to the establishment of true mutualistic associations in several groups of hemipterans, dipterans, blattids, and coleopterans, among others [9, 13-17]. Lepidopteran larvae, however, have been thought not to have established mutualistic associations with their gut-associated bacteria. Some studies demonstrated the survival, development time, and weight gain were not affected in antibiotic-fed larvae [18]. Additionally, the lack of special regions in the gut to house microorganisms has been argued as a strong limitation for the establishment of true associations with free-living microbes [19]. The harshness of the extremely alkaline conditions of the gut to most microorganisms also represents an unfavorable condition for establishing microbial associations [20]. Finally, the high variation in the composition of the microbial community driven by host plants would difficult the occurrence of associations that could hold through the required evolutionary time in order to allow the selection and establishment of true gut residents [21]. Nevertheless, other studies have shown that even in hostile environments as the midgut of lepidopteran larvae, there are evidence of gut colonization by certain bacterial groups [22-24]. In addition, gut-resident bacteria of lepidopteran larvae were demonstrated to play

94 important physiological roles for their hosts [25, 26]; besides, the continuous association with their hosts
95 for some of these microbes has been proved as they are horizontally transmitted [27].

96 Controversial topics in the scientific literature are always an invitation to new studies aiming at better
97 understanding and clarification of the topic. The debated existence of true gut-associates in lepidoptera is a
98 subject that needs further clarification due to two important contexts it is placed in. First, its remarkable
99 relevance to the understanding of how microbial associations can influence host phenotypes [28], and
100 insects have provided simple models for the clarification of fundamental principles in host-microbe
101 interactions [29, 30], with a great potential to assist in unravelling complex systems such as in mammalians.
102 Second, lepidopterans are yet the major group of agricultural pests, causing severe losses in food
103 production, posing serious threats to food security [31-33]. Understanding the diversity and function of gut
104 - microbes associations can lead to the development of new strategies for herbivore control.

105 In the present study we have chosen a lepidopteran species that is important both in the ecological and in
106 the economic context to investigate the existence of true gut associates of lepidopteran larvae. *Spodoptera*
107 *frugiperda* is an important agricultural pest in the Western Hemisphere and is currently invasive to countries
108 in Africa, Asia, and Oceania [34-38]. *Spodoptera frugiperda* is highly polyphagous, feeding on more than
109 300 host plants [39]. This species is actually a complex composed of two distinct strains known as the rice
110 (RS) and corn (CS) strains. The two strains are morphologically identical, with clear differences in host
111 preference, susceptibility to insecticides and transgenic crops (*Bacillus thuringiensis*), composition of sex
112 pheromone and mating behavior [40-47]. Genomic analysis of the host-adapted strains of *S. frugiperda*
113 identified several genes involved in the chemodetection of non-volatile molecules and detoxification of
114 xenobiotics showing signatures of positive selection, suggesting their contribution to *S. frugiperda* host
115 plant preferences [48]. Some of these genomic variations between host strains of *S. frugiperda* were also
116 detected at the transcriptional level, including those involved in xenobiotic metabolism [49].

117 Genetic studies suggest that population structure of *S. frugiperda* in the Western Hemisphere shows more
118 variation within *S. frugiperda* populations than between populations of different locations, indicating a

119 significant gene flow [50, 51]. The Mexican populations, on the other hand, have proven to be the most
120 different, suggesting limited migratory interactions with foreign populations [52, 53]. The population
121 genetic structure of Brazilian populations of *S. frugiperda* is partially based on host plants, with rice
122 populations, which are basically represent by rice strain individuals, having a strong effect on the overall
123 genetic structure of fall armyworm populations in Brazil [54].

124 Therefore, in this study we aim to verify the existence of bacterial groups that remain associated with the
125 gut microbial community of *S. frugiperda* larvae regardless of the geographical region or host plant used.
126 So, we sampled and sequenced the gut microbiota of fall armyworm larvae from corn and rice fields across
127 the American continent. Larvae were genotyped as rice or corn strain, and the structure of the bacterial gut
128 community was checked based on the geographical origin of the larvae, host-adapted strain and/or host
129 plant used. Despite the variation expected due to uncontrolled and unforeseen environmental factors, the
130 field conditions may provide essential information on potential symbionts that could be ecologically
131 important to their hosts in their natural habitats.

132

133 **Material and Methods**

134 Sampling and strains identification

135 Larvae of *Spodoptera frugiperda* with 2.5-3.0 cm in length were collected from corn and/or rice fields
136 during 2016-17 in Brazil (13.8224° S, 56.0835° W), Colombia (4.5709° N, 74.2973° W), Mexico
137 (23.6345° N, 102.5528° W), Panama (8.5380° N, 80.7821° W), Paraguay (23.4425° S, 58.4438° W), and
138 Peru (9.1900° S, 75.0152° W), and stored in absolute ethanol. Once in the laboratory, larvae had the width
139 of the head capsule measured, and only those larvae with head capsule width within the limits of size of
140 5th and 6th instars [55] were further dissected for gut collection. Dissections were carried after surface
141 sterilization under aseptic conditions in a laminar flow hood. The larval digestive tract was carefully

142 removed, washed in sterile saline and further used in metabarcoding analysis of the gut microbiota. The
143 remaining carcass was used for host strain identification.

144 *Spodoptera frugiperda* were genotyped for strain identification using the mitochondrial *cytochrome*
145 *oxidase I* (*COI*) gene as a marker. DNA was extracted using the genomic DNA preparation protocol from
146 RNALater™, with modifications. The carcass obtained from dissected larvae was placed in 2 mL tubes
147 with 750 µL digestion buffer (60 mM Tris pH 8.0, 100 mM EDTA, 0.5% SDS) and proteinase K (500
148 µg/mL), macerated using pestle, and mixed well by inversion. Samples were incubated overnight at 55°C.
149 Afterwards, 750 µL of phenol:chloroform (1:1) was added and rapidly inverted for 2 min. Samples were
150 centrifuged at high speed for 10 min. The aqueous layer was collected and phenol:chloroform extraction
151 was repeated twice before a final extraction with chloroform. The aqueous layer was collected, added to
152 0.1 volume of 3M sodium acetate (pH 5.2) and an equal volume of 95% ethanol. Samples were then mixed
153 by inversion, incubated for 40 min at -80°C before centrifugation (27,238 *g* x 30 min x 4°C). The pellet
154 obtained was washed twice with 1 mL of 85% ice-cold ethanol, centrifuged for 10 min after each wash,
155 and dried at 60°C during 5-10 min in a SpeedVac. Finally, the pellet was resuspended in nuclease-free
156 water. DNA concentration and quality were estimated by spectrophotometry and standard DNA agarose
157 gel electrophoresis [56].

158 Polymerase chain reactions (PCR) for partial amplification of the mitochondrial *COI* gene was conducted
159 using the primer set JM76 (5´-GAGCTGAATTAGGRACTCCAGG-3´) and JM77 (5´-
160 ATCACCTCCWCCTGCAGGATC-3´), to produce an expected amplicon of 569 base pairs (bp) [57]. The
161 PCR mixture contained 100-150 ng of gDNA, 1.5 mM of $MgCl_2$, 1x PCR buffer, 0.2 mM of each dNTP,
162 0.32 µM of each primer and 0.5U of GoTaq® DNA Polymerase (Promega) in a total volume of 25 µL.
163 The thermocycling condition was 94°C x 1 min (1x), followed by 33 cycles at 92°C x 45 s, 56°C x 45 s,
164 and 72°C x 1 min, and one cycle at 72°C x 3 min for final extension. Amplicons were then subjected to
165 restriction analysis using the *Msp*I (HpaII) restriction endonuclease. Samples were gently mixed,
166 centrifuged for a few seconds and incubated overnight at 37°C. Subsequently, digestion and the resulting

products were verified using a 1.5% agarose gel electrophoresis. The corn strain (*CS*) was identified from restriction analyses yielding two fragments (497bp and 72bp), while restriction analyses that produced no digestion identified the rice strain (*RS*) [57].

DNA extraction, amplification and 16S rDNA sequencing

The midgut obtained from dissected larvae were individually powdered in liquid nitrogen, and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), following the manufacturer's recommendations. The quality, integrity and purity of the DNA obtained was measured by spectrophotometry and agarose gel electrophoresis as before. DNA samples were stored in -20°C and sent for library construction, normalization and sequencing in the Center for Functional Genomics (http://www.esalq.usp.br/genomicafuncional/), one of the multiusers laboratories of our institution. Paired-end reads were generated after amplifying the v3-v4 region of 16S rRNA gene (approximately 550 bp) using the Nextera XT DNA Library Preparation Kit (Illumina) for paired-end (2x 300 bp) sequencing in the Illumina MiSeq platform.

Sequences analyses

Illumina adapters at the 3' end of the reads were removed using *Cutadapt* [58]. The bioinformatics analyses of the gut microbiome were performed with QIIME2 v. 2020.2.0 [59]. Raw sequence data were quality filtered with *q2-dada2* plugin for filtering phiX reads and chimeric sequences [60]. In order to remove low quality regions from quality filter reads, *dada2 denoise-single* method trimmed off the first 18 nucleotides of the forward reads and 22 nucleotides from the reverse reads. It also truncated each sequence at position 290 in the forward and 220 in the reversed reads. These positions were chosen based on visual inspection of plotted quality scores from demultiplexed reads. A phylogeny was estimated with *SEPP* [61] as implemented in the *q2-fragment-insertion* QIIME2 plugin. All amplicon sequence variants (ASVs) were

aligned with *feature-classifier classify-sklearn* against the SILVA-132-99 database [62] that was trained with a Naïve Bayes classifier [63] on the Illumina 16S rRNA gene primers targeting the V3–V4 region.

