Peer Community Journal

Section: Microbiology

Research article

Published
2024-01-29

Cite as
Lucio Navarro-Escalante, Pablo Benavides and Flor E. Acevedo (2024) *Diversity of bacterial symbionts associated with the tropical plant bug* Monalonion velezangeli *(Hemiptera: Miridae) revealed by high-throughput 16S-rRNA sequencing*, Peer Community Journal, 4: e7.

Correspondence
lucio.navarro@austin.utexas.edu

Peer-review
Peer reviewed and recommended by PCI Microbiology, https://doi.org/10.24072/pci.microbiol.100004

Diversity of bacterial symbionts associated with the tropical plant bug *Monalonion velezangeli* (Hemiptera: Miridae) revealed by high-throughput 16S-rRNA sequencing

Lucio Navarro-Escalante [1], Pablo Benavides [1], and Flor E. Acevedo [2]

Volume 4 (2024), article e7

https://doi.org/10.24072/pcjournal.362

Abstract

Insects and microbes have developed complex symbiotic relationships that evolutionarily and ecologically play beneficial roles for both, the symbiont and the host. In most Hemiptera insects, bacterial symbionts offer mainly nutritional, defensive, and reproductive roles in addition to promoting the adaptive radiation of several hemipteran phytophagous lineages. The tropical plant bug *Monalonion velezangeli* (Hemiptera: Miridae) is a polyphagous herbivore considered an important insect pest for several economically relevant tropical crops, but information about the composition of its bacterial microbiota was missing. In this study, we describe the diversity and structure of the bacterial microbiota in the nymph and adult life stages of *M. velezangeli* using Illumina high-throughput sequencing of 16S ribosomal RNA gene amplicons (meta-barcoding). We found that both insect life stages share a similar microbiota in terms of bacterial diversity and community structure. The intracellular symbiont *Wolbachia* dominated the overall microbiome composition (~92%) in these life stages. Members of the core microbiota include *Wolbachia*, *Romboutsia*, *Ignavibacterium*, *Clostridium*, *Allobaculum*, *Paracoccus*, *Methylobacterium*, *Faecalibacterium*, *Collinsella*, *Rothia*, *Sphingomonas* and 4 other undetermined bacterial genera. Based on PCR screening and DNA sequencing of the *wsp* gene, *Wolbachia* infection was confirmed in almost 80% of samples, and represented by two different isolates or strains within the supergroup B. This data offers opportunities for studying the contribution of symbiotic bacteria in the biological performance of this insect pest, and provides a base to explore other insect control methods.

[1]Disciplina de Entomología, Centro Nacional de Investigaciones de Café - CENICAFÉ. Manizales, Colombia, [2]Department of Entomology, Pennsylvania State University, University Park, PA, United States

Peer Community Journal is a member of the Centre Mersenne for Open Scientific Publishing
http://www.centre-mersenne.org/

e-ISSN 2804-3871

Introduction

Most insects harbor diverse microbiota inside their body that collectively perform important biological roles for the insect-host in processes such as nutrition, reproduction, immunity, and development. These symbiotic interactions involve microbes adapted to live inside specialized host cells (intracellular symbionts), or outside cells (extracellular symbionts). The vast majority of insect-associated microbes reside in the gut lumen, and some are adapted to live within specialized structures in the insect posterior midgut. Insect gut-associated microorganisms have been proposed as key players in the adaptive radiation of herbivorous insects by allowing them to metabolize or assimilate recalcitrant plant compounds, or to exploit low-nutrient plant contents by providing additional nutritious molecules (Janson et al., 2008; Sudakaran et al., 2017; Motta et al., 2022; Ge et al., 2023). In some cases the outcomes of this symbiotic interplay in plant-feeding insects has also extended to the control of host-plant defense responses for the benefit of the insect (Chung et al., 2013; Acevedo et al., 2017; Schausberger, 2018; Li et al., 2019). Moreover, insect-associated microbial symbionts have been shown to confer resistance to chemical insecticides in various pest insects (Kikuchi et al., 2012; Blanton & Peterson, 2020; Sato et al., 2021).

Equally important, intracellular symbionts (e.g. *Wolbachia*) that frequently reside within the reproductive tissues of most insects are well known as manipulators of insect reproduction. *Wolbachia* are maternally inherited bacterial symbionts that infect at least 65% of insect species (Hilgenboecker et al., 2008), and are capable of altering host reproduction and fitness in order to achieve high frequency of infection in the host populations (Stouthamer et al., 1999). This manipulation can involve cytoplasmic incompatibility (CI) (Sinkins, 2004; Shropshire et al., 2020), parthenogenesis (Werren, 1997; Vavre et al., 2004; Zhou et al., 2021), male-killing (Hurst et al., 1999; Fukui et al., 2015), and feminization (O'Neill et al., 1998; Hiroki et al., 2002; Narita et al., 2007). Additionally, several lines of evidence show that *Wolbachia* can affect behavioral patterns in their hosts by altering mating, feeding, locomotion, or aggressive behavior in addition to learning and memory capacity (reviewed by Bi & Wang, 2020).

Several Hemiptera plant-feeding insect species in the suborders Sternorrhyncha, Auchenorrhyncha and Heteroptera display a variety of insect-microbial symbiosis. Phytophagous Sternorrhyncha and Auchenorrhyncha species have piercing and sucking mouthparts for stylet-sheath feeding (phloem and xylem sap-suckers) as in aphids and leafhoppers. Phytophagous Heteroptera species have macerate-and-flush feeding mouthparts (sucking of extra-orally digested plant tissues) as seen in stink bugs and plant bugs. In consequence, several of these insect species are agricultural pests of economic importance. Most members of Sternorrhyncha and Auchenorrhyncha harbor intracellular obligate symbionts within specialized cells or bacteriocytes that provide essential amino-acids and vitamins to the insect (Moran & Telang, 1998). However, most phytophagous Heteroptera members lack intracellular symbionts, but instead have developed relationships with extracellular symbionts in special midgut compartments such as midgut crypts and caeca. These extracellular symbionts are mainly found in stink bugs, flat bugs and seed bugs, within the infraorder Pentatomomorpha. Nonetheless, special symbiont-harboring midgut compartments seems to be absent in most phytophagous species in the infraorder Cimicomorpha which includes true plant bugs such as *Monalonion velezangeli*.

The plant bug *M. velezangeli* (Hemiptera: Miridae: Bryocorinae) is a neotropical polyphagous insect native to Central and South America. This insect feeds on 21 plant species in 14 families (Giraldo-Jaramillo & Machado, 2012; Rodas et al., 2014; Ocampo Flórez et al., 2018). It is considered as a strict phytophagous insect based on the lack of reports of other feeding habits, and the fact that all known members of the mirid subfamily Bryocorinae are herbivorous as well (Jung & Lee, 2012; Namyatova & Cassis, 2016). This plant bug is a notorious agricultural pest of cacao (*Theobroma cacao*, Malvaceae), avocado (*Persea americana*, Lauraceae), guava (*Psidium guava*, Myrtaceae), and tea (*Camellia sinensis*, Theaceae) (Jaimes et al., 2015; Ramírez-Gil et al., 2020). *Monalonion velezangeli* is also an emerging pest for coffee crops in Colombia especially in the southern coffee-producing regions of the country (Ramírez-Cortés et al., 2008). The immature (nymph) and adult stages of this plant bug feed on terminal shoot tips, young leaves or fruits causing cell-death at the feeding sites as the main direct damage. Severe plant damage is mainly caused by nymphal stages when they inject enzyme-rich saliva into the plant tissues for extra-oral digestion of the cell contents. Current recommendations for pest management vary according

to host crops. Common methods include cultural practices (e.g. manual collection of insects in the field or flaming), biological control with fungal entomopathogens, and insecticides. Despite its significance as an agricultural pest, several aspects of the biology of *M. velezangeli* remain poorly studied including the composition of its microbiota.

Diversity and functional characterization of symbiotic microbiota in Miridae plant bugs have been poorly studied except for the strictly phytophagous cotton fleahopper *Pseudatomoscelis seriatus* and the omnivorous *Adelphocoris suturalis* to the best of our knowledge (Xue et al., 2021; Fu et al., 2021; Luo et al., 2021). Knowledge of *M. velezangeli* associated microbiota is fundamental not only to better understand its biology, but also could provide new opportunities for the development of insect management methods. For example, symbiont-mediated RNA interference (smRNAi) is emerging as a potential approach for control of pest insects in agriculture (Dyson et al., 2022), and as an efficient tool for insect gene functional analysis (Lariviere et al., 2023). It is necessary to characterize the taxonomic composition of microbes within the insect body before any study on the role of the microbiota in insect biology or exploration of symbiont-based methods for pest control can be carried out. In this study, we analyzed for the first time the diversity and structure of the symbiotic microbiota within *M. velezangeli* nymph and adult life stages using high-throughput DNA amplicon sequencing of bacterial 16S rRNA gene (DNA meta-barcoding). Here we discovered a diverse microbiota across all life stages that is dominated by few bacterial genera, highlighting the presence of the endosymbiont *Wolbachia*.

Materials and methods

Insect collection, DNA isolation and 16S rRNA sequencing

Samples of immature and adult individuals of *M. velezangeli* feeding on leaves of multiple coffee plants (*Coffea arabica* var. Castillo) were collected from a coffee plantation in the Department of Huila (Segovianas, Coordinates: 2.3784, -75.88291), Colombia. Insects were externally sterilized by washing three times with 75% ethanol and immediately conserved in 96% ethanol for DNA isolation. Three independent samples of immature (pools of 5 nymphal stages, one per instar) and three independent samples of adults (pools of one female and one male) of *M. velezangeli* were used for microbiota analysis. Total DNA was isolated from whole-body insects using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) including a lysozyme treatment according to the manufacturer protocol. DNA integrity was checked on agarose gel and quantified on Nanodrop (Invitrogen, Waltham, MA, USA). PCR amplification of the hyper-variable region V3V4 of the bacterial 16S rRNA gene was performed using primers 341F (5'-CCT AYG GGR BGC ASC AG-3') and 806R (5'-GAC TAC NNG GGT ATC TAA T-3') (Caporaso et al., 2011; Klindworth et al., 2013). Illumina sequencing libraries were generated with NEBNext® UltraTM DNA Library Prep Kit (New England BioLabs, Ipswich, MA, USA). The 16S rRNA amplicon Illumina 250PE libraries were sequenced using NovaSeq platform (Illumina, San Diego, CA, USA) at Novogene Corporation Inc. (Sacramento, CA, USA).

