Microbiome and parasitome of *Aedes albopictus* and *Culex pipiens* mosquitoes in urban and sub-urban areas of South Korea

Chavarria Bayot, Xavier Bernardo

Department of Medical Science

Graduate School

Yonsei University

Microbiome and parasitome of *Aedes albopictus* and *Culex pipiens* mosquitoes in urban and sub-urban areas of South Korea

Advisor Ju Yeong Kim

A Master's Thesis Submitted
to the Department of Medical Science
and the Committee on Graduate School
of Yonsei University in Partial Fulfillment of the
Requirements for the Degree of
Master of Medical Science

Chavarria Bayot, Xavier Bernardo

June 2025

Microbiome and parasitome of Aedes albopictus and Culex pipiens mosquitoes in urban and sub-urban areas of South Korea

This Certifies that the Master's Thesis of Xavier Chavarria is Approved

Committee Chair _____
Shin, Myeong Heon

Committee Member _____
Kim, Ju Yeong

Committee Member _____
Yoon, Bo Kyung

Department of Medical Science
Graduate School
Yonsei University
June 2025

ACKNOWLEDGEMENTS

I thank the Government of South Korea, the National Institute for International Education (NIIED) and the Department of Medical Science of Yonsei University College of Medicine. I am thankful to my advising professor Ju Yeong Kim for his guidance, as well as the coauthors of the published version of this work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Microbiome and parasitome of *Aedes albopictus* and *Culex pipiens* mosquitoes in urban and sub-urban areas of South Korea

The mosquito microbiome interacts with their host's biology influencing their fitness and vector competence. Future mosquito biocontrol strategies will require descriptive knowledge of these interactions as well as constant and comprehensive microbiome surveillance. Metabarcoding offers a scalable method for such monitoring efforts. In this study, 16S and 18S rRNA gene metabarcoding was used via iSeq100 sequencing to profile the prokaryotic and eykaryotic communities of three population of *Aedes albopictus* and *Culex pipiens* in in South Korea. *Ae. Albopictus* and *Cx. pipiens* are two globally-distributed mosquito vectors with medical importance in South Korea. Bacterial alpha and beta diversity was significantly different among populations, suggesting that microbial composition varied by population. Pseudomonadota dominated the microbiota of both species. *Wolbachia* and *Aeromonas* dominated *Cx. pipiens*, while *Enterococcus* dominated *Ae. albopictus*. *Ascogregarina taiwanensis,* a common *Ae. Albopictus* parasite, was highly abundant in this species but not present in *Cx. pipiens*. Linear discriminant analysis Effect Size (LEfSe) tested on the microbiome of *A. Taiwanensis*–infected and uninfected *Ae. albopictus* showed its absence was associated with seven bacterial taxa.

Key words : microbiome, parasites, metabarcoding, *Aedes albopictus*, *Culex pipiens*, *Ascogregarina Taiwanensis*

1. INTRODUCTION

Mosquitoes (Diptera: Culicidae) are a global public health concern as they serves as vectors of multiple pathogens [1]. These pathogens do not live in isolation, but interact closely with the microbiome and the host [2]. These pathogen-microbiome-host interactions are known to exert direct effects on the mosquito vector competence [3]. The microbiome composition to mosquitoes depends on multiple factors, such as the environment, their food sources, the life stage, as well as the mosquito species and population [1,4]. Interactions withing the microbiome also regulate the mosquito microbiome composition [5]. In mosquito species, examples of these interactions are well documented: the bacteria *Asaia* and *Wolbachia* are known to antagonize each other and compete for colonization in *Anopheles* [6]. However, not only other bacteria can determine microbiome composition and body fitness. Rather, parasites are important pathogens that can affect competitive ability, body fitness, and influence microbiome composition [7]. For example, *Plasmodium* colonization in *Aedes aegypti* is known to be sensitive to other parasitic infections [8].

As certain bacterial taxa, such as *Wolbachia*, *Asaia* and *Serratia* will become relevant in the future for the implementation of biocontrol strategies for mosquito-borne diseases [2], the microbiome composition of different mosquito species needs to be comprehensively explored. Certain bacteria such as *Wolbachia,* are already in use to control mosquito reproduction [2]. Given that the mosquito microbiome changes not only across species, but also populations [9], information of the microbiome interactions within vectors from different geographic locations is needed [3] for the correct development of microbiota-based control of mosquito populations [1].