The downstream analysis was performed in the MicrobiomeAnalyst web platform (https://www.microbiomeanalyst.ca/) [64] and in R (version 4.0.4) [65]. Data were filtered keeping ASV with minimum count of four (4) per library and low count filter based on 20% prevalence across samples. Data were rarefied to the minimum library size (1155 reads), before any statistical comparisons. Rarefaction curves were based on the relationship between number of ASVs and number of sequences. Alpha diversity analysis was measured by the observed species and Shannon index. The results were plotted across samples and showed as box plots for each group. Beta diversity was investigated through principal components analysis (PCoA) using unweighted and weighted UniFrac distances, and through hierarchical clustering analysis using unweighted UniFrac distances.

We used PERMANOVA to test the strength and statistical significance of sample groupings based on generalized weighted UniFrac distances. This distance contains an extra parameter α (set at α=0.5) to control the weight of abundant lineages, so the distance is not dominated by highly abundant lineages. When differences were found between samples distances, a *post-hoc* analysis was performed with the package *pairwise.adonis* to identify differences among treatments and verify the adjusted *p* value [66]. As PERMANOVA assumes homogeneity of variances, we used *betadisper*, a multivariate analogue of Levene's test, as implemented in R to verify whether differences between groups in terms of their centroids are not due to differences in variances. Analysis of similarity (ANOSIM) was used when there was heterogeneity of variance among groups. In our sample set we had basically 3 groups: (i) countries that presented both strains in corn plants, (ii) countries with only the corn strain in corn plants and (iii) Panama with both strains in corn plants and only the rice strain in the rice plant. Since our design is unbalanced, we performed separate analyses to properly grasp our data. First, we excluded the samples that had rice as host plant, thus only the variables "strain" and "country" were considered. To test the effect of country and host plant, we excluded the corn strain from the analysis, considering only the rice strain, and performed

multilevel pairwise comparison using Adonis (PERMANOVA) from package *vegan* with adjusted *p*-values.

To visualize taxa abundance across the different groups, taxa plots were constructed based on phyla and genera. The core microbiome analysis was defined as the genera present in 50% or more of the samples and showing a relative abundance of 0.05% in each library. The differential abundance analysis was also analyzed using *DESeq2* methods [67]. Pattern Search was used to identify which features were correlated with the core microbiome in the gut microbial community. Pearson r was the distance measure used using the MicrobiomeAnalyst tool [64].

To cluster our samples groups into distinct 'metacommunities', we performed Dirichlet multinomial mixtures using the *get.communitytype* function [68] after exportation of biom ASV table from qiime2 to Mothur (v.1.44.3) and the selection of subsamples with *subsample=1000*, excluding low abundance samples that might be a result of artifact operational units and/or variation due to rare taxons ("singletons"). The best fitting number of metacommunities was obtained by selecting the minimum local Laplace value obtained after five iterations.

Results

A total of 63 *S. frugiperda* individuals, 8 *RS* and 45 *CS* were used in our analyses. Except for 8 specimens from Panama that were collected on rice, all other samples were collected in corn fields. Out of the 63 specimens analyzed, 21 were from Brazil (*CS*=18; *RS*=3), nine from Colombia (*CS*=8; *RS*=1), eight from Mexico (*CS*=8), six from Paraguay (*CS*=3; *RS*=3), five from Peru (*CS*=3; *RS*=2), and 13 from Panama (6 from corn fields; *CS*=5, *RS*=1; and 8 from rice fields; *RS*=8).

Rarefaction analysis (Fig. S1) showed that sampling was adequate for an accurate characterization of the diversity and richness of the larval gut microbiota of *S. frugiperda*. Samples that failed to achieve adequate sampling depth were excluded from further analyses. There was no difference in alpha-diversity values

between strains or among countries (Fig. 1) measure by observed species and Shannon diversity indices. The beta diversity measured by weighted Unifrac distances did not exhibit specific clustering based on the country of origin or *S. frugiperda* strains (Fig. 2).

When considering samples collected in maize, no differences in the composition of the gut microbial community between strains (p=0.215) (Table 1) nor among different countries considering the adjusted p-values (p-values < 0.05) were detected (Table 2). Betadisper showed that groups had the same dispersion, failing to reject the null hypothesis of homogeneous multivariate dispersions, meeting the assumption for Adonis (Table 1). It thus provided confidence to the PERMANOVA results, meaning the values obtained were not an artifact of heterogeneity of dispersions. Likewise, no differences were found between host plants (p=0.344) or country (p=0.0709) when considering only the rice strain (Table 3). Additionally, all replicates of metacommunity analyses resulted in the same pattern (K=1), meaning that according to the Dirichlet model there is not a clear pattern of grouping ASVs across samples.

At the phylum level, the midgut of *S. frugiperda* was composed by *Proteobacteria*, *Firmicutes* and *Actinobacteria* (Fig. 3). There was no significant difference at the phylum level among countries or between strains. Taxa bar plots at the genus level indicated that individuals from the same country exhibited a high degree of variability in terms of bacteria taxa abundance (Fig. 4). *Klebsiella* and *Erysipelatoclostridium* were the taxa that differed among countries (Fig. 5), and the abundance of *Erysipelatoclostridium* also differed between *RS* and *CS* (Fig. 6).

The bacterial core of the larval midgut of *S. frugiperda* at the genus level was composed by *Pseudomonas* and *Enterococcus*. Correlation analysis identified 10 genera that were positively correlated and 10 genera negatively correlated with *Pseudomonas*. However, only three genera were positively correlated, while 18 were negatively correlated with *Enterococcus* (Fig. 7).

Discussion

Our results indicate that bacterial communities of the Fall armyworm larval midgut do not differ between strains collected from the same country nor among countries. These findings follow the pattern of the population genetic structure of *S. frugiperda* in the Western Hemisphere, where the majority of the genetic variability is within individual populations and not between populations, suggesting that populations of *S. frugiperda* functions as a panmictic population [50, 51].

As expected, we detected high variations in the composition of the gut microbiota among larvae. Such differences are likely to occur due to differences in corn varieties and associated endophytes, and soil type and associated microbiota, which also interact with plants and affect the plant endophyte community, ultimately interfering with the microbial composition of herbivores [69-71]. Variation in the microbiota from individual samples within treatments is commonly reported to several organisms, including species of Lepidoptera [72-74]. In humans, interindividual variation in the populations of gut microbes can be higher than 90% [75].

Obadia and collaborators [76] exploring the colonization of bacteria in the *Drosophila melanogaster* gut found that several strains of different species can maintain a stable association with the fly gut under laboratory conditions. They demonstrated that the establishment of bacteria in the gut works like a lottery and that stochastic factors generate alternative, stable states of gut colonization. Moreover, the resident species that have colonized the larval gut earlier, reduced the chances of subsequent colonization. Another interesting point raised concerning our study was that the peritrophic matrix in the midgut prevents the bacteria from attaching to the epithelial cells. Therefore, the lack of tissue attachment potentially makes these luminal populations less stable within the gut.

But regardless the high variation observed in the gut microbiota associated with the larval midgut of *S. frugiperda*, our analysis identified a core of bacteria despite the geographical origin of fall armyworm samples. The maintenance of a core independently of any interfering systemic effects points to the existence of bacterial associates with specific functions. In addition, the high variability in the composition of the midgut microbiota may allow for rapid host adaptation through rapid selection of microbiota suitable for

289 contributing to the host under different stress conditions, such as abiotic factors, dietary resources, and risk
290 of natural enemy attack [77].

291 The ASVs *Pseudomonas* and *Enterococcus* identified in this study as core members of the microbiota of
292 the fall armyworm were also identified before as part of the core taxa associated with the gut of *S.*
293 *frugiperda* larvae from corn fields [10, 77-80]. The high abundance of *Pseudomonas* in our samples
294 suggests that this genus of bacteria could assist *S. frugiperda* larvae to overcome environmental stressors,
295 particularly by aiding larvae to degrade natural and/or synthetic toxic xenobiotics. *Pseudomonas* capable
296 to degrade several pesticides were recovered from the gut of laboratory-selected resistant lines (Almeida,
297 Moraes, Trigo, Omoto, & Cônsoli, 2017), but also from field populations of *S. frugiperda* collected from
298 several corn-producing areas in Brazil (Gomes, Omoto, & Cônsoli, 2020). *Pseudomonas* have also been
299 demonstrated to degrade secondary metabolites in the gut of a coleopteran host [81]. Additionally,
300 *Pseudomonas* abundance increased in the gut of *Plutella xylostella* resistant to prothiofos when compared
301 to susceptible larvae, and was also shown to have antagonistic activity to several species of
302 entomopathogenic fungi through siderophore production as demonstrated in culture plates [82].