Processing of 16S rRNA sequence data and taxonomic classification

Demultiplexed raw 16S rRNA sequences were processed using QIIME2 v.2020.8 (Bolyen et al., 2019) as follows. Paired-end read sequences were quality-filtered, denoised and clustered using DADA2 (Callahan et al., 2016) (dada2 denoise-paired) to produce Amplicon Sequence Variants (ASV). The ASVs were taxonomically classified using the plugin feature-classifier classify-sklearn with the GreenGenes database (version 13_8) using default confidence threshold (≥ 0.7). The ASVs that could not be identified to genus with Greengenes were blasted against the NCBI Microbial Genome sequences (Bacteria and Archaea) to identify best hits and also compared with the EzBioCloud Database (version 2021.07.07) for assignation of genus using 97% identity threshold on both searches. The original GreenGenes identification taxon level was maintained when NCBI-BLAST and EzBiocloud resulted in contradictory genus best-hits at ≥97% identify respectively. Contaminant sequences identified as chloroplast or mitochondria were removed from processed data tables and excluded from further analyses.

Diversity analysis and taxon abundance comparisons

The ASV tables for raw abundance and taxonomy classification were exported from QIIME2 and processed through the MicrobiomeAnalyst tool (Dhariwal et al., 2017; Chong et al., 2020) using the

Marker Data Profiling (MDP) pipeline as follows. The ASV abundances were brought to the total sum scaling for data normalization and further analysis of diversity. Alpha-diversity was estimated using the number of observed taxa (Observed), Chao1, ACE, Fisher and Shannon (H') indexes. Statistical differences between groups (Nymph vs Adult) were assessed with Mann-Whitney U test. Beta-diversity was assessed using Bray-Curtis distance between groups and their ordination visualized with Principal Coordinate Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS). Statistical differences in community structure between groups was tested with the permutational multivariate analysis of variance (PERMANOVA, one-way) and the analysis of similarities (ANOSIM, one-way). Both were based on Bray-Curtis distance as implemented on Past v.4.08 (Hammer et al., 2001). Differences in dispersion within each group was tested using PERMDISP (Anderson & Walsh, 2013). Bacteria taxon abundance bar-plots were built with the MicrobiomeAnalyst tool and the heatmap plots using Matrix2png (Pavlidis & Noble, 2003). Statistical differences for taxon abundances between groups were tested with the Mann-Whitney U test.

Molecular screening of *Wolbachia* endosymbiont

Abdomen samples were separately dissected from nymphs or adults under sterile conditions in a stereoscope from the *M. velazangeli* individuals collected in this study and individually used for DNA isolation with DNeasy Kit (Qiagen) as described above. Detection and classification of *Wolbachia* was performed following the *wsp* gene (*Wolbachia* surface protein) PCR-based method established by Zhou et al. (Zhou et al., 1998) as follows. PCR screening was done with the *wsp*-specific primers wsp81F (5'-TGG TCC AAT AAG TGA TGA AGA AAC-3') and wsp691R (5'-AAA AAT TAA ACG CTA CTC CA-3') in 20 µL reactions containing 1x Green GoTaq® reaction buffer (Promega, USA), 250 µM dNTPs, 0.5 µM of each primer, 0.5 u of GoTaq® polymerase (Promega, Madison, WI, USA) and 1 µL of DNA template. PCR cycling involved one initial step of denaturation at 95°C for 2 min, and then followed by 35 cycles of three steps including 95°C for 40 sec, 55°C for 30 sec and 72°C for 40 sec. The cycle ends with a final extension of 72°C for 5 min. DNA template integrity was additionally tested by PCR with universal primers for arthropod 28S rRNA gene sequences (28sF3633: 5'-TAC CGT GAG GGA AAG TTG AAA-3', and 28sR4076: 5'-AGA CTC CTT GGT CCG TGT TT-3') using the same PCR reaction conditions and cycling described above. Total DNA from a naturally *Wolbachia*-infested fruit fly (*Drosophila melanogaster*) strain was used as positive control in the PCR screening experiments. PCR amplicons were visualized with agarose gel electrophoresis.

Sanger DNA sequencing and phylogenetic analysis of *Wolbachia wsp* amplicons

A group of 10 randomly selected *wsp* PCR amplicons (wsp81F/691R primers) derived from the *M. velezangeli* DNA samples were further purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer protocol. Purified amplicons were directly submitted to ABI automated bidirectional sequencing with wsp81F and wsp691R primers. DNA sequence chromatograms were processed in Chromas v2.6.6 (https://technelysium.com.au/wp/chromas/) for quality and primer-sequence trimming. Bidirectional sequences for each sample were clustered into single DNA consensus sequences (isolates) using GeneStudio v.2.2.0 (https://sourceforge.net/projects/genestudio/). The DNA consensus sequences were compared to available sequence data at GeneBank nt database using BLASTn search algorithm and were deposited at GeneBank under accession numbers OR129441-OR129450.

A phylogenetic analysis of the *M. velezangeli*-derived *wsp* sequences was performed using the web-based Phylogeny.fr platform (Dereeper et al., 2008) along with *wsp* sequences from insect-derived *Wolbachia* isolates at the GeneBank database as representatives of major *Wolbachia* subgroups found in insects according to Zhou et al. (Zhou et al., 1998). Sequences were aligned using ClustalW (v2.1) (Thompson et al., 1994). After alignment, positions with gaps were removed from the alignment. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT) (Guindon & Gascuel, 2003). The default substitution model was selected assuming an estimated proportion of invariant sites (of 0.003) and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma=0.398). Reliability for internal branches was assessed using the aLRT test (SH-Like) (Anisimova & Gascuel, 2006).

Results

Microbial 16S rRNA sequence data

A total of 491,802 denoised non-chimeric merged sequences for the 16S rRNA V3V4 variable region were produced among all samples (nymph and adult) after removing putative contaminant sequences. Sequence clustering produced 123 ASVs, with an average number of ASVs for adult and nymph samples of 57 and 79 respectively. The number of Illumina reads and ASV sequences for each sample are detailed in Table 1. Rarefaction curves showed that all samples reached richness saturation (Figure 1A) indicating that sequencing effort was enough to capture total diversity (Good's coverage > 99.99% for all samples, Table 1).

Table 1 - Overview of Illumina 16S rRNA gene amplicon sequencing of the bacterial microbiota in *Monalonion velezangeli*.

Sample	Raw PE reads	Clean PE reads	Raw merged sequences	Clean merged sequences	Total ASVs	Good's coverage
Adult 1	173,233	96,265	74,667	69,782	63	100%
Adult 2	162,775	109,344	90,923	90,168	62	100%
Adult 3	167,579	109,789	91,180	90,009	45	100%
Nymph 1	170,239	110,169	87,856	86,212	82	99,99%
Nymph 2	172,490	85,101	60,756	58,026	85	100%
Nymph 3	169,525	115,394	97,798	97,605	71	100%

Diversity of bacterial community

The bacterial diversity associated with the nymph and adult life stages of *M. velezangeli* was analyzed through five alpha-diversity indices (Figure 1B and Table 2). No statistical differences for species richness (Observed species), abundance (Chao1 and ACE) and abundance distribution indices (Fisher and Shannon) were detected between nymph and adult stages (Observed species: U = 0, p = 0.1; Chao1: U = 0, p = 0.1; ACE: U = 0, p = 0.1; Fisher: U = 0, p = 0.1; Shannon: U = 3, p = 0.7).

Differences in microbial community structure (beta-diversity) between nymph and adult was assessed with PERMANOVA and ANOSIM analyses, and their ordinal distances (Bray-Curtis dissimilarity) visualized with PCoA and NMSD plotting (Figure 1C,D). PERMANOVA tests whether distance in community structure differs between groups (e.g. nymph vs adult) (Anderson 2001) whereas ANOSIM tests whether distances between groups are greater than within groups (Clarke 1993). Both analyses indicated no significant differences in microbial community structure between nymphs and adults (PERMANOVA: F-value: 0.4774; R-squared: 0.1135; p-value = 0.5016; ANOSIM: R: -0.1111; p-value = 0.7019). We assessed the differences in dispersion (variance) within groups with PERMDISP (Anderson & Walsh, 2013) considering that PERMANOVA and ANOSIM are sensitive to variance within groups. This analysis showed that there is homogeneity of multivariate dispersions between nymph and adult samples (PERMDISP: F-value: 0.1958; p-value: 0.681).

Table 2 - Alpha diversity indices for 16S rRNA-based microbiota in *Monalonion velezangeli*.