In Korea, mosquito-borne pathogens continue to raise public health concerns [10], especially due to imported cases of diseases like malaria, dengue, and chikungunya [11], associated with mosquito populations from different geographic regions. Additionally, anthropological factors such as climate change and increased international travel pose the risk of the recrudescence of pathogens such as Malaria parasites, making the continued surveillance of mosquitoes and their microbiome a priority in South Korea [12].

The two species studied here are invasive mosquitoes with the potential to carry and transmit pathogens. First, *Aedes albopictus* is a species that thrives in urban habitats and serves as vector for

important arboviruses worldwide. Among these, the most relevant are the Dengue Virus (DENV) [13], the West Nile virus (WNV) [14], the Japanese encephalitis virus (JEV) [15], and the chikungunya virus (CHIKV) [16]. Recent imported cases of arboviral infections such as CHIKV, are believed to be linked to this vector [11]. *Culex pipiens* is also an important urban mosquito [17] as studies have shown this species can be a competent vector for WNV [18] and JEV in South Korea [19]. The *Culex* species complex presents two subspecies that are present in South Korea [20]: *Cx. pipiens pallens* and *Cx. pipiens* f. *molestus*.

In this study, the microbiome and parasitome compositions of *Ae. albopictus* from one region and *Cx. pipiens* populations from two regions in South Korea was characterized by 16S and 18S ribosomal RNA metabarcoding. Although metabarcoding is commonly employed to investigate microbial communities in environmental contexts, its use in vector surveillance has gained recent attention [21].

2. MATERIALS AND METHODS

2.1. Sample collection

Sampling was performed for adult mosquitoes in the early autumn (September 2023) from an urban area located in Seoul, Gyeonggi, and the semi-urban area of Asan city, Chungnam (Table 1). In the Seoul location (37° 33’ 44.06’’ N, 126° 56’ 0.96’’ E), mosquitoes were collected around the same area during five collection days (n = 74). Two collection batches were carried out in Asan. A first collection of n = 20 in the locality of Oncheon (36° 47’ 5.31’’ N, 127° 0’ 7.0’’ E) where we collected Cx. pipiens individuals, and a second collection of n = 30 in the locality of Sinchang (36° 46’ 42.11’’ N, 126° 56’ 9.62’’ E) where we collected *Ae. albopictus* individuals (Figure 1). Sampling took place outdoors during the early morning and near sources of vegetation in all locations. Resting adult mosquitoes were collected alive from natural or artificial surfaces with a mouth aspirator and stored in sterile conical plastic tubes. We avoided swarm collection and human landing collection to prevent contamination. Samples were stored at -81 °C until DNA extraction. Samples were frozen without the use of a fast-freezing agent.

Table 1. Mosquitoes captured in Seoul and Asan for this study

Mosquito species	Stage	Seoul	Asan
Ae. albopictus	Adults	0	30
	Pupae	0	2
	3rd instar larvae	0	10
Cx. pipiens	Adults	74	20
	Pupae	0	3
	3rd instar larvae	0	3
Total		74	68

Figure 1. Study Area. Samplin areas in South Korea (A). Surrounding areas of the Seoul location (B-E). Surrounding area of Oncheon, Asan (F). Surrounding area of Sinchang, Asan (G). Stagnant water environment of mosquito larvae in Sinchang, Asan (H-I). Mosquito larvae from stagnant water collected in Oncheon, Asan (J).

2.2. Sampling of other life stages

Additionally, the larval (n = 13) and pupal (n = 5) stages were also collected from stagnant water sources like flower pots and discarded tires in the Asan location. Larvae and pupae were preserved in 2 mL RNAlater solution (Invitrogen, Vilnius, Lithuania) at room temperature (approximately 20 °C) and then frozen at -81 °C until DNA extraction. The life stage of each mosquito was assessed based on morphology. Species typing was performed in the same way as for adults. However, all diversity calculations and statistical comparisons of the related microbial community were performed only on adult samples.