303 It is noteworthy that *Enterococcus* is the most prevalent and abundant group identified in the gut microbiota
304 of *Spodoptera* species [78, 79, 83], and also the most active in the gut of *S. frugiperda* [84]. Additionally,
305 it has been demonstrated that *Enterococcus mundtii* is effective in colonizing and forming biofilm in the
306 gut of *Spodoptera littoralis* [22, 27]. There is also evidence that *E. mundtii* can be inherited by *S. littoralis*
307 through vertical transmission [23]. Some species of *Enterococcus* produce antimicrobial peptides with high
308 level of inhibitory activity against potential bacterial competitors [27], which may explain its prevalence
309 when compared to other phylotypes in *S. frugiperda* gut communities, but also the high negative correlation
310 of *Enterococcus* with the other bacterial species of the gut microbiota community of *S. frugiperda* in this
311 study.

312 Overall, this study provided an extended view of the fall armyworm gut microbiota and supported the
313 hypothesis that bacterial taxonomic compositions across different localities in the Western Hemisphere are

similar to each other, presenting high inter-individual variance, and that there are no significant differences in gut microbiota composition between the host-adapted strains of *S. frugiperda*. Nevertheless, our findings provide further evidence that *Pseudomonas* and *Enterococcus* are true symbionts of *S. frugiperda* as they were identified in the gut microbiota of *S. frugiperda* larvae regardless the host plant and site of collection. Further investigations on the functional contribution of these species as members of the gut bacterial community of fall armyworm larvae is required for a deeper understanding of the nature of this relationship.

Acknowledgements

We are grateful to the São Paulo Research Foundation (FAPESP) (process 2011/50877-0) and the Ministry of Science, Technology and Innovation (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq: process 462140-2014/8) for the grant provided to the senior author. The HPC resources made available by the Superintendence of Information Technology of the University of São Paulo. We also thank FAPESP for the PhD student fellowship (2017/24377-7) provided to the first author. We also would like to thank our collaborators from Panama, Peru, Colombia, Paraguay and Brazil who helped us with the field collection of larval samples. This manuscript is one of the chapters of the PhD Thesis of the first author.

References

1. Hammer TJ, Sanders JG, Fierer N (2019) Not all animals need a microbiome. FEMS Microbiol Lett 366: fnz117. https://doi.org/10.1093/femsle/fnz117

2. Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proc Natl Acad Sci U S A 107: 20051-20056. https://doi.org/10.1073/pnas.1009906107

3. Sharon G, Segal D, Zilber-Rosenberg I, Rosenberg E (2011) Symbiotic bacteria are responsible for diet-induced mating preference in *Drosophila melanogaster*, providing support for the hologenome concept of evolution. Gut Microbes 2: 190-192. https://doi.org/10.4161/gmic.2.3.16103

4. Frago E, Dicke M, Godfray HCJ (2012) Insect symbionts as hidden players in insect–plant interactions. Trends Ecol Evol 27: 705-711. https://doi.org/10.1016/j.tree.2012.08.013

5. Douglas AE (2009) The microbial dimension in insect nutritional ecology. Funct Ecol 23: 38-47. https://doi.org/10.1111/j.1365-2435.2008.01442.x

6. Engel P, Moran NA (2013) The gut microbiota of insects–diversity in structure and function. FEMS Microbiol Rev 37: 699-735. https://doi.org/10.1111/1574-6976.12025

7. Azambuja P, Feder D, Garcia ES (2004) Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. Exp Parasitol 107: 89-96. https://doi.org/10.1016/j.exppara.2004.04.007

8. Oliveira NC de, Cônsoli FL (2020) Beyond host regulation: Changes in gut microbiome of permissive and non-permissive hosts following parasitization by the wasp *Cotesia flavipes*. FEMS Microbiol Ecol 96(2): fiz206. https://doi.org/10.1093/femsec/fiz206

353 9. Kikuchi Y, Hosokawa T, Fukatsu T (2011) An ancient but promiscuous host–symbiont association
354 between *Burkholderia* gut symbionts and their heteropteran hosts. ISME J 5: 446-460.
355 https://doi.org/10.1038/ismej.2010.150

356 10. Gomes AFF, Omoto C, Cônsoli FL (2020) Gut bacteria of field-collected larvae of *Spodoptera*
357 *frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than
358 laboratory-selected resistant strains. J Pest Sci 93: 833-851. https://doi.org/10.1007/s10340-020-01202-0

359 11. Chen B, Zhang N, Xie S, Zhang X, He J, Muhammad A, Sun C, Lu X, Shao Y (2020) Gut bacteria of
360 the silkworm *Bombyx mori* facilitate host resistance against the toxic effects of organophosphate
361 insecticides. Environ Int 143: 105886. https://doi.org/10.1016/j.envint.2020.105886

362 12. Almeida LGd, Moraes LABd, Trigo JR, Omoto C, Cônsoli FL (2017) The gut microbiota of insecticide-
363 resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation.
364 PLoS One 12: e0174754. https://doi.org/10.1371/journal.pone.0174754

365 13. Chu C-C, Spencer JL, Curzi MJ, Zavala JA, Seufferheld MJ (2013) Gut bacteria facilitate adaptation to
366 crop rotation in the western corn rootworm. Proc Natl Acad Sci U S A 110: 11917-11922.
367 https://doi.org/10.1073/pnas.1301886110

368 14. Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T (2006) Strict host-symbiont co-speciation
369 and reductive genome evolution in insect gut bacteria. PLoS Biol 4: e337.
370 https://doi.org/10.1371/journal.pbio.0040337

371 15. Koch H, Schmid-Hempel P (2011) Socially transmitted gut microbiota protect bumble bees against an
372 intestinal parasite. Proc Natl Acad Sci U S A 108: 19288-19292. https://doi.org/10.1073/pnas.1110474108

373 16. Cheng D, Guo Z, Riegler M, Xi Z, Liang G, Xu Y (2017) Gut symbiont enhances insecticide resistance
374 in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome 5: 13.
375 https://doi.org/10.1186/s40168-017-0236-z

376 17. Salcedo-Porras N, Umaña-Diaz C, Bitencourt RdOB, Lowenberger C (2020) The role of bacterial
377 symbionts in Triatomines: an evolutionary perspective. Microorganisms 8: 1438.
378 https://doi.org/10.3390/microorganisms8091438

379 18. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N (2017) Caterpillars lack a resident gut
380 microbiome. Proc Natl Acad Sci U S A 114: 9641-9646. https://doi.org/10.1073/pnas.1707186114

381 19. Appel HM (2017) The chewing herbivore gut lumen: physicochemical conditions and their impact on
382 plant nutrients, allelochemicals, and insect pathogens. In: Bernays EA (Ed.), Insect-Plant Interactions. CRC
383 Press, pp. 209-224

384 20. Dow JA (1984) Extremely high pH in biological systems: a model for carbonate transport. Am J Physiol
385 246: R633-R636. https://doi.org/10.1152/ajpregu.1984.246.4.R633

386 21. Gayatri Priya N, Ojha A, Kajla MK, Raj A, Rajagopal R (2012) Host plant induced variation in gut
387 bacteria of *Helicoverpa armigera*. PLoS One 7: e30768. https://doi.org/10.1371/journal.pone.0030768

388 22. Mazumdar T, Teh BS, Murali A, Schmidt-Heck W, Vogel H, Schlenker Y, Boland W (2021) Survival
389 strategies of *Enterococcus mundtii* in the gut of *Spodoptera littoralis*: a live report. J Chem Ecol 47: 227-
390 241. https://doi.org/10.1007/s10886-021-01246-1.

391 23. Teh B-S, Apel J, Shao Y, Boland W (2016) Colonization of the intestinal tract of the polyphagous pest
392 *Spodoptera littoralis* with the GFP-tagged indigenous gut bacterium *Enterococcus mundtii*. Front Microbiol
393 7: 928. https://doi.org/10.3389/fmicb.2016.00928

394 24. Mason CJ, St. Clair A, Peiffer M, Gomez E, Jones AG, Felton GW, Hoover K (2020) Diet influences
395 proliferation and stability of gut bacterial populations in herbivorous lepidopteran larvae. PLoS One 15:
396 e0229848. https://doi.org/10.1371/journal.pone.0229848

25. Shao Y, Arias-Cordero E, Guo H, Bartram S, Boland W (2014) In vivo pyro-SIP assessing active gut microbiota of the Cotton Leafworm, *Spodoptera littoralis*. PLoS One 9: e85948. https://doi.org/10.1371/journal.pone.0085948

26. Xia X, Lan B, Tao X, Lin J, You M (2020) Characterization of *Spodoptera litura* gut bacteria and their role in feeding and growth of the host. Front Microbiol 11: 1492. https://doi.org/10.3389/fmicb.2020.01492

27. Shao Y, Chen B, Sun C, Ishida K, Hertweck C, Boland W (2017) Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. Cell Chem Biol 24: 66-75. https://doi.org/10.1016/j.chembiol.2016.11.015

28. Moya A, Pereto J, Gil R, Latorre A (2008) Learning how to live together: genomic insights into prokaryote-animal symbioses. Nature Rev Genet 9: 218-229. https://doi.org/10.1038/nrg2319

29. Douglas AE (2011) Lessons from studying insect symbioses. Cell Host Microbe 10: 359-367. https://doi.org/10.1016/j.chom.2011.09.001

30. Kostic AD, Howitt MR, Garrett WS (2013) Exploring host–microbiota interactions in animal models and humans. Genes Dev 27: 701-718. https://doi.org/10.1101/gad.212522.112

31. Scoble MJ (1992) The Lepidoptera. Form, function and diversity. Oxford University Press.

32. McCaffery AR (1998) Resistance to insecticides in Heliothine Lepidoptera: a global view. Philos Trans R Soc Lond B Biol Sci 353: 1735-1750. https://doi.org/10.1098/rstb.1998.0326

33. Riegler M (2018) Insect threats to food security. Science 361: 846. https://doi.org/10.1126/science.aau7311

34. Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11: e0165632. https://doi.org/10.1371/journal.pone.0165632

419 35. Johnson SJ (1987) Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda*
420 in the Western Hemisphere. Int J Trop Insect Sci 8: 543-549. https://doi.org/10.1017/S1742758400022591

421 36. Otim MH, Tay WT, Walsh TK, Kanyesigye D, Adumo S, Abongosi J, Ochen S, Sserumaga J, Alibu S,
422 Abalo G, Asea G, Agona A (2018) Detection of sister-species in invasive populations of the fall armyworm
423 *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from Uganda. PLoS One 13: e0194571.
424 https://doi.org/10.1371/journal.pone.0194571

425 37. Padhee AK, Prasanna BM (2019) The emerging threat of Fall Armyworm in India. Indian Farm 69: 51-
426 54.