Sample	Observed	Chao1 (±se)	ACE (±se)	Fisher	Shannon (H')
Adult1	63	63 (±0.0)	63 (±1.69)	6.82	0.81
Adult2	62	62 (±0.0)	62 (±2.90)	6.50	0.41
Adult3	45	45 (±0.0)	45 (±1.91)	4.55	0.37
Nymph1	82	83 (±2.33)	82.5 (±3.25)	8.94	0.46
Nymph2	85	85 (±0.0)	85 (±2.97)	9.78	1.12
Nymph3	71	71 (±0.0)	71 (±2.66)	7.49	0.39

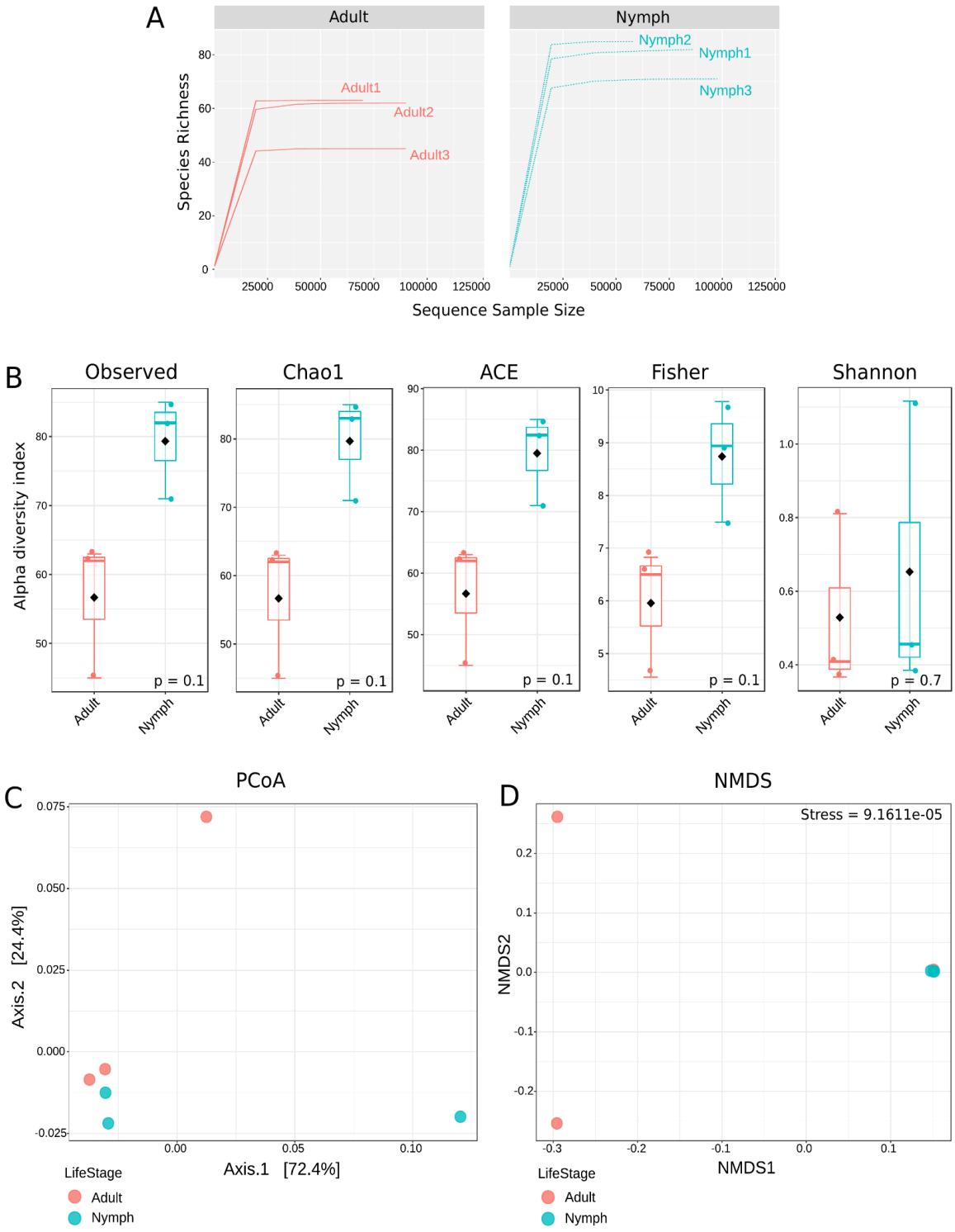

Figure 1 - Diversity and community structure of the bacterial microbiota in adult and nymph life stages of *Monalonion velezangeli*. (A) Rarefaction curves. (B) Alpha diversity indices and their corresponding p-value of the Mann-Whitney U test. (C) Principal coordinate analysis (PCoA) plot based on Bray-Curtis dissimilarity of bacterial communities in nymphs and adults. (D) Non-metric multidimensional scaling (NMDS) ordination analysis plot based on Bray-Curtis dissimilarity of bacterial communities in nymphs and adults. Stress value represents the goodness-of-fit for the NMDS analysis.

Removal of *Wolbachia*-associated sequences is a regular practice for microbiome analysis in insects when they are detected in bacterial 16S rRNA libraries (Chandler et al., 2014; Rudman et al., 2019). We compared the overall microbial community structure in our samples when *Wolbachia* sequences are kept or removed from the data. The removal of *Wolbachia* reads did not alter the similarities in alpha diversity indices between nymphs and adults (Supplementary Table S1) (Observed species: U = 0, p = 0.1; Chao1: U = 0, p = 0.1; ACE: U = 0, p = 0.1; Fisher: U = 0, p = 0.1; Shannon: U = 3, p = 0.1). Similarity in microbial community structure between life stages also remained unchanged (PERMANOVA: F-value: 1.698; R-squared: 0.5142; p-value = 0.2028; ANOSIM: R: 0.2593; p-value = 0.2992; PERMDISP: F-value: 0.0993; p-value = 0.7684).

Taxonomic composition of bacterial community

From the 123 ASV, 107 (87%) were taxonomically assigned to at least the Phylum level. Taxonomic distribution of ASVs included 10 bacteria phyla, 18 classes, 22 orders, 33 families and 36 genera. Distribution of relative abundances for phylum, order and genus levels are shown in Figure 2 and fully detailed for all taxonomic levels in Supplementary Tables S2 to S6 (Navarro-Escalante et al., 2024). Overall, the Phylum Proteobacteria (92.6%) and Firmicutes (5.2%) represented almost the full microbiota detected in this study (Supplementary Table S2, Figure 2A). The orders Rickettsiales (Phylum Proteobacteria: Class Alphaproteobacteria) and Clostridiales (Phylum Firmicutes: Class Clostridia) with abundances 91.9% and 4.8% respectively dominated the bacterial community. To a lesser extent, other 20 orders were present at or below 1% overall abundance (Supplementary Table S4, Figure 2B).

From the total 123 ASVs, 95 (77.2%) were assigned to the genus level where 66 ASVs (53.7%) were annotated using GreenGenes (≥ 0.7 confidence level) and 29 ASVs (23.6%) annotated using BLASTn and EzBioCloud (≥ 97% identity to top-hit for both algorithms). The remaining 28 ASVs (22.8%) were considered as undetermined at genus level (Not Assigned). At the genus level, *Wolbachia* (Rickettsiaceae) dominated the overall abundance (91.9%) across nymph and adult samples followed by *Romboutsia* (1.8%), *Ignavibacterium* (0.8%), *Clostridium* (0.70%), *Mycoplasma* (0.5%), *Allobaculum* (0.4%), *Blautia* (0.4%), *Eubacterium_g23* (0.3%), *Sporobacter* (0.3%), *Paracoccus* (0.3), *Methylobacterium* (0.2%), *Dorea* (0.2%), *Sediminibacterium* (0.1%), *Faecalibacterium* (0.1%), and *Ruminococcus* (0.1%) as the top 15 taxa. Other 34 genera were present at abundances below 0.1% across all life stages (Supplementary Table S6, Figure 2C). The relative abundances for bacteria taxa in all taxonomic levels (Phylum to Genus) were similar between both insect life stages (Mann-Whitney *U* test, p-values > 0.05, Supplementary Tables S2 to S6). Similarly, no statistical differences were found at bacterial ASV level between both life stages (Mann-Whitney *U* test, p-values > 0.05).

Removal of *Wolbachia* sequences from this analysis did not alter the similarities in the overall relative abundances at ASV or genus levels between life stages (Mann-Whitney *U* test, p-values > 0.05) as estimated above despite changes in the proportions of total reads counts and relative taxon abundances across the individual samples. Additionally, apart from *Wolbachia*, the list of the top ten most abundant genus remained unchanged; and in all cases the microbiota was dominated by *Romboutsia* with few changes in the order of the remaining genera (Figure 3B). However, the exclusion of *Wolbachia* resulted in Firmicutes (69.3%) as the overall dominant Phylum, followed by Proteobacteria (10.4%), Chlorobi (6.2%) and other seven Phylum to a lesser extent (Figure 3A).

Core microbiota

According to the data collected in this study, the core microbiota of *M. velezangeli* is composed of 21 bacterial ASVs (17% of all ASVs) that were consistently shared between the nymph and adult life stages (ASVs present in all samples in this study) (Figure 2D). These core ASVs were identified by analyzing 42 ASVs present in all samples of both life stages. The remaining 21 ASVs were present at either nymph or adult. Other 81 ASVs (65.9% of all ASVs) were not consistently detected in all samples of each life stage and may represent transient or non-resident microbes within the microbiome of *M. velezangeli*.

The bacterial genus assignments for the core 21 ASVs based on 16S GreenGenes database are shown in Table 3. These core bacterial genera, listed in decreasing order of abundance, included: *Wolbachia*, *Romboutsia*, *Ignavibacterium*, *Clostridium*, *Allobaculum*, *Paracoccus*, undetermined Anaerolineaceae, *Methylobacterium*, *Faecalibacterium*, undetermined Lachnospiraceae, *Collinsella*, *Rothia*, undetermined Peptostreptococcaceae, *Sphingomonas* and undetermined Coriobacteriaceae.