2.3. DNA extraction and mosquito species identification

The mosquito cuticle was washed with absolute ethanol and dried at room temperature (approximately 20 °C) prior to DNA extraction. Whole genomic DNA was extracted from individual mosquitoes using the entire insect with newly opened NucleoSpin DNA Insect extraction kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions and then stored at -41 °C. Mosquito species was assessed by morphology and confirmed by Sanger sequencing (Bionics, Seoul, Republic of Korea) of the mitochondrial cytochrome c oxidase subunit I (*MT-CO1*) partial sequence using LCO1490 and HCO2198 primers by following a protocol described earlier (Table 2). Amplification was performed using the AccuPower Taq PCR PreMix (BIONEER, Daejeon, Republic of Korea) with 2 μL of template DNA and 1 μL of 10 μM of each primer for a 20-μL final volume. PCR-grade water was used as the negative control.

2.4. Microbiome molecular identification

The bacterial community was identified using the 16S_V4_515F and 16S_V4_806R primers to amplify the V4 region of the 16S rRNA bacterial gene (Table 2). Parasites were identified using the 18S_V9_1391f and 18S_V9_EukBr primers to amplify the V9 region of the 18S rRNA eukaryotic gene. All the targets were amplified using the PCR protocol described by Kim at al. [22]. An eight cycle-PCR amplification step was used to attach Illumina multiplexing indices for library preparation using the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). Amplicon clean-

up was performed using AMPure XP (Beckman Coulter, Brea, CA, USA) after each amplification step. For the multiplexing step, DNA concentration was measured for every sample using the QuantiFluor ONE dsDNA System (Madison, WI, USA). Samples were pooled in Tris-HCl buffer for a 2 nM pool and then diluted for a final pooling concentration of 50 pM. Metabarcoding was performed according to the manufacturer's instructions, using the iSeq100 i1 Reagent Cartridge v2 (Illumina) on the iSeq100 Sequencing System platform. PhiX Control v3 (Illumina) was used as the control for all runs.

All 142 samples (124 adult samples composed of 74 adult *Cx. pipiens* from Seoul, 20 adult *Cx. pipiens* from Asan, 30 adult *Ae. albopictus* from Asan, and 18 samples from other life stages) were processed for 18S rRNA metabarcoding. A total of 131 samples (composed of 74 adult *Cx. pipiens* from Seoul, 20 adult *Cx. pipiens* from Asan, 30 adult *Ae. albopictus* from Asan and 7 samples from other life stages) were processed for 16S rRNA metabarcoding sequencing.

Since *Ascogregarina taiwanensis* amplicon sequence variants (ASVs) were highly prevalent in *Ae. albopictus*, we confirmed the infection status of positive *Ae. albopictus* by sequencing (Bionics, South Korea) with specific primers (Table 2) using the same PCR kit used for COI gene amplification. PCR conditions were the same as described by Seabourn et al (2020) [9].

Table 2. Primers used in this study

Primer	Sequence	Annealing temperature	Band Size	Reference
LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	48°C	700 bp	[23]
HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′			
16S_V4_515F	5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A-3'	55 °C	350 bp	[24]
16S_V4_806R	5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT-3'			
18S_V9_1391f	5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTA CAC ACC GCC CGT C-3'	55 °C	250 bp	[25]
18S_V9_EukBr	5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTG ATC CTT CTG CAG GTT CAC CTA C-3'			
12S_L1085_F	5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCC AAA CTG GGA TTA GAT AAC CC3'	55 °C	215 bp	[26]
12S_H1259_R	5'-GTC TCG TG GGC TCG GAG ATG TGT ATA AGA GAC AGG TTT GCT GAA GAT GGC GGT A-3'			
AT_SHORT_2_F	5'-TCG ATG AAG GAC GCA GCT TA-3'	56 °C	100 bp	[9]
AT_SHORT_2_R	5'-AGG CAC TGA ACT GGA CAT ACT-3'			

2.5. Bioinformatics and statistical analyses

All statistical analyses were performed only on the 124 adult mosquito samples (composed of 74 adult *Cx. pipiens* from Seoul, 20 adult *Cx. pipiens* from Asan, 30 adult *Ae. albopictus* from Asan). Microbiota analyses were performed using the QIIME 2 pipeline v. 2022.11.1 (https://qiime2.org/). Raw reads were demultiplexed and trimmed using the q2-cutadapt plugin [27]. The trimmed sequences were denoised using the DADA2 package with the consensus method for chimera exclusion and minimum overlap of 4bp [28]. Taxonomic classification of the ASVs was performed using the classify-consensus-blast classifier of the q2-feature-classifier plugin [29] against the SILVA v. 138.99 database for 16S rRNA ASVs and an in-house NCBI-RefSeq database for 18S rRNA ASVs.