427 38. Piggott MP, Tadle FPJ, Patel S, Gomez KC, Thistleton B (2021) Corn-strain or rice-strain? Detection
428 of fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), in northern Australia. Int J
429 Trop Insect Sci 41: 2607-2615. https://doi.org/10.1007/s42690-021-00441-7

430 39. Montezano DG, Specht A, Sosa-Gómez DR, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SVd,
431 Peterson JA, Hunt TE (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the
432 Americas. African Entomol 26: 286-300. https://doi.org/10.4001/003.026.0286

433 40. Adamczyk Jr JJ, Holloway JW, Leonard BR, Graves JB (1997) Susceptibility of fall armyworm
434 collected from different plant hosts to selected insecticides and transgenic Bt cotton. J Cotton Sci 1: 21-28.

435 41. Cruz-Esteban S, Rojas JC, Sánchez-Guillén D, Cruz-López L, Malo EA (2018) Geographic variation
436 in pheromone component ratio and antennal responses, but not in attraction, to sex pheromones among fall
437 armyworm populations infesting corn in Mexico. J Pest Sci 91: 973-983. https://doi.org/10.1007/s10340-
438 018-0967-z

439 42. Lima ER, McNeil JN (2009) Female sex pheromones in the host races and hybrids of the fall armyworm,
440 *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Chemoecology 19: 29-36.
441 https://doi.org/10.1007/s00049-009-0005-y

442 43. Schöfl G, Heckel DG, Groot AT (2009) Time-shifted reproductive behaviours among fall armyworm
443 (Noctuidae: Spodoptera frugiperda) host strains: evidence for differing modes of inheritance. J Evol Biol
444 22: 1447-1459. https://doi.org/10.1111/j.1420-9101.2009.01759.x

445 44. Veenstra KH, Pashley DP, Ottea JA (1995) Host-plant adaptation in fall armyworm host strains:
446 comparison of food consumption, utilization, and detoxification enzyme activities. Ann Entomol Soc Am
447 88: 80-91. https://doi.org/10.1093/aesa/88.1.80

448 45. Pashley DP, Hardy TN, Hammond AM (1995) Host effects on developmental and reproductive traits
449 in fall armyworm strains (Lepidoptera: Noctuidae). Ann Entomol Soc Am 88: 748-755.
450 https://doi.org/10.1093/aesa/88.6.748

451 46. Orsucci M, Mone Y, Audiot P, Gimenez S, Nhim S, Nait-Saidi R, Frayssinet M, Dumont G, Boudon J-
452 P, Vabre M (2020) Transcriptional differences between the two host strains of *Spodoptera frugiperda*
453 (Lepidoptera: Noctuidae). bioRxiv: 263186. https://doi.org/10.1101/263186

454 47. Ingber DA, Mason CE, Flexner L (2018) Cry1 Bt susceptibilities of fall armyworm (Lepidoptera:
455 Noctuidae) host strains. J Econ Entomol 111: 361-368. https://doi.org/10.1093/jee/tox311

456 48. Gouin A, Bretaudeau A, Nam K, Gimenez S, Aury JM, Duvic B, Hilliou F, Durand N, Montagné N,
457 Darboux I, Kuwar S, Chertemps T, Siaussat D, Bretschneider A, Moné Y, Ahn SJ, Hänniger S, Grenet
458 ASG, Neunemann D, Maumus F, Luyten I, Labadie K, Xu W, Koutroumpa F, Escoubas JM, Llopis A,
459 Maïbèche-Coisne M, Salasc F, Tomar A, Anderson AR, Khan SA, Dumas P, Orsucci M, Guy J, Belser C,
460 Alberti A, Noel B, Couloux A, Mercier J, Nidelet S, Dubois E, Liu NY, Boulogne I, Mirabeau O, Le Goff
461 G, Gordon G, Oakeshott J, Consoli FL, Volkoff AN, Fescemyer HW, Marden JH, Luthe DS, Herrero S,
462 Heckel DG, Wincker P, Kergoat GJ, Amselem J, Quesneville H, Groot AT, Jacquin-Joly E, Nègre N,
463 Lemaitre C, Legeai F, d'Alençon E, Fourniere P (2017) Two genomes of highly polyphagous lepidopteran
464 pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. Sci Rep 7: 11816.
465 https://doi.org/10.1038/s41598-017-10461-4

466 49. Silva-Brandão KL, Horikoshi RJ, Bernardi D, Omoto C, Figueira A, Brandão MM (2017) Transcript
467 expression plasticity as a response to alternative larval host plants in the speciation process of corn and rice
468 strains of Spodoptera frugiperda. BMC Genomics 18: 792. https://doi.org/10.1186/s12864-017-4170-z

469 50. Clark PL, Molina-Ochoa J, Martinelli S, Skoda SR, Isenhour DJ, Lee DJ, Krumm JT, Foster JE (2007)
470 Population variation of the fall armyworm, *Spodoptera frugiperda,* in the Western Hemisphere. J Insect Sci
471 7: 1-10. https://doi.org/10.1673/031.007.0501

472 51. Arias O, Cordeiro E, Corrêa AS, Domingues FA, Guidolin AS, Omoto C (2019) Population genetic
473 structure and demographic history of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): implications for
474 insect resistance management programs. Pest Manag Sci 75: 2948-2957. https://doi.org/10.1002/ps.5407

475 52. Nagoshi RN, Rosas-García NM, Meagher RL, Fleischer SJ, Westbrook JK, Sappington TW, Hay-Roe
476 M, Thomas JMG, Murúa GM (2015) Haplotype profile comparisons between *Spodoptera frugiperda*
477 (Lepidoptera: Noctuidae) populations from Mexico with those from Puerto Rico, South America, and the
478 United States and their implications to migratory behavior. J Econ Entomol 108: 135-144.
479 https://doi.org/10.1093/jee/tou044

480 53. Tay WT, Rane R, Padovan A, Walsh T, Elfekih S, Downes S, Nam K, d'Alençon E, Zhang J, Wu Y,
481 Nègre N, Kunz D, Kriticos DJ, Czepak C, Otim M, Gordon KHJ (2020) Whole genome sequencing of
482 global *Spodoptera frugiperda* populations: evidence for complex, multiple introductions across the Old
483 World. bioRxiv: 2020.2006.2012.147660. https://doi.org/10.1101/2020.06.12.147660

484 54. Silva-Brandão KL, Peruchi A, Seraphim N, Murad NF, Carvalho RA, Farias JR, Omoto C, Cônsoli FL,
485 Figueira A, Brandão MM (2018) Loci under selection and markers associated with host plant and host-
486 related strains shape the genetic structure of Brazilian populations of *Spodoptera frugiperda* (Lepidoptera,
487 Noctuidae). PLoS One 13: e0197378. https://doi.org/10.1371/journal.pone.0197378

488 55. Montezano DG, Specht A, Sosa-Gomez DR, Roque-Specht VF, de Paula-Moraes SV, Peterson JA,
489 Hunt TE (2019) Developmental parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) immature

490 stages under controlled and standardized conditions. J Agric Sci 11(8): 76.
491 https://doi.org/10.5539/jas.v11n8p76 .

492 56. Sambrook J (2001) Molecular cloning: a laboratory manual/Joseph Sambrook, David W. Russell. Cold
493 Spring Harbor, NY: Cold Spring Harbor Laboratory.

494 57. Levy HC, Garcia-Maruniak A, Maruniak JE (2002) Strain identification of Spodoptera frugiperda
495 (Lepidoptera: Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase C subunit I gene. Fla
496 Entomol 85: 186-191. https://doi.org/10.1653/0015-4040(2002)085[0186:SIOSFL]2.0.CO;2

497 58. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads.
498 EMBnet.journal 17(1): 10-12. https://doi.org/10.14806/ej.17.1.200..