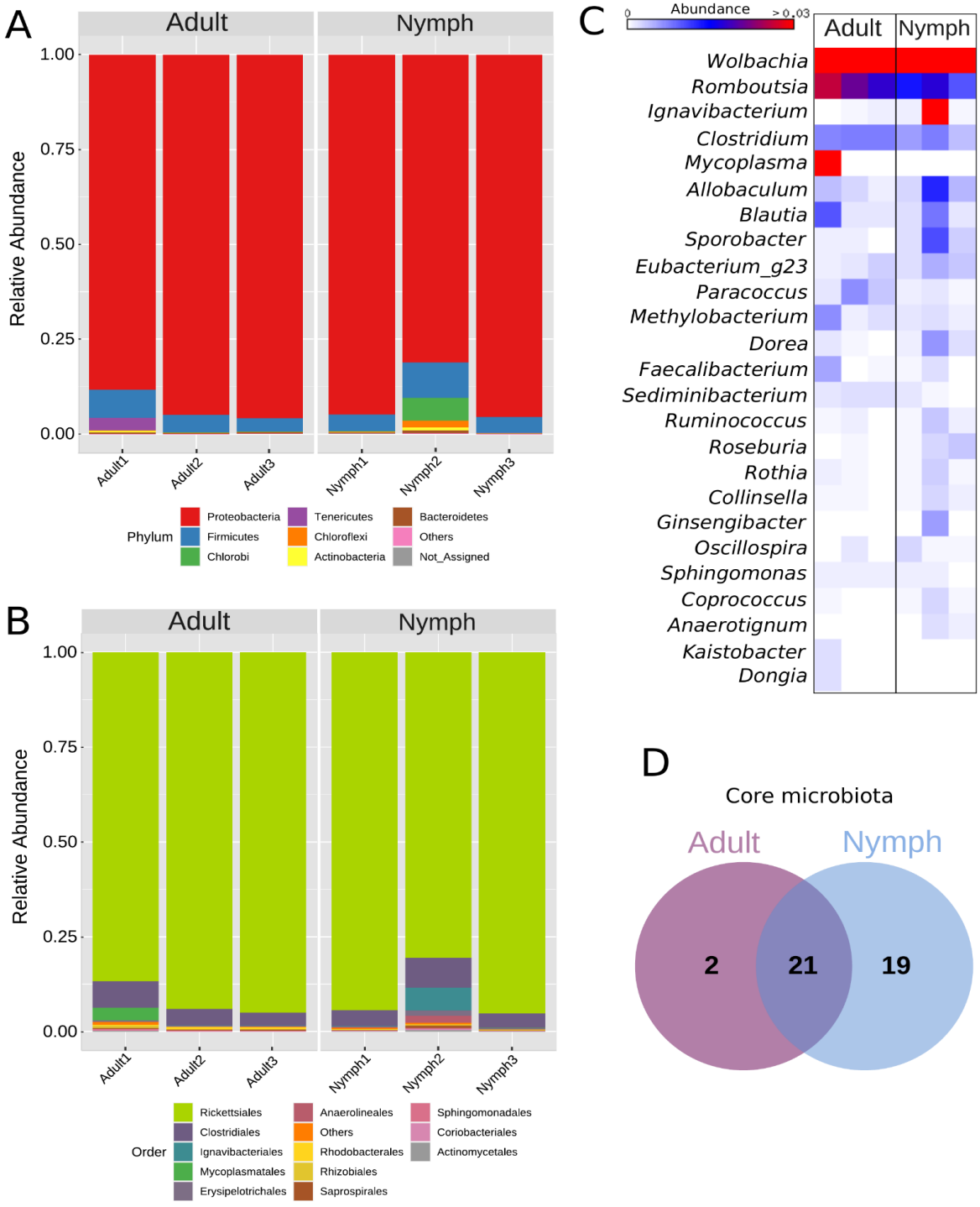

Figure 2 - Taxonomic composition of the bacterial microbiota in nymphs and adults of *Monalonion velezangeli*. (A) Relative abundance at Phylum level. (B) Relative abundance at Order level. (C) Heatmap for relative abundances at genus level. (D) Number of ASV sequences consistently detected on either adults of nymphs and number of shared ASVs (circle intersection) as members of the core microbiota.

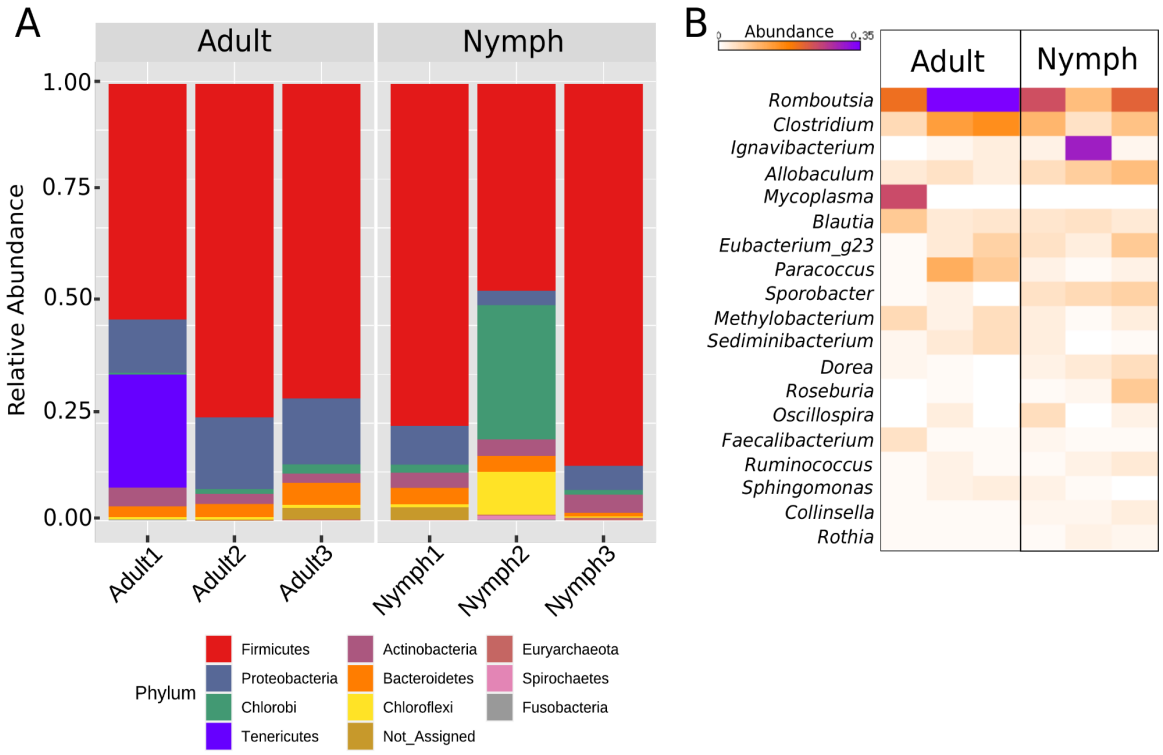

Figure 3 - Relative abundance of the bacterial microbiota, with the exclusion of *Wolbachia*-associated sequences, for nymphs and adults of *Monalonion velezangeli*. (A) Relative abundances at Phylum level. (B) Heatmap of relative abundances at genus level.

Wolbachia PCR detection and profiling

Taking into account the large proportion of *Wolbachia*-associated ASVs found in our samples, we decided to further investigate the presence of *Wolbachia* endosymbiont in *M. velezangeli* by PCR screening of the *wsp* gene. About 79% of the insect individuals tested (22 out of 28) from the Segovianas locality resulted positive for *Wolbachia* infection according to the amplification of a ~600 bp DNA band (Figure 4). A PCR test for DNA template integrity showed that all 28 (100%) *M. velezangeli* DNA samples were PCR quality grade based on the successful amplification of a DNA band for the arthropod 28S rRNA gene target. This indicates that the no *wsp* amplification in 21% (6/28) of the samples could be explained by the absence of *Wolbachia* infection and not because of a low DNA template quality.

DNA Sanger sequencing was performed for 10 randomly selected *wsp* DNA amplicons derived from *M. velezangeli* samples. All DNA sequences had clearly defined single-pick chromatograms, which suggested the presence of single *Wolbachia*-strain infections in each sequenced sample. A multiple sequence alignment showed that these isolates can be grouped in two distinct *wsp* sequence haplotypes that share 78% similarity between them (Supplementary Figure S1). Haplotype 1 (hereafter wMvel1) was represented by 80% (8/10) of the sequence isolates in this study, whereas haplotype 2 (hereafter wMvel2) was represented by the remaining 20% (2/10) isolates. A BLASTn search against the GeneBank database showed that wMvel1 *wsp* sequence was 99.46% identical (top hit) to a *Wolbachia wsp* isolate from the butterfly *Acraea equitorialis* (GenBank accession: AJ271195) whereas wMvel2 *wsp* was 98.91% identical (top hit) to a *Wolbachia wsp* isolate from the planthopper *Perkinsiella saccharicida* (GenBank accession: GU190768) (Hughes et al., 2011). Phylogenetic analysis clustered all wMvel *wsp* sequences within the *Wolbachia wsp* B supergroup clade (Figure 5) and assigned the distinct wMvel *wsp* haplotypes within two distant subclades respectively along with their corresponding *wsp* BLASTn top-hits.

Table 3 - Bacterial genus annotations for ASVs considered as members of the core microbiota in *Monalonion velezangeli*.

ID	Overall abundance	Genus (Family) rank annotation#
ASV01	91.7%	*Wolbachia* (Rickettsiaceae)
ASV02	0.97%	*Romboutsia* (Peptostreptococcaceae)*
ASV03	0.79%	*Romboutsia* (Peptostreptococcaceae)*
ASV04	0.73%	*Ignavibacterium* (Ignavibacteriaceae)*
ASV05	0.47%	*Clostridium* (Clostridiaceae)*
ASV06	0.26%	*Paracoccus* (Rhodobacteraceae)
ASV07	0.25%	Undetermined (Anaerolineaceae)*
ASV08	0.22%	*Allobaculum* (Erysipelotrichaceae)
ASV09	0.19%	*Methylobacterium* (Methylobacteriaceae)
ASV10	0.14%	*Sediminibacterium* (Chitinophagaceae)
ASV11	0.13%	*Allobaculum* (Erysipelotrichaceae)
ASV12	0.12%	*Faecalibacterium* (Ruminococcaceae)
ASV13	0.11%	*Clostridium* (Clostridiaceae)
ASV14	0.09%	Undetermined (Lachnospiraceae)
ASV15	0.09%	*Collinsella* (Coriobacteriaceae)
ASV16	0.09%	*Rothia* (Micrococcaceae)
ASV17	0.06%	*Clostridium* (Clostridiaceae)
ASV18	0.06%	Undetermined (Peptostreptococcaceae)
ASV19	0.06%	*Allobaculum* (Erysipelotrichaceae)
ASV20	0.05%	*Sphingomonas* (Sphingomonadaceae)
ASV21	0.04%	Undetermined (Coriobacteriaceae)

The taxonomic classification was determined using the 16S GreenGenes (GG) database with a confidence level of ≥0.7. For ASVs where GG failed to assign a Genus taxon, the Genus identification was performed using the BLASTn and EzBioCloud search algorithms with a concomitant ≥97% sequence identity for their top hits (taxa denoted with asterisk [*]). Further details can be found in the Methods section.