Alpha (Shannon index and richness, defined as observed features) and beta diversity (unweighted UniFrac distances and Bray–Curtis dissimilarity) metrics were obtained for bacterial ASVs using the core-metrics-phylogenetic plugin after rarefaction to a 1,000-read depth. Alpha diversity metrics were compared among the three adult mosquito populations—*Cx. pipiens* from Seoul and Asan and *Ae. albopictus* from Asan—using the Kruskal–Wallis rank sum test with the stats package in the R software v. 4.3.1. (https://www.R-project.org/). Post-hoc pairwise Wilcoxon's tests with Benjamini–Hochberg correction from the stats package were used to assess specific pairwise differences in the Shannon index, and the Dunn's test with Bonferroni correction was applied as a post-hoc test to evaluate the differences in richness using the FSA package in R. Beta diversity metrics were compared between, and within the three populations, using permutational multivariate analysis of variance (PERMANOVA) in QIIME 2. Beta diversity metrics were visualised through principal coordinate analysis (PCoA) in R.

Linear discriminant analysis (LDA) effect size (LEfSe) was performed in R using the microbiomeMarker package [30]**.** LDA is a dimensionality reduction statistical tool that separates classes by selecting the best linear combinations of features. LEfSe implements LDA and differential abundance test to find indicator taxa that are representative from each group [27]. Histograms of the ASVs LDA scores were constructed with a 2.0 cutoff and p-value < 0.05 to characterise the representative bacterial taxa from the three populations. The same analysis was repeated for *Ae. albopictus* population to test representative taxa of gregarine infected and non-infected hosts. The microeco package [31] was used to visualize the dominant bacterial taxa in the microbiome of the

three populations and to construct a co-occurrence network of the ASVs identified in this study at the family and genus levels. Co-occurrence was calculated using a Spearman correlation matrix. The cal_network function was used for the construction of the network with a 0.05 *p*-value threshold and the Spearman's correlation coefficient optimisation option. All raw sequencing data were uploaded to the NCBI Short Read Archive under the BioProject number PRJNA1104456.

3. RESULTS

3.1. Alpha diversity of the mosquito microbiome

Differences in the alpha diversity and richness of the microbiome were observed among the three adult mosquito populations (Seoul Cx. pipiens, Asan Cx. pipiens, and Asan *Ae. albopictus;* Figure 2). Specifically, Cx. pipiens from Asan had lower Shannon diversity and lower richness compared to those from Seoul (Shannon diversity: $p = 0.01$; richness: $p = 0.0002$) and Ae. albopictus from Asan (Shannon diversity: $p < 0.001$; richness: $p = 0.003$). Additionally, the Cx. pipiens Seoul population exhibited lower Shannon diversity compared to the Ae. albopictus population in Asan ($p < 0.001$), although the richness of ASVs did not vary significantly between these two populations ($p = 0.767$).

3.2. Beta diversity of the mosquito microbiome

Beta diversity distances PCoA showed distinct microbiome compositions by population (Figure 3). PERMANOVA of the unweighted UniFrac distances showed a difference in the beta diversity of the microbiota between the three adult populations (p = 0.001). The microbiome of *Cx. pipiens* and *Ae. Albopictus* differed within Asan (p = 0.003), and the *Cx. pipiens* from Asan were different than those from Seoul (p = 0.001; Figure 4). Distances of *Ae. albopictus* from Asan were smaller within the same Asan population compared to *Cx. pipiens* from Asan, with the largest distances observed when comparing them with *Cx. pipiens* from Seoul. The differences in beta diversity show that the *Ae. albopictus* from Asan has a more similar microbiome composition within its own population and other species in the same region than in comparison to *Cx. pipiens* from Seoul. Additionally, the distances of *Cx. pipiens* from Asan were closer to *Ae. albopictus* from Asan than to *Cx. pipiens* from Seoul, pointing that geographic proximity influences the microbiome similarities between these populations (Figure 4). PERMANOVA results based on the Bray–Curtis dissimilarity matrix showed the same beta diversity differences (p = 0.001).