499 59. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ,
500 Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan
501 BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM,
502 Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson
503 DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen
504 S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I,
505 Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz
506 C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT,
507 Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E,
508 Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ,
509 Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der
510 Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren
511 J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso
512 JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat
513 Biotechnol 37: 852-857. https://doi.org/10.1038/s41587-019-0209-9

514 60. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-
515 resolution sample inference from Illumina amplicon data. Nat Methods 13: 581-583.
516 https://doi.org/10.1038/nmeth.3869

517 61. Mirarab S, Nguyen N, Warnow T (2012) SEPP: SATé-enabled phylogenetic placement. Pac Symp
518 Biocomput 2012: 247-258. https://doi.org/10.1142/9789814366496_0024

519 62. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The
520 SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic
521 Acids Res 41: D590-D596. https://doi.org/10.1093/nar/gks1219

522 63. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JG (2018)
523 Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-
524 classifier plugin. Microbiome 6: 90. https://doi.org/10.1186/s40168-018-0470-z

525 64. Chong J, Liu P, Zhou G, Xia J (2020) Using MicrobiomeAnalyst for comprehensive statistical,
526 functional, and meta-analysis of microbiome data. Nat Protoc 15: 799-821. https://doi.org/10.1038/s41596-
527 019-0264-1

528 65. Team RC (2020) R: A language and environment for statistical computing. Version 4.0. 2 (Taking Off
529 Again). R Foundation for Statistical Computing, Vienna, Austria.

530 66. Arbizu PM (2020). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version
531 0.4.

532 67. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq
533 data with DESeq2. Genome Biol 15: 550. https://doi.org/10.1186/s13059-014-0550-8

534 68. Holmes I, Harris K, Quince C (2012) Dirichlet multinomial mixtures: generative models for microbial
535 metagenomics. PLoS One 7: e30126. https://doi.org/10.1371/journal.pone.0030126

536 69. Correa-Galeote D, Bedmar EJ, Arone GJ (2018) Maize endophytic bacterial diversity as affected by
537 soil cultivation history. Front Microbiol 9: 484. https://doi.org/10.3389/fmicb.2018.00484

538 70. Liu Y, Yan H, Zhang X, Zhang R, Li M, Xu T, Yang F, Zheng H, Zhao J (2020) Investigating the
539 endophytic bacterial diversity and community structures in seeds of genetically related maize (*Zea mays*
540 L.) genotypes. 3 Biotech 10: 27. https://doi.org/10.1007/s13205-019-2034-8

541 71. Meliani A, Bensoltane A, Mederbel K (2012) Microbial diversity and abundance in soil: related to plant
542 and soil type. Am J Plant Nutr FertTechnol 2: 10-18. https://doi.org/10.3923/ajpnft.2012.10.18

543 72. Martínez-Solís M, Collado MC, Herrero S (2020) Influence of diet, sex, and viral infections on the gut
544 microbiota composition of *Spodoptera exigua* caterpillars. Front Microbiol 11: 753.
545 https://doi.org/10.3389/fmicb.2020.00753

546 73. Mach N, Ruet A, Clark A, Bars-Cortina D, Ramayo-Caldas Y, Crisci E, Pennarun S, Dhorne-Pollet S,
547 Foury A, Moisan M-P (2020) Priming for welfare: gut microbiota is associated with equitation conditions
548 and behavior in horse athletes. Sci Rep 10:8311. https://doi.org/10.1038/s41598-020-65444-9

549 74. Hisada T, Endoh K, Kuriki K (2015) Inter- and intra-individual variations in seasonal and daily
550 stabilities of the human gut microbiota in Japanese. Arch Microbiol 197: 919-934.
551 https://doi.org/10.1007/s00203-015-1125-0

552 75. Dorrestein Pieter C, Mazmanian Sarkis K, Knight R (2014) Finding the missing links among
553 metabolites, microbes, and the host. Immunity 40: 824-832. https://doi.org/10.1016/j.immuni.2014.05.015

554 76. Obadia B, Güvener ZT, Zhang V, Ceja-Navarro JA, Brodie EL, Ja WW, Ludington WB (2017)
555 Probabilistic invasion underlies natural gut microbiome stability. Curr Biol 27: 1999-2006. e1998.
556 https://doi.org/10.1016/j.cub.2017.05.034

557 77. Paniagua Voirol LR, Frago E, Kaltenpoth M, Hilker M, Fatouros NE (2018) Bacterial symbionts in
558 Lepidoptera: their diversity, transmission, and impact on the host. Front Microbiol 9: 556.
559 https://doi.org/10.3389/fmicb.2018.00556

560 78. Jones AG, Mason CJ, Felton GW, Hoover K (2019) Host plant and population source drive diversity of
561 microbial gut communities in two polyphagous insects. Sci Rep 9: 2792. https://doi.org/10.1038/s41598-
562 019-39163-9

563 79. Gichuhi J, Sevgan S, Khamis F, Van den Berg J, du Plessis H, Ekesi S, Herren JK (2020) Diversity of
564 fall armyworm, *Spodoptera frugiperda* and their gut bacterial community in Kenya. PeerJ 8: e8701.
565 https://doi.org/10.7717/peerj.8701

566 80. Ugwu JA, Liu M, Sun H, Asiegbu FO (2020) Microbiome of the larvae of *Spodoptera frugiperda* (J.E.
567 Smith) (Lepidoptera: Noctuidae) from maize plants. J Appl Entomol 144: 764-776.
568 https://doi.org/10.1111/jen.12821

569 81. Ceja-Navarro JA, Vega FE, Karaoz U, Hao Z, Jenkins S, Lim HC, Kosina P, Infante F, Northen TR,
570 Brodie EL (2015) Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. Nat
571 Commun 6: 7618. https://doi.org/10.1038/ncomms8618

572 82. Indiragandhi P, Anandham R, Madhaiyan M, Poonguzhali S, Kim GH, Saravanan VS, Sa T (2007)
573 Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-
574 caught populations of diamondback moth, *Plutella xylostella* and their potential for, antagonism towards
575 entomopathogenic fungi and host insect nutrition. J Appl Microbiol 103: 2664-2675.
576 https://doi.org/10.1111/j.1365-2672.2007.03506.x

577 83. Chen B, Teh B-S, Sun C, Hu S, Lu X, Boland W, Shao Y (2016) Biodiversity and activity of the gut
578 microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Sci Rep 6: 29505.
579 https://doi.org/10.1038/srep29505

580 84. Rozadilla G, Cabrera NA, Virla EG, Greco NM, McCarthy CB (2020) Gut microbiota of *Spodoptera*

581 *frugiperda* (J.E. Smith) larvae as revealed by metatranscriptomic analysis. J Appl Entomol 144: 351-363.

582 https://doi.org/10.1111/jen.12742

583

FIGURES CAPTIONS

Fig. 1 Alpha diversity index of Shannon index (A, C) and observed taxa (B, D) obtained for samples from the gut microbiota of the corn and rice strains of *Spodoptera frugiperda* larvae (C, D) from different countries (A, B). The statistical values from the Test t (pairwise comparison) and ANOVA (group comparison) are shown in which box.

Fig. 2 Principal coordinates analysis (PCoA) based on unweighted (A, B) and weighted (C, D) unifrac analysis of the midgut microbial community of the corn and rice strains of *Spodoptera frugiperda* larvae (B, D) from different countries (A, C). The statistical values from PERMANOVA are shown in each box.

Fig. 3 Taxonomic composition of the microbial community associated with the midgut of corn and rice strains of *Spodoptera frugiperda* larvae sampled in different countries at the phylum level.

Fig. 4 Taxonomic composition of the microbial community of the larval midgut of corn and rice strains of *Spodoptera frugiperda* at the genus level.

Fig. 5 The abundance of *Klebsiella* and *Erysipelatoclostridium* as a differential feature of the microbiota associated with the larval midgut of *Spodoptera frugiperda* from different countries.

Fig. 6 The abundance of *Erysipelatoclostridium* as a differential feature of the microbiota associated with the larval midgut of the corn and rice strains of *Spodoptera frugiperda*.

Fig. 7 The core gut microbiota of *Spodoptera frugiperda* at the genus level identified by MicrobiomeAnalyst using the parameters sample prevalence (50 %) and relative abundance (0.5 %).

Fig. 8 Pattern correlation analysis of the larval gut bacteria of *Spodoptera frugiperda* at the genus level. Red indicates positive correlation and blue indicates negative correlations with the presence of *Enterococcus* (A) or *Pseudomonas* (B).

606 **Table 1.** PERMANOVA and BETADISPER results from comparisons of the gut microbial communities
607 among countries and *Spodoptera frugiperda* strains (corn and rice strains) excluding samples
608 from rice plants using UniFrac (alpha 0.5) values.
609

	PERMANOVA		ANOSIM		BETADISPER	
	R^2	*p* value	R	*p* value	F value	Pr(>F)
Country	0.11698	0.044 *	-	-	0.2444	0.9406
Strain	0.02181	0.215	-	-	3.3965	0.07093
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