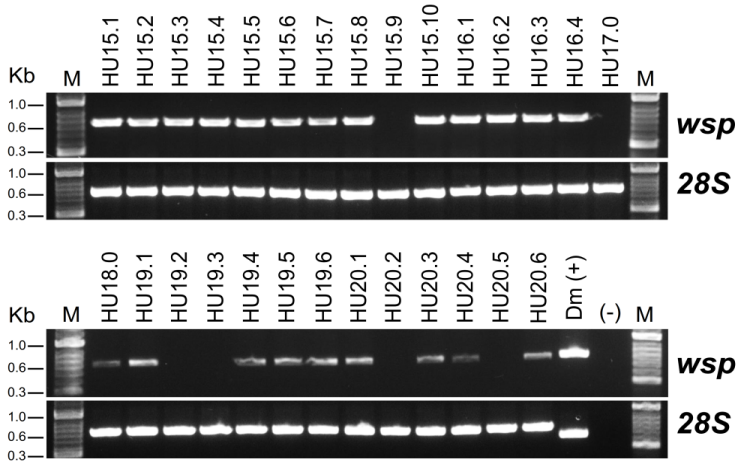

Figure 4 - Molecular screening for presence of *Wolbachia* endosymbiont in *Monalonion velezangeli* samples. DNA samples from single insects (HU15.1 to HU20.6) were tested for PCR amplification of the *Wolbachia wsp* gene using wsp81F and wsp691R primers. Quality of DNA was tested by amplification of the 28S rRNA (28S) gene fragment (~700 bp). DNA from a *Drosophila melanogaster* (Dm) population was used as positive control for *Wolbachia* infection, and water (-) as negative control.

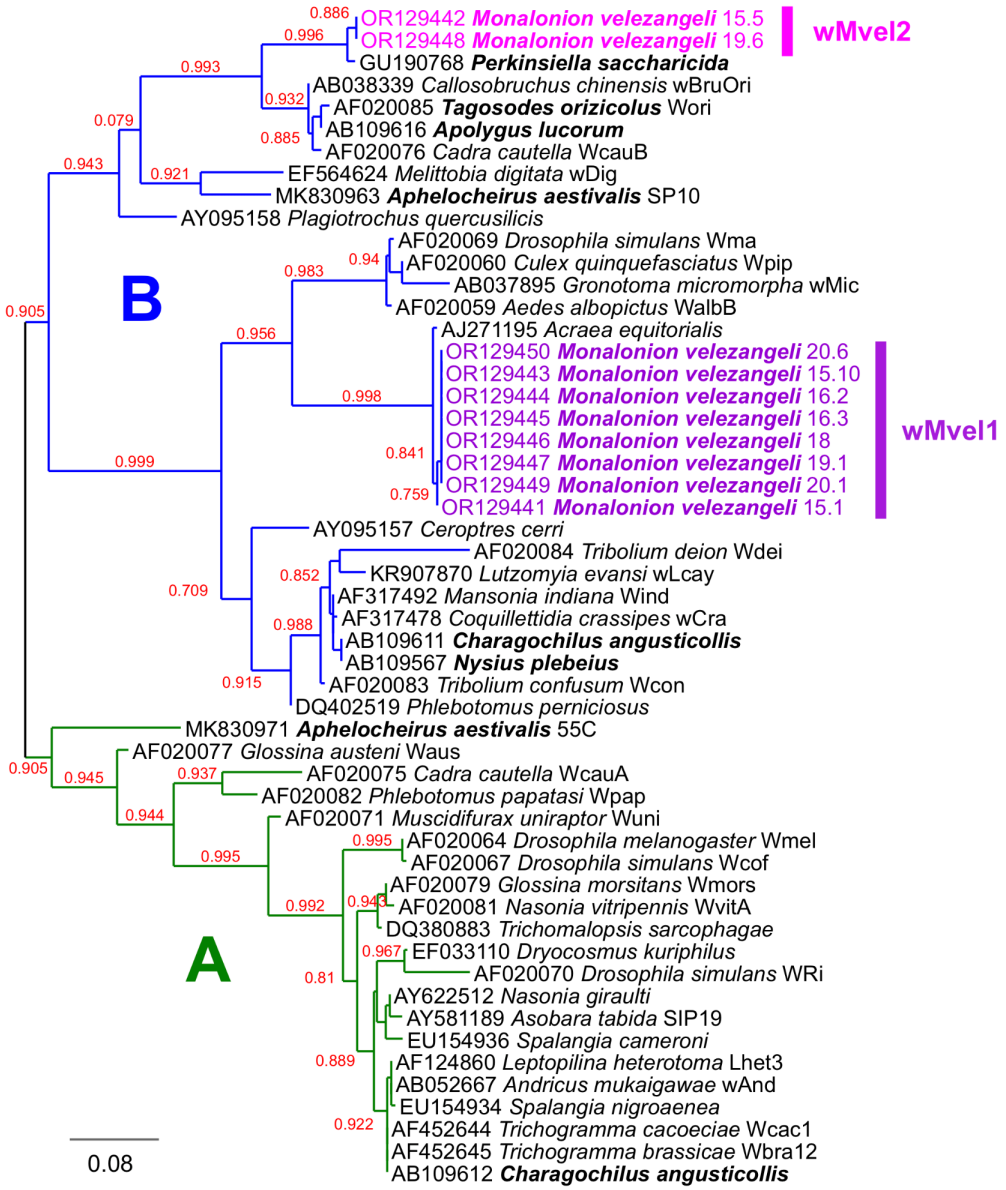

Figure 5 - Maximum Likelihood phylogenetic tree of *Wolbachia wsp* sequences from *Monalonion velezangeli* and representative *Wolbachia* strains from other host insects at the GenBank database. *Wolbachia* supergroups A (green branch) and B (blue branch) clusters based on *wsp* sequences are shown. Sequence haplotypes clustering of the *M. velezangeli wsp* isolates, wMvel1 and wMvel2, are shown in purple and pink colors respectively. Hemiptera species are highlighted in bold letters. The aLRT branch supports are indicated as red numbers. Genbank accession numbers precede each sequence name.

Discussion

We used 16S rRNA amplicon high-throughput sequencing to investigate for the first time the diversity of the symbiotic bacteria community associated with the tropical plant bug *M. velezangeli*. Here, we found a relatively diverse core microbiota dominated by genera *Wolbachia*, *Romboutsia*, *Ignavibacterium* and *Clostridium*. Although this plant bug is a polyphagous herbivore considered a pest for various tropical crops in America, in this study we focused the bacteria screening on a population feeding on coffee plants in Colombia. We found that overall bacteria diversity (Alpha diversity, Figure 1B) was similar between the nymph and the adult life stages. Based on the most abundant taxa (ASVs with overall abundance >0.01%), the bacterial community composition (Beta diversity) is conserved between these two developmental

stages. However, there is a degree of variability regarding the presence of bacteria with low abundance within and between life stages. The immature forms of *M. velezangeli* go through 5 nymphal instars that differ among them mainly in body size (Giraldo-Jaramillo et al., 2010). The microbial composition we present in this work for the nymph is based on pooled individuals from all instars. Hence, whether the overall bacterial community diversity and structure experience any changes along nymphal development needs to be addressed in future analyses.

The bacterial 16S rRNA gene sequence has been used historically as a gold standard genetic marker to infer bacteria taxonomic identity and community diversity in high-throughput microbiome studies especially with the use of the partial sequencing of some of its nine hypervariable sequence regions (V1 to V9) (Van de Peer et al., 1996). In our study, we used the sequences of the combined V3-V4 variable regions, a 16S sequence section commonly utilized in microbiome analysis; however, it must be noticed that the used of partial sequences of this gene marker can result in overestimation of microbial diversity due to bacterial intragenomic heterogeneity (Sun et al., 2013), and does not offer enough accuracy for bacteria identification at the species or strain level (Johnson, Spakowicz, et al., 2019). Being aware of this bias, we mainly describe the microbial taxonomic diversity in this study at genus level as the deepest taxonomic rank.

We found that the intracellular symbiont *Wolbachia* dominated the full microbiota associated with *M. velezangeli* which represent about 92% of the bacterial load within the body of nymph and adult stages. The observed high abundance of *Wolbachia* in our samples may indicate a proportionally elevated titer of this endosymbiont in the analyzed insects as well. Presence of *Wolbachia* was also confirmed by PCR screening in *M. velezangeli* samples. Additionally, DNA sequence analysis of wMvel *wsp* isolates indicates that they belong to the *Wolbachia* B supergroup. Insect-infecting *Wolbachia* strains with major biological effects have been mostly associated with host reproductive disturbances such as CI, parthenogenesis, male-killing and feminization (Serbus et al., 2008; Werren et al., 2008; Kaur et al., 2021). Furthermore, recent studies suggest that *Wolbachia* infections may also influence other behavioral and physiological processes including nutrition, defense and insecticide-resistance (Hosokawa et al., 2010; Nikoh et al., 2014; Zug & Hammerstein, 2015; Zhang et al., 2020; Soh & Veera Singham, 2022). In other mirid species the presence of *Wolbachia* has been associated with reproductive alterations and nutritional roles. For example in the predatory mirid bug *Macrolophus pygmaeus* this parasitic bacteria induces strong CI (Machtelinckx et al., 2009). *Wolbachia* infection in the hematophagous bed bugs *Cimex lectularius* and *Cimex hemipterus* (Hemiptera: Miridae) creates an obligate mutualism that is essential for normal insect growth and reproduction via provision of B vitamins (Hosokawa et al., 2010; Laidoudi et al., 2020). *Wolbachia* infections in insects have been mainly associated with host reproductive tissues, but it is also commonly found in several insect somatic organs or tissues including brain, salivary glands, gut, malpighian tubules, muscles, fat bodies and also as habitant of bacteriocytes (Hosokawa et al., 2010; Casper-Lindley et al., 2011; Pietri et al., 2016; Diouf et al., 2018). The presence of this parasitic endosymbiont in *M. velezangeli* raises new questions about the possible biological implications for this plant bug. The detection of two distinct wMvel *wsp* haplotypes in our analysis suggest that multiple *Wolbachia* strains are present in the insect population tested here. However, insect individuals seem to be infected by single *Wolbachia* strains. Additionally, the prevalence of infection is not 100% across all insect individuals, which seems to indicate that an obligate mutualism is not the proper characterization of the *M. velezangeli* - *Wolbachia* relationship.