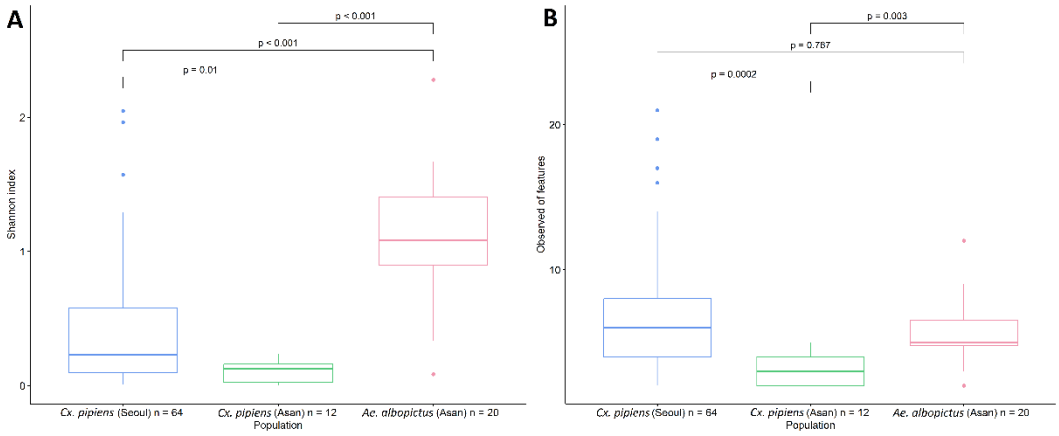

Figure 2. Comparisons of alpha diversity metrics of the microbiome of three adult mosquito populations in Korea. Comparison of diversity of bacterial ASVs assessed using the Shannon index between populations (A) and comparison of bacterial ASV richness between populations (B). Only comparisons between adult individuals were made.

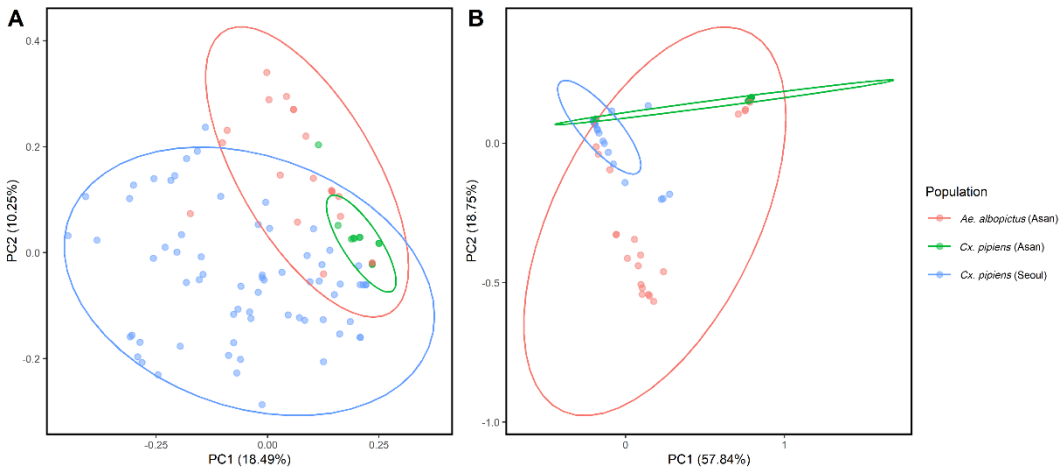

Figure 3. Comparisons of beta diversity metrics of the microbiome of three adult mosquito populations in Korea. Principal component analysis (PCoA) of the unweighted UniFrac distances between populations (A). PERMANOVA *p*-value = 0.001. Principal component analysis (PCoA) of the Bray–Curtis dissimilarity index between populations (B). PERMANOVA *p*-value = 0.001. Ellipses represent t-distribution 95% confidence intervals.