610

611

612

613

614

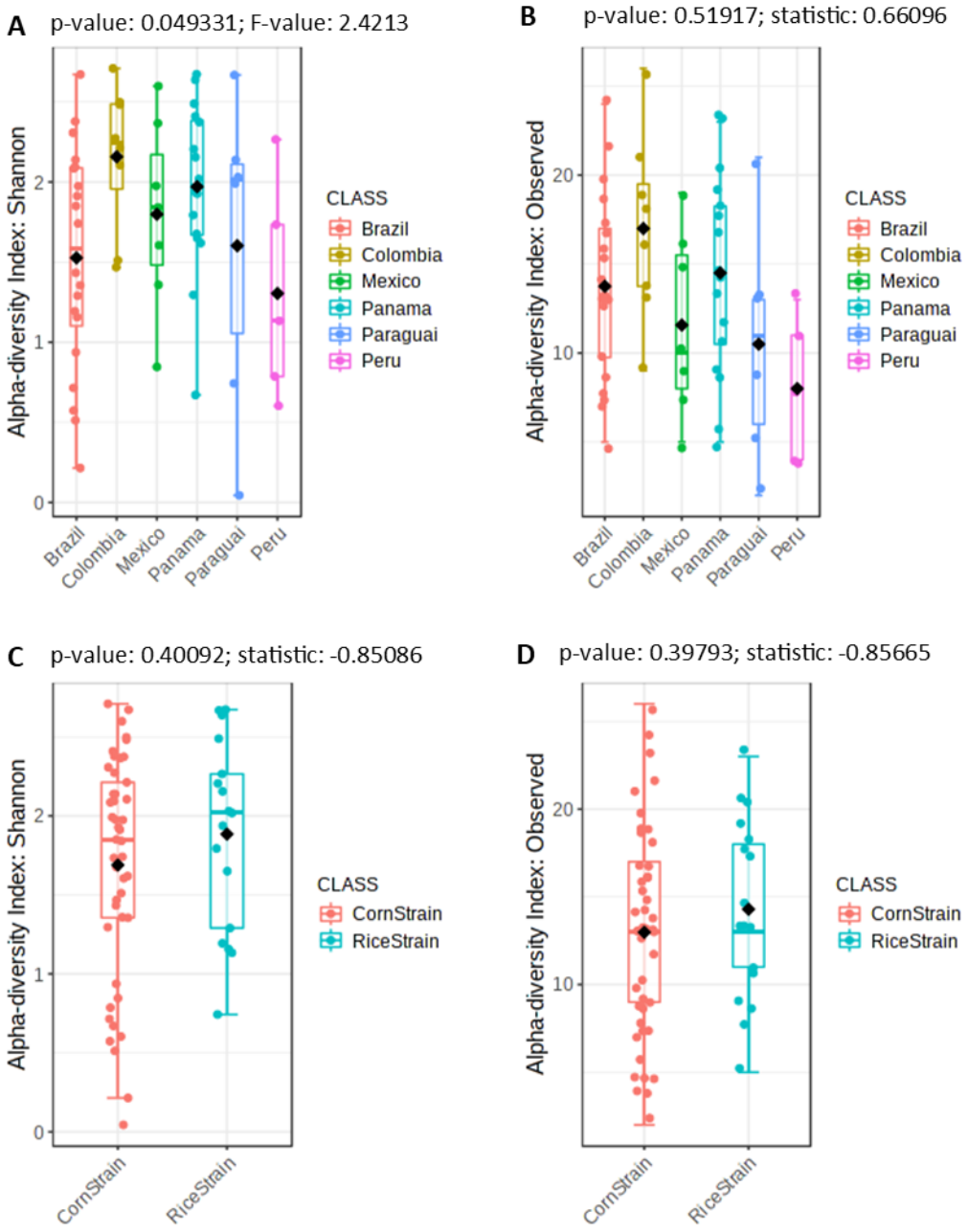
615

Table 2. Post-hoc analysis of comparisons of the *Spodoptera frugiperda* gut microbial communities among countries using UniFrac (alpha 0.5) values.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
Colombia vs Brazil	1	0.3806171	2.0309445	0.07245366	0.011	0.165
Colombia vs Mexico	1	0.2728639	1.3648260	0.08882795	0.080	1.000
Colombia vs Panama	1	0.2279787	1.1800870	0.07773915	0.196	1.000
Colombia vs Paraguai	1	0.3238154	1.6384779	0.12013642	0.095	1.000
Colombia vs Peru	1	0.3136700	1.6244547	0.12867524	0.060	0.900
Brazil vs Mexico	1	0.2544575	1.2752619	0.04675526	0.147	1.000
Brazil vs Panama	1	0.3235731	1.6516793	0.05973161	0.044	0.660
Brazil vs Paraguai	1	0.1472816	0.7425192	0.03000985	0.722	1.000
Brazil vs Peru	1	0.2553736	1.3015037	0.05355651	0.151	1.000
Mexico vs Panama	1	0.2217735	1.0281323	0.06841384	0.390	1.000
Mexico vs Paraguai	1	0.2483650	1.1092582	0.08461640	0.292	1.000
Mexico vs Peru	1	0.2227645	1.0045716	0.08368242	0.383	1.000
Panama vs Paraguai	1	0.2516586	1.1648641	0.08848281	0.254	1.000
Panama vs Peru	1	0.2287484	1.0730526	0.08887998	0.328	1.000
Paraguai vs Peru	1	0.1878847	0.8404890	0.08541130	0.553	1.000

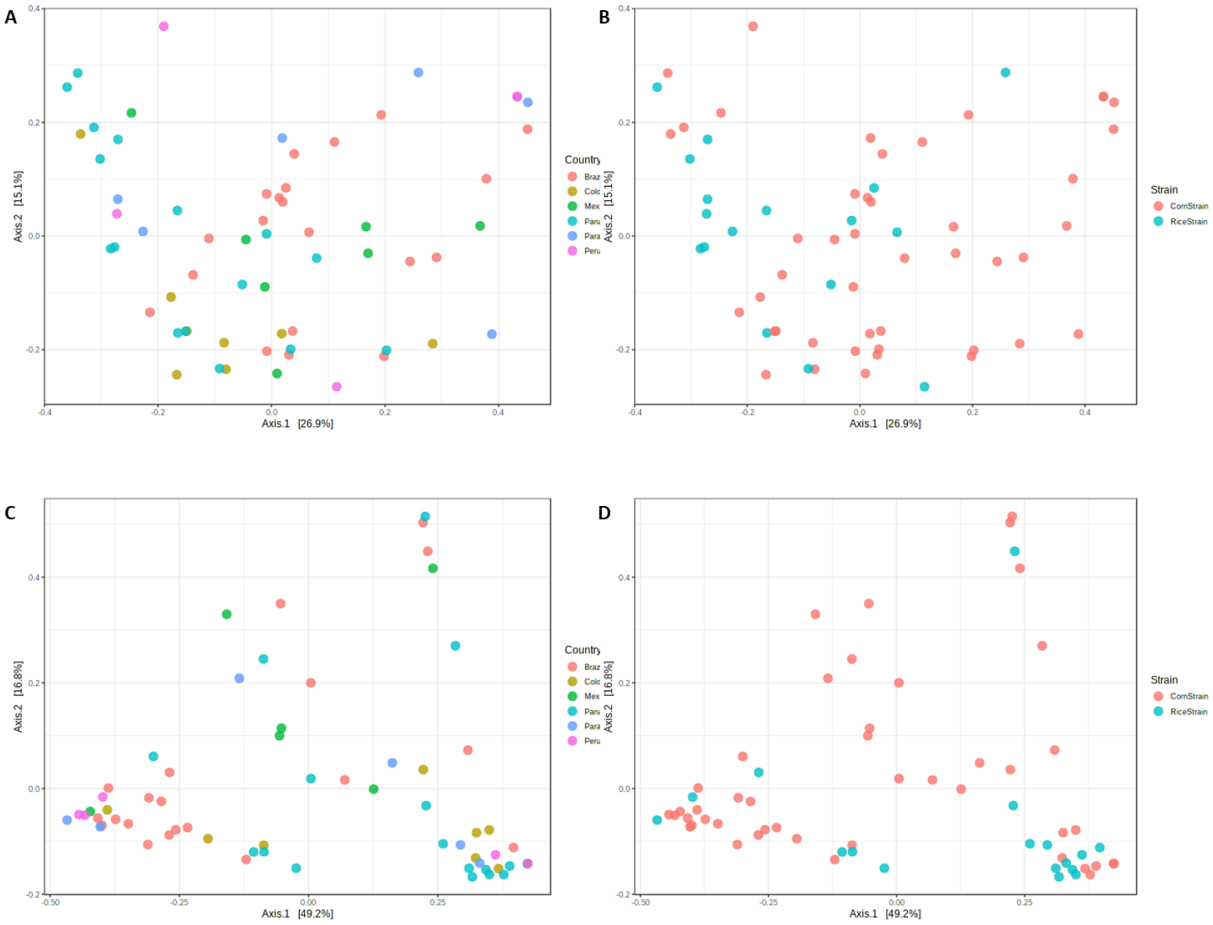
Table 3. PERMANOVA, ANOSIM and BETADISPER results from comparisons of the gut microbial communities of the *Spodoptera frugiperda* rice strain among countries and host plants using UniFrac (alpha 0.5) values.