The extremely high abundance of ASV sequences identified as *Wolbachia* in our samples (~92% overall abundance) could introduce a potential confounding effect in the estimation of relative abundances for the actual gut-associated bacterial taxa. This possible issue was recently analyzed by Wilches et al. (Wilches et al., 2021) using the spotted-wing drosophila (*Drosophila suzukii*) as a case of study when high-throughput sequencing is applied to investigate the microbiome in *Wolbachia*-infected insect samples. The authors detected large discrepancies in the measures of alpha and beta diversity as well as in the relative abundances of several bacteria taxa in the microbiome between *Wolbachia*-infected fly samples (mean abundance of 98.8% for *Wolbachia* sequences) and non-infected. This and other work has shown that in some cases removing the *Wolbachia*-associated reads from the analyses could also have major impacts in the interpretation of the study results which may be especially relevant when comparing infected samples versus non-infected (Henry & Ayroles, 2021; Wilches et al., 2021). We addressed the impact of removing *Wolbachia* reads in microbiota diversity and structure in *M.*

velezangeli. Here, the exclusion of *Wolbachia*-associated sequences did not affect the similarity in microbiota composition between the life stages.

Apart from *Wolbachia* (Proteobacteria) the remaining top 10 most abundant bacterial genera detected in *M. velezangeli* include members of Phylum Firmicutes (5.2% overall abundance) such as *Romboutsia*, *Clostridium*, *Allobaculum*, *Blautia*, *Eubacterium_g 2 3*, *Sporobacter*, *Dorea* and *Faecalibacterium*, as well as the Proteobacteria genera *Paracoccus*, *Methylobacterium* and the Chlorobi genus *Ignavibacterium*. Members of these Firmicutes genera have been previously found in the alimentary canals of other arthropods (Grech-Mora et al., 1996; Husseneder et al., 2017; Li et al., 2020; Fang et al., 2020; Shukla & Beran, 2020; Mejía-Alvarado et al., 2021). In our study, *Romboutsia* (1.75%) (Firmicutes: Peptostreptococcaceae) was the second most abundant bacterial genus across all samples. Members of this genus have been mainly found in the gut microbiota from several vertebrate animals and insects (Gerritsen et al., 2014; Ricaboni et al., 2016; Johnson, Sylte, et al., 2019; Shukla & Beran, 2020). There is no information about the functional roles of the *Romboutsia* members as gut symbionts; however, they seem to be well adapted to live within animal guts (Gerritsen et al., 2017, 2019). Similarly, members of *Paracoccus*, *Methylobacterium* and *Ignavibacterium* are regular habitants of arthropod guts (Zhang et al., 2016, 2018; Sajnaga et al., 2022). We infer that most abundant bacteria genera found in this study, except *Wolbachia*, are likely residents of the *M. velezangeli* gut lumen and may be involved in important biological processes for this plant bug. Several of these symbionts (*Romboutsia*, *Ignavibacterium*, *Clostridium*, *Paracoccus*, *Allobaculum*, *Methylobacterium*, *Faecalibacterium*, *Collinsella*, *Rothia* and *Sphingomonas*) were found to be consistently present in all our samples of nymph and adult stages and we consider them as members of the insect gut-associated core microbiota. Most of these genera, except for *Paracoccus*, *Methylobacterium* and *Sphingomonas*, are primarily anaerobic bacteria taxa. Compared with the microbiota associated with the cotton fleahopper *P. seriatus* (Hemiptera: Miridae) (Fu et al., 2021) and *A. suturalis* (Hemiptera: Miridae) (Xue et al., 2021) the composition at the genus level within *M. velezangeli* is clearly different. In *P. seriatus*, the gut microbiome is dominated by bacteria *Diaphorobacter*, *Lactococcus*, *Pseudomonas*, *Pantoea* and *Izhakiella*; whereas in *A. suturalis*, the gut microbiome is dominated by *Erwinia*, *Acinetobacter*, *Staphylococcus*, and *Lactococcus*. These differences in microbiota composition could be associated with environmental differences due to host-plant species, feeding habits and geographical origins.

Several bacteria isolates found in *M. velezangeli*'s microbiota that belong to genera *Paracoccus*, *Methylobacterium* and *Sphingomonas* are potential culturable strains and may also represent candidate symbionts for paratransgenic approaches such as symbiont-mediated RNAi (Dyson et al., 2022). The use of bacteria within paratransgenesis applications requires a culturable symbiont genetically manipulable and especially amenable under aerobic culturable conditions (Ratcliffe et al., 2022). Conditions like these make easier bacterial engineering and experimentation processes. Future attempts for selection of culturable bacterial isolates from the *M. velezangeli* gut will reveal what microbes have these characteristics.

Conclusions

The tropical plant bug *M. velezangeli* harbors a diverse microbiota, and in some cases it can be dominated by the intracellular symbiont *Wolbachia*. The *M. velezangeli* microbiota also contain potential gut-associated members of the genera *Romboutsia*, *Ignavibacterium*, *Clostridium*, *Paracoccus*, *Allobaculum*, *Methylobacterium*, *Faecalibacterium*, *Collinsella*, *Rothia* and *Sphingomonas*. The persistent detection of these bacteria genera in nymphal and adult life stages indicates they seem to be part of the core microbiome and likely play important biological roles in the normal development of *M. velezangeli*. Additionally, our observations suggest that multiple *Wolbachia* strains are present in *M. velezangeli* populations, but insect individuals seem to harbor single-strain infections. The findings reported by this study offer new avenues to improve our understanding of the microbiome contribution in the biology of Miridae plant bugs such as the tropical insect pest *M. velezangeli*.

Acknowledgements

The authors thank the National Federation of Coffee Growers of Colombia (FNC) and the personnel of the Extension Service of FNC at the Department of Huila (Colombia) for their support and logistic arrangements for insect sample collection. The authors also thank the coffee farmers at the Municipality of La Argentina (Huila, Colombia) who kindly allowed the insect sampling in their coffee plantations. Preprint version 7 of this article has been peer-reviewed and recommended by Peer Community In Microbiology (https://doi.org/10.24072/pci.microbiol.100004; Volland, 2024).

Funding

This work was supported by the National Coffee Research Center at the National Federation of Coffee Growers of Colombia [Funder ID 100019597, project number ENT101006]; and Sistema General de Regalías del Departamento de Huila, Colombia [convenio 0244 del 2014].

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

Data, script, code and supplementary material

The datasets generated or analyzed during the current study are included in this article and its supplementary data (https://doi.org/10.5281/zenodo.10524900; Navarro-Escalante et al., 2024). The raw sequence data is accessible at the NCBI Sequence Read Archive (SRA) database under the bioproject number PRJNA875474 (http://www.ncbi.nlm.nih.gov/bioproject/875474).

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. *Molecular Plant-Microbe Interactions*, **30**, 127–137. https://doi.org/10.1094/MPMI-11-16-0240-R

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, **83**, 557–574. https://doi.org/10.1890/12-2010.1

Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology*, **55**, 539–552. https://doi.org/10.1080/10635150600755453

Bi J, Wang Y-F (2020) The effect of the endosymbiont *Wolbachia* on the behavior of insect hosts. *Insect Science*, **27**, 846–858. https://doi.org/10.1111/1744-7917.12731

Blanton AG, Peterson BF (2020) Symbiont-mediated insecticide detoxification as an emerging problem in insect pests. *Frontiers in Microbiology*, **11**. https://doi.org/10.3389/fmicb.2020.547108

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR,

Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, **37**, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, **13**, 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, **108 Suppl 1**, 4516–4522. https://doi.org/10.1073/pnas.1000080107

Casper-Lindley C, Kimura S, Saxton DS, Essaw Y, Simpson I, Tan V, Sullivan W (2011) Rapid fluorescence-based screening for *Wolbachia* endosymbionts in *Drosophila* germ line and somatic tissues. *Applied and Environmental Microbiology*, **77**, 4788–4794. https://doi.org/10.1128/AEM.00215-11

Chandler JA, James PM, Jospin G, Lang JM (2014) The bacterial communities of *Drosophila suzukii* collected from undamaged cherries. *PeerJ*, **2**, e474. https://doi.org/10.7717/peerj.474

Chong J, Liu P, Zhou G, Xia J (2020) Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nature Protocols*, **15**, 799–821. https://doi.org/10.1038/s41596-019-0264-1

Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW (2013) Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences*, **110**, 15728–15733. https://doi.org/10.1073/pnas.1308867110

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard J-F, Guindon S, Lefort V, Lescot M, Claverie J-M, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, **36**, W465-469. https://doi.org/10.1093/nar/gkn180

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 Research*, **45**, W180–W188. https://doi.org/10.1093/nar/gkx295

Diouf M, Miambi E, Mora P, Frechault S, Robert A, Rouland-Lefèvre C, Hervé V (2018) Variations in the relative abundance of *Wolbachia* in the gut of *Nasutitermes arborum* across life stages and castes. *FEMS microbiology letters*, **365**. https://doi.org/10.1093/femsle/fny046

Dyson P, Figueiredo M, Andongma AA, Whitten MMA (2022) Symbiont-mediated RNA interference (SMR): using symbiotic bacteria as vectors for delivering RNAi to insects. *Methods in Molecular Biology (Clifton, N.J.)*, **2360**, 295–306. https://doi.org/10.1007/978-1-0716-1633-8_21