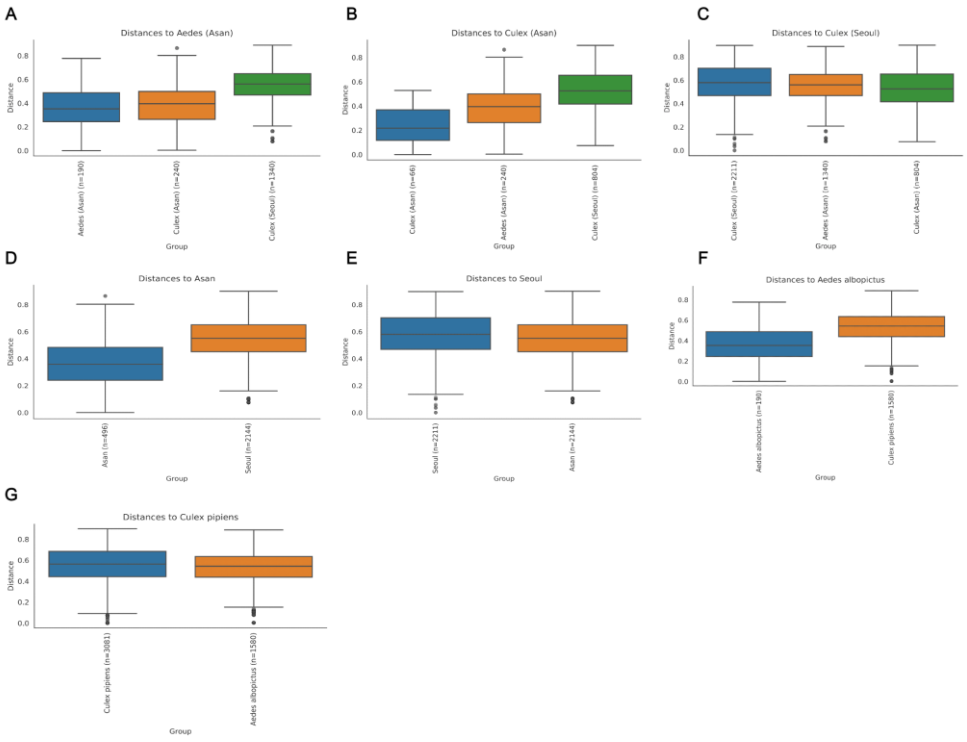

Figure 4. Microbiome beta diversity (unweighted UniFrac distances) differences in the present study. Unweighted UniFrac distances from the three populations to the *Ae. Albopictus* population from Asan (A). Unweighted UniFrac distances from the three populations to the *Cx. pipiens* population from Asan (B). Unweighted UniFrac distances from the three populations to the *Cx. pipiens population* from Seoul (C). Unweighted UniFrac distances of all samples from the two locations to the Asan location (D). Unweighted UniFrac distances of all samples from the two locations to the Seoul location (E). Unweighted UniFrac distances of all samples from the two species to *Ae. albopictus* (F). Unweighted UniFrac distances of all samples from the two species to *Cx. pipiens* (G).

3.3. Mosquito microbiome composition

An average of 4,859 reads per sample and 508 ASVs were identified from the V4 region of the 16S rRNA gene from the 131 mosquitoes sequenced. After rarefaction, the minimum and maximum number of reads per sample ranged from 1,000 to 72,969 reads. In adult *Cx. pipiens*, the most abundant reads belonged to the phyla Pseudomonadota (95.6%), Bacteroidota (1.6%), Cyanobacteriota (1.3%), Spirochaetota (0.7%), Actinobacteriota (0.01%), and Campilobacterota (0.01%). The most abundant reads in adult *Ae. albopictus* were associated with the phyla Pseudomonadota (73.1%), Bacillota (10.88%), Actinobacteriota (4.92%), Planctomycetota (0.64%), Actinobacteriota (4.9%), Bacteroidota (0.4%), and Acidobacteriota (0.05%). The bacterial composition of the three adult populations is shown in Figure 5, and the composition of the larvae and pupae is shown in Figure 6. The genus *Wolbachia* and the family Comamonadaceae dominated the microbiome of the three populations. However, both groups from Asan were also dominated by *Aeromonas*, and *Ae. albopictus* individuals in particular, by *Enterococcus* (Figure 7).