	PERMANOVA		ANOSIM		BETADISPER	
	R^2	*p* value	R	*p* value	F value	Pr(>F)
Country	-	-	0.2664	0.070929	5.6096	0.00755 **
Host Plant	0.06149	0.344	-	-	2.1328	0.1635
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						



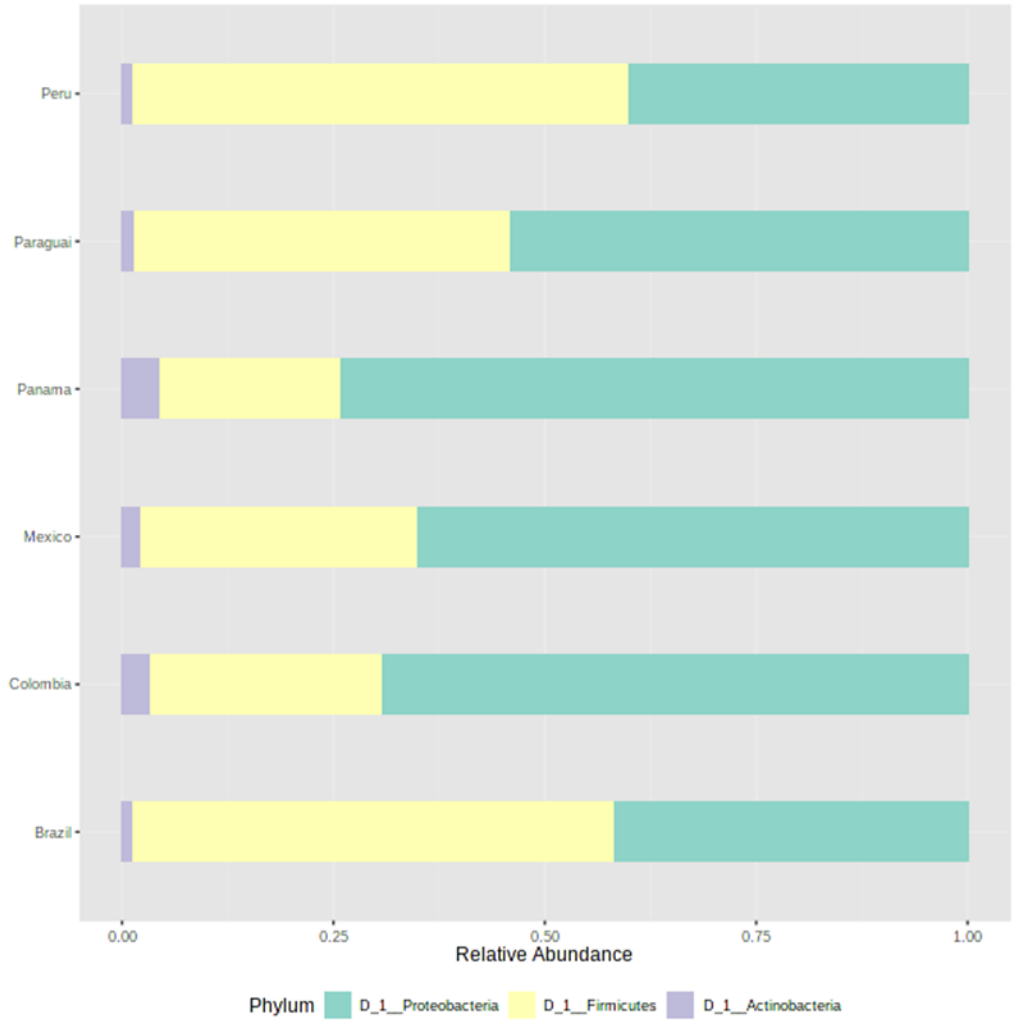
628

629 Fig 1



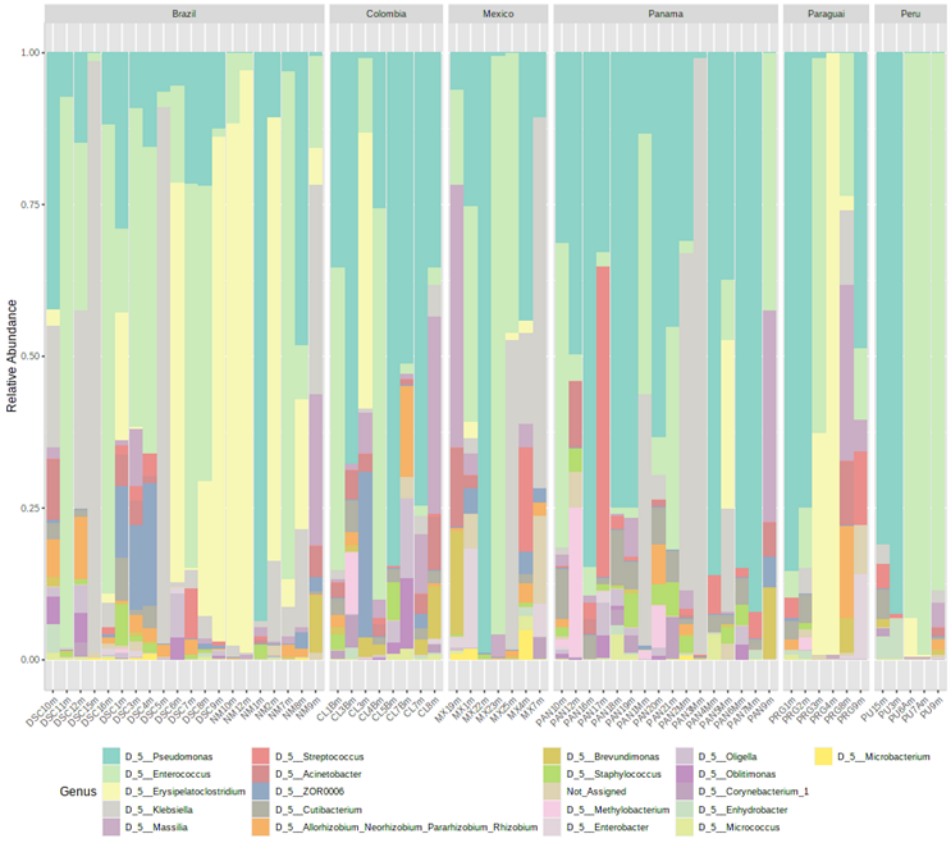
630

631 Fig2



632

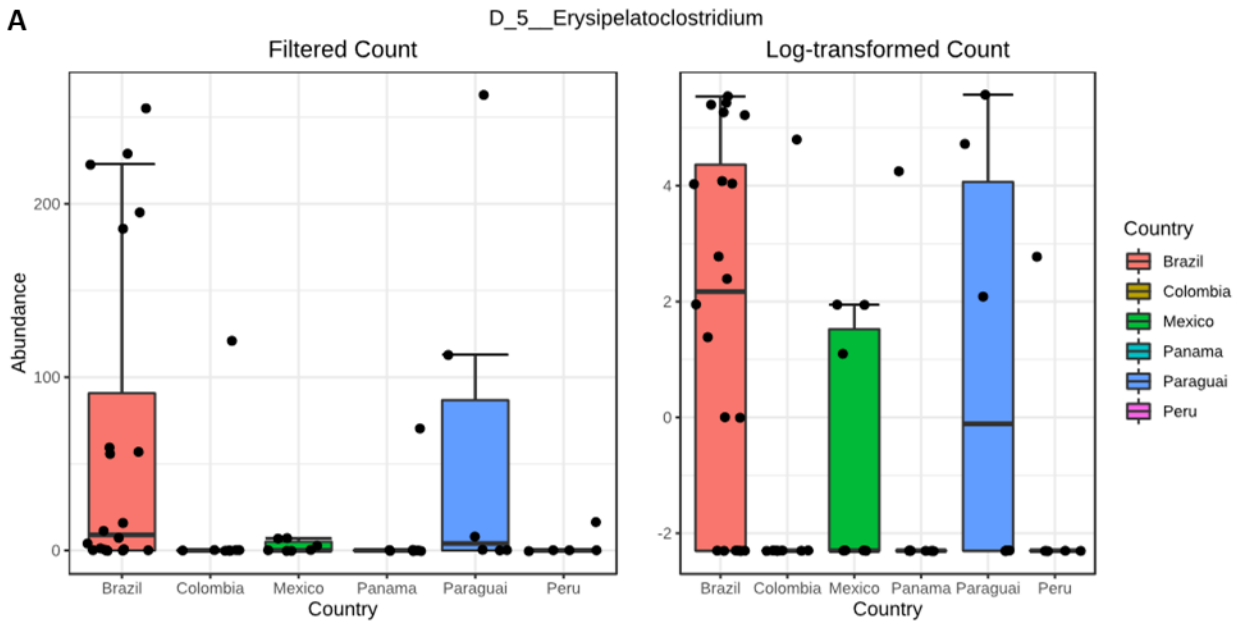
633 Fig 3



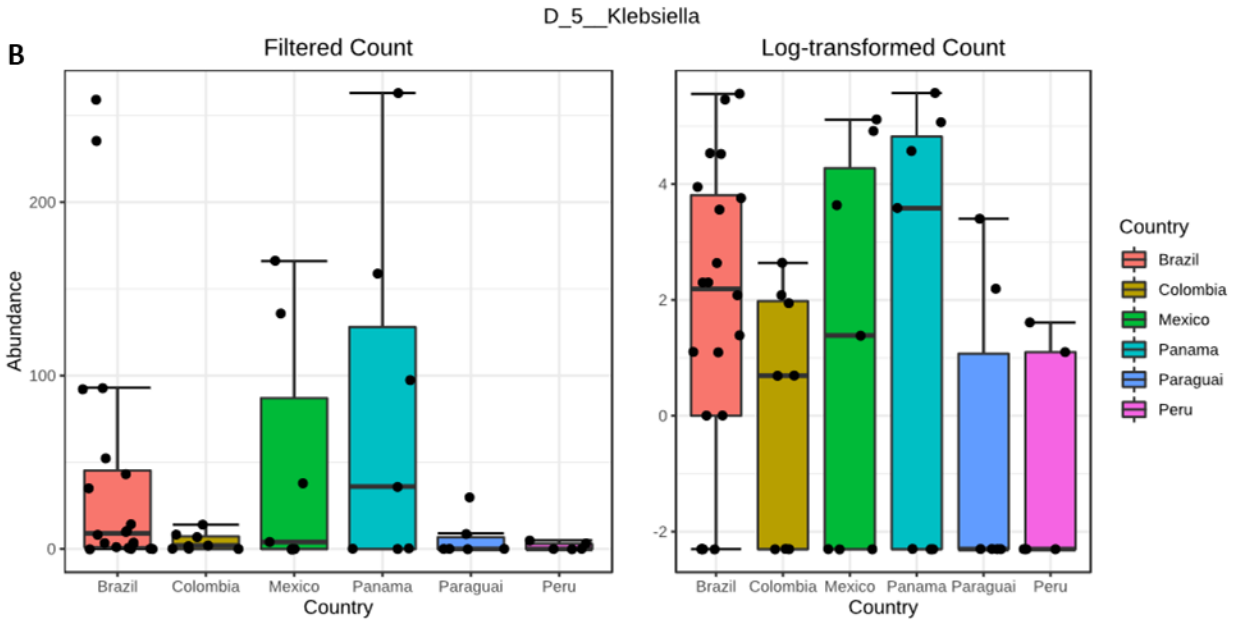
634

635

636 Fig 4



Log2FC -7.3779; lfcSE: 2.2887; p-value: 0.0012659; FDR: 0.012659