Fang J-X, Zhang S-F, Liu F, Zhang X, Zhang F-B, Guo X-B, Zhang Z, Zhang Q-H, Kong X-B (2020) Differences in gut bacterial communities of *Ips typographus* (coleoptera: curculionidae) induced by enantiomer-specific α-pinene. *Environmental Entomology*, **49**, 1198–1205. https://doi.org/10.1093/ee/nvaa098

Fu Z, Antwi JB, Sword GA, Barman AK, Medina RF (2021) Geographic variation of bacterial communities associated with cotton fleahopper, *Pseudatomoscelis seriatus*. *Southwestern Entomologist*, **46**, 17–32. https://doi.org/10.3958/059.046.0102

Fukui T, Kawamoto M, Shoji K, Kiuchi T, Sugano S, Shimada T, Suzuki Y, Katsuma S (2015) The endosymbiotic bacterium *Wolbachia* selectively kills male hosts by targeting the masculinizing gene. *PLOS Pathogens*, **11**, e1005048. https://doi.org/10.1371/journal.ppat.1005048

Ge S-X, Li T-F, Ren L-L, Zong S-X (2023) Host-plant adaptation in xylophagous insect-microbiome systems: Contributionsof longicorns and gut symbionts revealed by parallel metatranscriptome. *iScience*, **26**, 106680. https://doi.org/10.1016/j.isci.2023.106680

Gerritsen J, Fuentes S, Grievink W, van Niftrik L, Tindall BJ, Timmerman HM, Rijkers GT, Smidt H (2014) Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastro-intestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus *Clostridium* into the genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov. *International Journal of Systematic and Evolutionary Microbiology*, **64**, 1600–1616. https://doi.org/10.1099/ijs.0.059543-0

Gerritsen J, Hornung B, Renckens B, van Hijum SAFT, Martins Dos Santos VAP, Rijkers GT, Schaap PJ, de Vos WM, Smidt H (2017) Genomic and functional analysis of *Romboutsia ilealis* CRIBT reveals adaptation to the small intestine. *PeerJ*, **5**, e3698. https://doi.org/10.7717/peerj.3698

Gerritsen J, Hornung B, Ritari J, Paulin L, Rijkers GT, Schaap PJ, Vos WM de, Smidt H (2019) A comparative and functional genomics analysis of the genus *Romboutsia provides* insight into adaptation to an intestinal lifestyle. , 845511. https://doi.org/10.1101/845511

Giraldo-Jaramillo M, Benavides-Machado P, Villegas-Garcia. C (2010) Aspectos morfológicos y biológicos de *Monalonion velezangeli* Carvalho and Costa Hemiptera : Miridae en café. *Cenicafé*, **61**, 195–205. https://www.cenicafe.org/es/publications/arc061(03)195-2052.pdf

Giraldo-Jaramillo M, Machado PB (2012) Conozca los hospedantes, sitios de alimentación y oviposición de la chinche de la chamusquina del café. *Avances Técnicos Cenicafé*, 1–8. https://publicaciones.cenicafe.org/index.php/avances_tecnicos/article/view/500/553

Grech-Mora I, Fardeau M-L, Patel BKC, Ollivier B, Rimbault A, PRENSIER G, GARCIA J-L, GARNIER-SILLAM E (1996) Isolation and characterization of *Sporobacter termitidis* gen. nov., sp. nov., from the digestive tract of the wood-feeding termite *Nasutitermes lujae*. *International Journal of Systematic and Evolutionary Microbiology*, **46**, 512–518. https://doi.org/10.1099/00207713-46-2-512

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704. https://doi.org/10.1080/10635150390235520

Hammer Ø, Harper D, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, **4**, 1–9.

Henry LP, Ayroles JF (2021) Meta-analysis suggests the microbiome responds to evolve and resequence experiments in *Drosophila melanogaster*. *BMC microbiology*, **21**, 108. https://doi.org/10.1186/s12866-021-02168-4

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH (2008) How many species are infected with *Wolbachia*?-A statistical analysis of current data. *FEMS microbiology letters*, **281**, 215–220. https://doi.org/10.1111/j.1574-6968.2008.01110.x

Hiroki M, Kato Y, Kamito T, Miura K (2002) Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Pieridae). *Die Naturwissenschaften*, **89**, 167–170. https://doi.org/10.1007/s00114-002-0303-5

Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T (2010) *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences*, **107**, 769–774. https://doi.org/10.1073/pnas.0911476107

Hughes GL, Allsopp PG, Brumbley SM, Woolfit M, McGraw EA, O'Neill SL (2011) Variable infection frequency and high diversity of multiple strains of *Wolbachia pipientis* in *Perkinsiella* planthoppers. *Applied and Environmental Microbiology*, **77**, 2165–2168. https://doi.org/10.1128/AEM.02878-10

Hurst GDD, Jiggins FM, Schulenburg JHG von der, Bertrand D, West SA, Goriacheva II, Zakharov IA, Werren JH, Stouthamer R, Majerus MEN (1999) Male-killing *Wolbachia* in two species of insect. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 735. https://doi.org/10.1098/rspb.1999.0698

Husseneder C, Park J-S, Howells A, Tikhe CV, Davis JA (2017) Bacteria associated with *Piezodorus guildinii* (Hemiptera: Pentatomidae), with special reference to those transmitted by feeding. *Environmental Entomology*, **46**, 159–166. https://doi.org/10.1093/ee/nvw112

Jaimes LFT, Valenzuela JRC, Londoño GAC, García DAM, Zuluaga MEL (2015) Relación entre la presencia y el daño de *Monalonion velezangeli* Carvalho & Costa y algunos factores climáticos en cultivos de aguacate cv. Hass. *Ciencia y Tecnología Agropecuaria*, **16**, 79–85. https://doi.org/10.21930/rcta.vol16_num1_art:381

Janson EM, Stireman JO, Singer MS, Abbot P (2008) Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution; International Journal of Organic Evolution*, **62**, 997–1012. https://doi.org/10.1111/j.1558-5646.2008.00348.x

Johnson JS, Spakowicz DJ, Hong B-Y, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M, Sodergren E, Weinstock GM (2019) Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, **10**, 5029. https://doi.org/10.1038/s41467-019-13036-1

Johnson TA, Sylte MJ, Looft T (2019) In-feed bacitracin methylene disalicylate modulates the turkey microbiota and metabolome in a dose-dependent manner. *Scientific Reports*, **9**, 8212. https://doi.org/10.1038/s41598-019-44338-5

Jung S, Lee S (2012) Molecular phylogeny of the plant bugs (Heteroptera: Miridae) and the evolution of feeding habits. *Cladistics: The International Journal of the Willi Hennig Society*, **28**, 50–79. https://doi.org/10.1111/j.1096-0031.2011.00365.x

Kaur R, Shropshire JD, Cross KL, Leigh B, Mansueto AJ, Stewart V, Bordenstein SR, Bordenstein SR (2021) Living in the endosymbiotic world of *Wolbachia*: A centennial review. *Cell Host & Microbe*, **29**, 879–893. https://doi.org/10.1016/j.chom.2021.03.006

Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T (2012) Symbiont-mediated insecticide resistance. *Proceedings of the National Academy of Sciences*, **109**, 8618–8622. https://doi.org/10.1073/pnas.1200231109

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, **41**, e1. https://doi.org/10.1093/nar/gks808

Laidoudi Y, Levasseur A, Medkour H, Maaloum M, Ben Khedher M, Sambou M, Bassene H, Davoust B, Fenollar F, Raoult D, Mediannikov O (2020) An earliest endosymbiont, *Wolbachia massiliensis* sp. nov., strain PL13 from the bed bug (*Cimex hemipterus*), type strain of a new supergroup T. *International Journal of Molecular Sciences*, **21**, 8064. https://doi.org/10.3390/ijms21218064

Lariviere PJ, Leonard SP, Horak RD, Powell JE, Barrick JE (2023) Honey bee functional genomics using symbiont-mediated RNAi. *Nature Protocols*, **18**, 902–928. https://doi.org/10.1038/s41596-022-00778-4

Li Q, Fan J, Sun J, Zhang Y, Hou M, Chen J (2019) Anti-plant defense response strategies mediated by the secondary symbiont *Hamiltonella defensa* in the wheat aphid *Sitobion miscanthi*. *Frontiers in Microbiology*, **10**. https://doi.org/10.3389/fmicb.2019.02419

Li R, Li M, Yan J, Zhang H (2020) Composition and function of the microbiotas in the different parts of the midgut of *Pyrrhocoris sibiricus* (Hemiptera: Pyrrhocoridae) revealed using high-throughput sequencing of 16S rRNA. *EJE*, **117**, 352–371. https://doi.org/10.14411/eje.2020.040

Luo J, Cheng Y, Guo L, Wang A, Lu M, Xu L (2021) Variation of gut microbiota caused by an imbalance diet is detrimental to bugs' survival. *The Science of the Total Environment*, **771**, 144880. https://doi.org/10.1016/j.scitotenv.2020.144880

Machtelinckx T, Van Leeuwen T, Vanholme B, Gehesquière B, Dermauw W, Vandekerkhove B, Gheysen G, De Clercq P (2009) *Wolbachia* induces strong cytoplasmic incompatibility in the predatory bug *Macrolophus pygmaeus*. *Insect Molecular Biology*, **18**, 373–381. https://doi.org/10.1111/j.1365-2583.2009.00877.x

Mejía-Alvarado FS, Ghneim-Herrera T, Góngora CE, Benavides P, Navarro-Escalante L (2021) Structure and dynamics of the gut bacterial community across the developmental stages of the coffee berry borer, *Hypothenemus hampei*. *Frontiers in Microbiology*, **12**. https://doi.org/10.3389/fmicb.2021.639868

Moran NA, Telang A (1998) Bacteriocyte-associated symbionts of insects. *BioScience*, **48**, 295–304. https://doi.org/10.2307/1313356