Differentially enriched taxa in the microbiomes of the three adult populations were identified using LEfSe analysis (Figure 8). *Cx. pipiens* mosquitoes from Seoul were significantly enriched with *Wolbachia* sp. having an LDA score of 5.53. Other significantly enriched taxa from this population were the families Spirochaetaceae (4.11), Erwiniaceae (3.80), Arcobacteraceae (2.56), and the genera *Comamonas* sp. (3.84), *Acinetobacter* sp. (3.63) and *Thiovirga* sp. (2.95). In *Cx. pipiens* from Asan, the family Xanthobacteraceae (3.11), and the genera *Aeromonas* sp. (5.60) and *Methylophilus* sp. (2.91) were differentially enriched. Finally, representative taxa of *Ae. albopictus* populations in Asan were the family Comamonadaceae (3.88), and the genera *Enterococcus* sp. (4.98), *Limnobacter* sp. (3.50), *Variorovax* sp. (2.47), *Nevskia* sp. (2.46) and *Dysgonomonas* sp. (2.45).

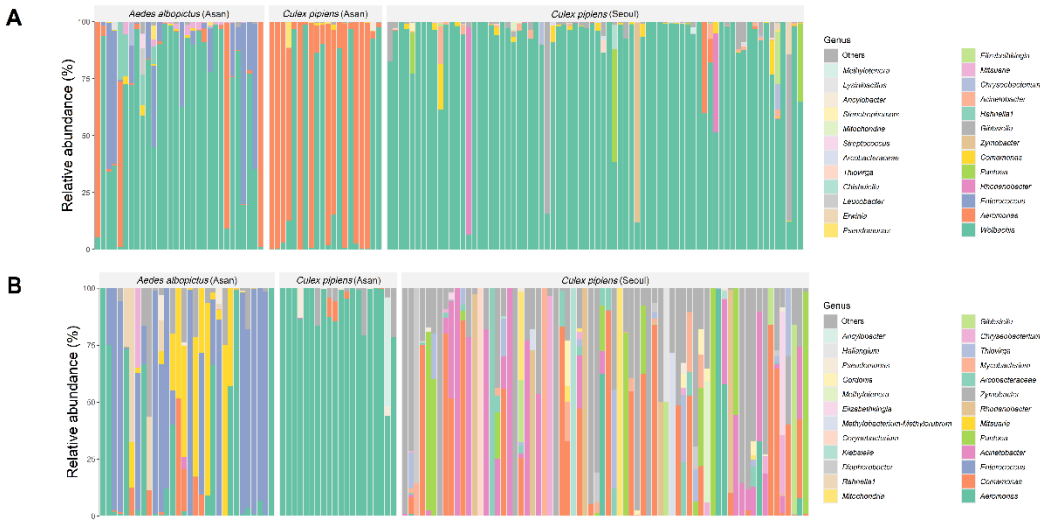

Figure 5. Microbiome composition of the three adult mosquito populations studied in South Korea. Relative abundance of the ASVs found in the three mosquito populations including Wolbachia ASVs (A) and excluding Wolbachia ASVs (B). Bar plots show the 25 most abundant ASVs. Only adult mosquito samples are included.