Log2FC -5.942; lfcSE:1.6946; p-value: 4.5431E-4; FDR: 0.0090863

637

638

639 Fig 5

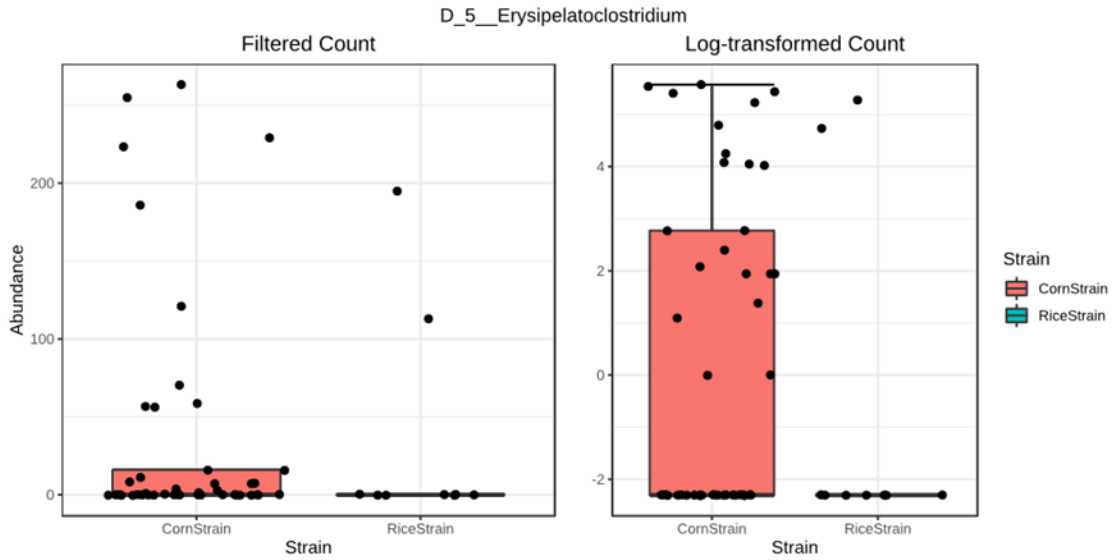
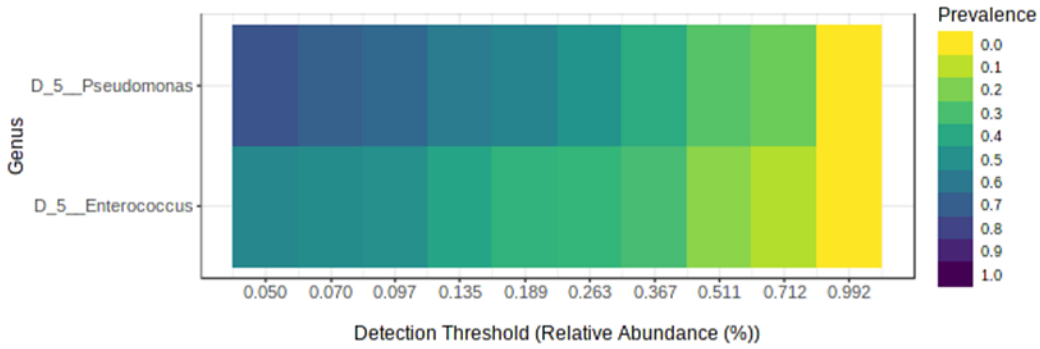


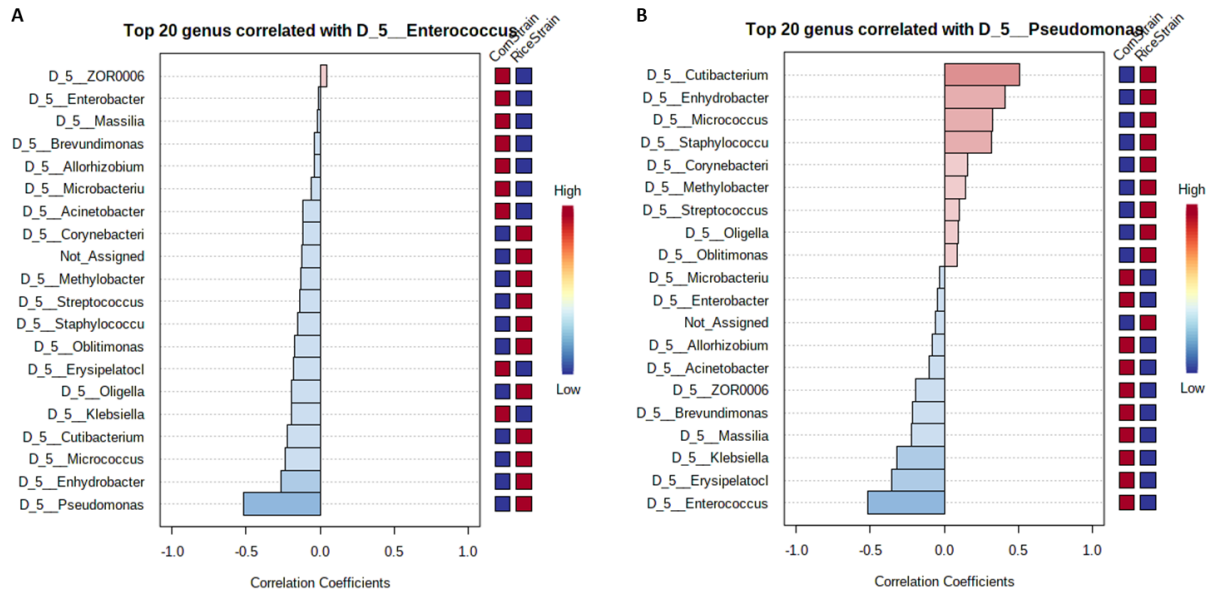
Fig 6



644

645

646 Fig 7



647

648

649

650 Fig 8

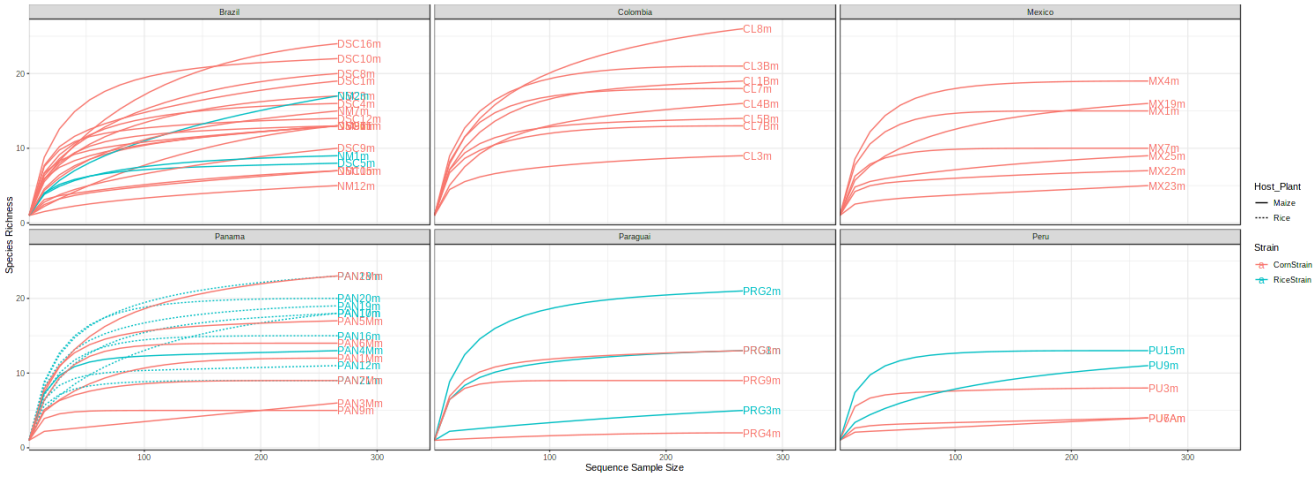


Fig. S1 Rarefaction curves showing the relationship between number of ASVs and number of sequences. The rarefaction curve for the midgut of *Spodoptera frugiperda* strains (RS= red and CS=blue) fed on and maize collected in different countries.