Motta EV, Gage A, Smith TE, Blake KJ, Kwong WK, Riddington IM, Moran N (2022) Host-microbiome metabolism of a plant toxin in bees (H Koch, WS Garrett, H Koch, Eds,). *eLife*, **11**, e82595. https://doi.org/10.7554/eLife.82595

Namyatova AA, Cassis G (2016) Systematic revision and phylogeny of the plant bug tribe Monaloniini (Insecta: Heteroptera: Miridae: Bryocorinae) of the world. *Zoological Journal of the Linnean Society*, **176**, 36–136. https://doi.org/10.1111/zoj.12311

Narita S, Kageyama D, Nomura M, Fukatsu T (2007) Unexpected mechanism of symbiont-induced reversal of insect sex: Feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development. *Applied and Environmental Microbiology*, **73**, 4332–4341. https://doi.org/10.1128/AEM.00145-07

Navarro-Escalante L, Benavides P, Acevedo FE (2024) Supplementary Data: Diversity of bacterial symbionts associated with the tropical plant bug Monalonion velezangeli (Hemiptera: Miridae) revealed by high-throughput 16S-rRNA sequencing. https://doi.org/10.5281/zenodo.10524900

Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T (2014) Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 10257–10262. https://doi.org/10.1073/pnas.1409284111

Ocampo Flórez V, Durán Prieto J, Albornoz M, Forero D (2018) New plant associations for *Monalonion velezangeli* (Hemiptera: Miridae) in green urban areas of Bogotá (Colombia). *Acta Biológica Colombiana*, **23**, 205–208. https://doi.org/10.15446/abc.v23n2.69374

O'Neill E by SL, Hoffmann AA, Werren and JH (Eds.) (1998) *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford University Press, Oxford, New York. https://doi.org/10.1093/oso/9780198577867.001.0001

Pavlidis P, Noble WS (2003) Matrix2png: a utility for visualizing matrix data. *Bioinformatics (Oxford, England)*, **19**, 295–296. https://doi.org/10.1093/bioinformatics/19.2.295

Pietri JE, DeBruhl H, Sullivan W (2016) The rich somatic life of *Wolbachia*. *MicrobiologyOpen*, **5**, 923–936. https://doi.org/10.1002/mbo3.390

Ramírez-Cortés HJ, Gil-Palacio ZN, Benavides-Machado P (2008) Monalonion velezangeli *La chinche de la chamusquina del café*. Canicafe, Chinchina, Caldas, Colombia. https://www.cenicafe.org/es/publications/avt0367.pdf

Ramírez-Gil JG, López JH, Henao-Rojas JC (2020) Causes of hass avocado fruit rejection in preharvest, harvest, and packinghouse: Economic losses and associated variables. *Agronomy*, **10**, 8. https://doi.org/10.3390/agronomy10010008

Ratcliffe NA, Furtado Pacheco JP, Dyson P, Castro HC, Gonzalez MS, Azambuja P, Mello CB (2022) Overview of paratransgenesis as a strategy to control pathogen transmission by insect vectors. *Parasites & Vectors*, **15**, 112. https://doi.org/10.1186/s13071-021-05132-3

Ricaboni D, Mailhe M, Khelaifia S, Raoult D, Million M (2016) Romboutsia timonensis, a new species isolated from human gut. *New Microbes and New Infections*, **12**, 6–7. https://doi.org/10.1016/j.nmni.2016.04.001

Rodas CA, Serna R, Hurley BP, Bolaños MD, Granados GM, Wingfield MJ (2014) Three new and important insect pests recorded for the first time in Colombian plantations. *Southern Forests: a Journal of Forest Science*, **76**, 245–252. https://doi.org/10.2989/20702620.2014.965983

Rudman SM, Greenblum S, Hughes RC, Rajpurohit S, Kiratli O, Lowder DB, Lemmon SG, Petrov DA, Chaston JM, Schmidt P (2019) Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 20025–20032. https://doi.org/10.1073/pnas.1907787116

Sajnaga E, Skowronek M, Kalwasińska A, Kazimierczak W, Lis M, Jach ME, Wiater A (2022) Comparative Nanopore sequencing-based evaluation of the midgut microbiota of the summer chafer (*Amphimallon solstitiale* l.) associated with possible resistance to entomopathogenic nematodes. *International Journal of Environmental Research and Public Health*, **19**, 3480. https://doi.org/10.3390/ijerph19063480

Sato Y, Jang S, Takeshita K, Itoh H, Koike H, Tago K, Hayatsu M, Hori T, Kikuchi Y (2021) Insecticide resistance by a host-symbiont reciprocal detoxification. *Nature Communications*, **12**, 6432. https://doi.org/10.1038/s41467-021-26649-2

Schausberger P (2018) Herbivore-associated bacteria as potential mediators and modifiers of induced plant defense against spider mites and thrips. *Frontiers in Plant Science*, **9**. https://doi.org/10.3389/fpls.2018.01107

Serbus LR, Casper-Lindley C, Landmann F, Sullivan W (2008) The genetics and cell biology of *Wolbachia*-host interactions. *Annual Review of Genetics*, **42**, 683–707. https://doi.org/10.1146/annurev.genet.41.110306.130354

Shropshire JD, Leigh B, Bordenstein SR (2020) Symbiont-mediated cytoplasmic incompatibility: What have we learned in 50 years? (D Weigel, Ed,). *eLife*, **9**, e61989. https://doi.org/10.7554/eLife.61989

Shukla SP, Beran F (2020) Gut microbiota degrades toxic isothiocyanates in a flea beetle pest. *Molecular Ecology*, **29**, 4692–4705. https://doi.org/10.1111/mec.15657

Sinkins SP (2004) *Wolbachia* and cytoplasmic incompatibility in mosquitoes. *Insect Biochemistry and Molecular Biology*, **34**, 723–729. https://doi.org/10.1016/j.ibmb.2004.03.025

Soh L-S, Veera Singham G (2022) Bacterial symbionts influence host susceptibility to fenitrothion and imidacloprid in the obligate hematophagous bed bug, *Cimex hemipterus*. *Scientific Reports*, **12**, 4919. https://doi.org/10.1038/s41598-022-09015-0

Stouthamer R, Breeuwer JA, Hurst GD (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology*, **53**, 71–102. https://doi.org/10.1146/annurev.micro.53.1.71

Sudakaran S, Kost C, Kaltenpoth M (2017) Symbiont acquisition and replacement as a source of ecological innovation. *Trends in Microbiology*, **25**, 375–390. https://doi.org/10.1016/j.tim.2017.02.014

Sun D-L, Jiang X, Wu QL, Zhou N-Y (2013) Intragenomic heterogeneity of 16S rRNA genes causes overestimation of prokaryotic diversity. *Applied and Environmental Microbiology*, **79**, 5962–5969. https://doi.org/10.1128/AEM.01282-13

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Van de Peer Y, Chapelle S, De Wachter R (1996) A quantitative map of nucleotide substitution rates in bacterial rRNA. *Nucleic Acids Research*, **24**, 3381–3391. https://doi.org/10.1093/nar/24.17.3381

Vavre F, de Jong JH, Stouthamer R (2004) Cytogenetic mechanism and genetic consequences of thelytoky in the wasp *Trichogramma cacoeciae*. *Heredity*, **93**, 592–596. https://doi.org/10.1038/sj.hdy.6800565

Volland J-M (2024) Shedding light on bacteria associated with an agricultural pest, the tropical plant bug *Monalonion velezangeli*: a foundational descriptive study. *Peer Community in Microbiology*, **1**, 100004. https://doi.org/10.24072/pci.microbiol.100004

Werren JH (1997) Biology of *Wolbachia*. *Annual Review of Entomology*, **42**, 587–609. https://doi.org/10.1146/annurev.ento.42.1.587

Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews. Microbiology*, **6**, 741–751. https://doi.org/10.1038/nrmicro1969

Wilches DM, Coghlin PC, Floate KD (2021) Next generation sequencing, insect microbiomes, and the confounding effect of *Wolbachia*: a case study using spotted-wing drosophila (*Drosophila suzukii*) (*Diptera: Drosophilidae*). *Canadian Journal of Zoology*, **99**, 588–595. https://doi.org/10.1139/cjz-2020-0260

Xue H, Zhu X, Wang L, Zhang K, Li D, Ji J, Niu L, Wu C, Gao X, Luo J, Cui J (2021) Gut bacterial diversity in different life cycle stages of *Adelphocoris suturalis* (Hemiptera: Miridae). *Frontiers in Microbiology*, **12**, 670383. https://doi.org/10.3389/fmicb.2021.670383

Zhang S, Gan L, Qin Q, Long X, Zhang Y, Chu Y, Tian Y (2016) Paracoccusacridae sp. nov., isolated from the insect *Acrida cinerea* living in deserted cropland. *International Journal of Systematic and Evolutionary Microbiology*, **66**, 3492–3497. https://doi.org/10.1099/ijsem.0.001222

Zhang D, Wang Y, He K, Yang Q, Gong M, Ji M, Chen L (2020) *Wolbachia* limits pathogen infections through induction of host innate immune responses. *PloS One*, **15**, e0226736. https://doi.org/10.1371/journal.pone.0226736

Zhang L, Yun Y, Hu G, Peng Y (2018) Insights into the bacterial symbiont diversity in spiders. *Ecology and Evolution*, **8**, 4899–4906. https://doi.org/10.1002/ece3.4051

Zhou W, Rousset F, O'Neil S (1998) Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 509–515. https://doi.org/10.1098/rspb.1998.0324

Zhou J-C, Zhao Q, Liu S-M, Shang D, Zhao X, Huo L-X, Dong H, Zhang L-S (2021) Effects of thelytokous parthenogenesis-inducing *Wolbachia* on the fitness of *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) in superparasitised and single-parasitised hosts. *Frontiers in Ecology and Evolution*, **9**. https://doi.org/10.3389/fevo.2021.730664

Zug R, Hammerstein P (2015) *Wolbachia* and the insect immune system: what reactive oxygen species can tell us about the mechanisms of *Wolbachia*-host interactions. *Frontiers in Microbiology*, **6**, 1201. https://doi.org/10.3389/fmicb.2015.01201