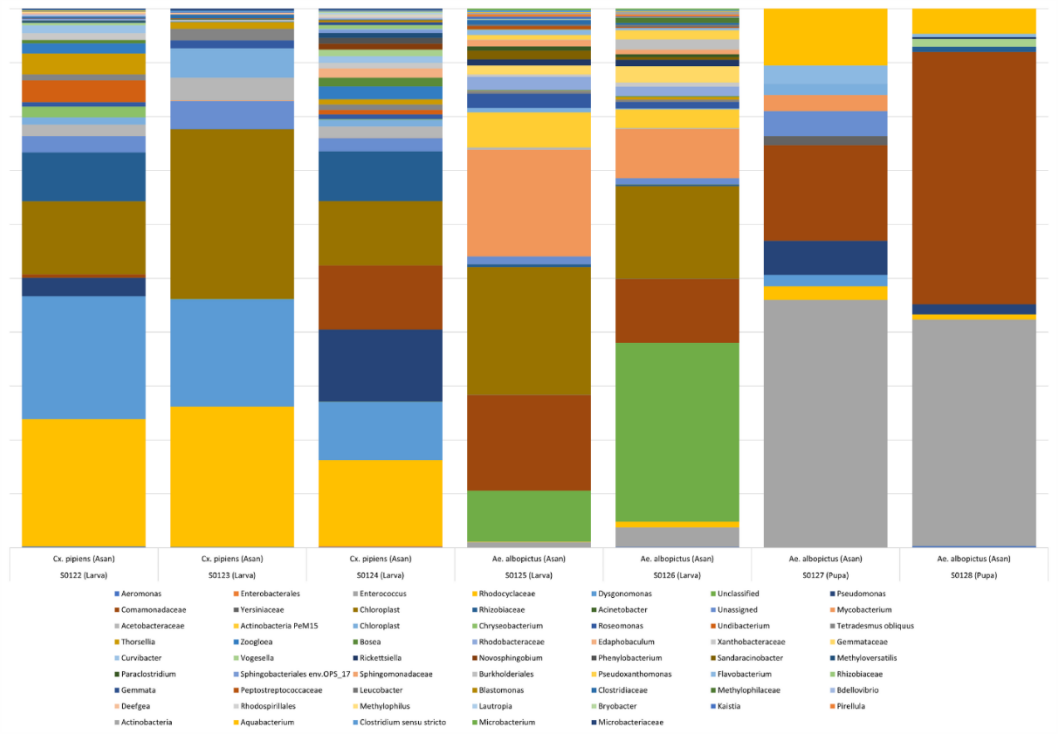

Figure 6. Microbiome composition of larva and pupa stages of *Ae. albopictus* and *Cx. pipiens* in Asan (Chungnam region). Only ASVs with more than 100 total reads are shown. ASVs from Wolbachia are not shown.

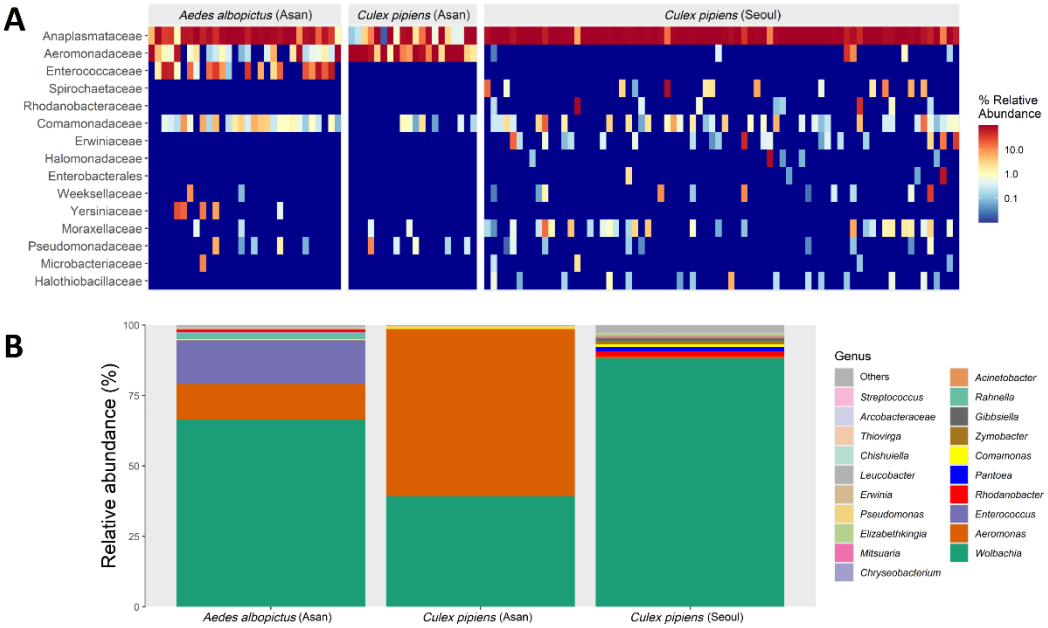

Figure 7. Relative abundance of dominant bacterial taxa in three adult mosquito populations studied in South Korea. Heatmap of the 15 most abundant Families of the bacterial ASVs found in the three adult mosquito populations (A). Mean relative abundance of the 20 most common bacterial ASVs by adult mosquito population (B).

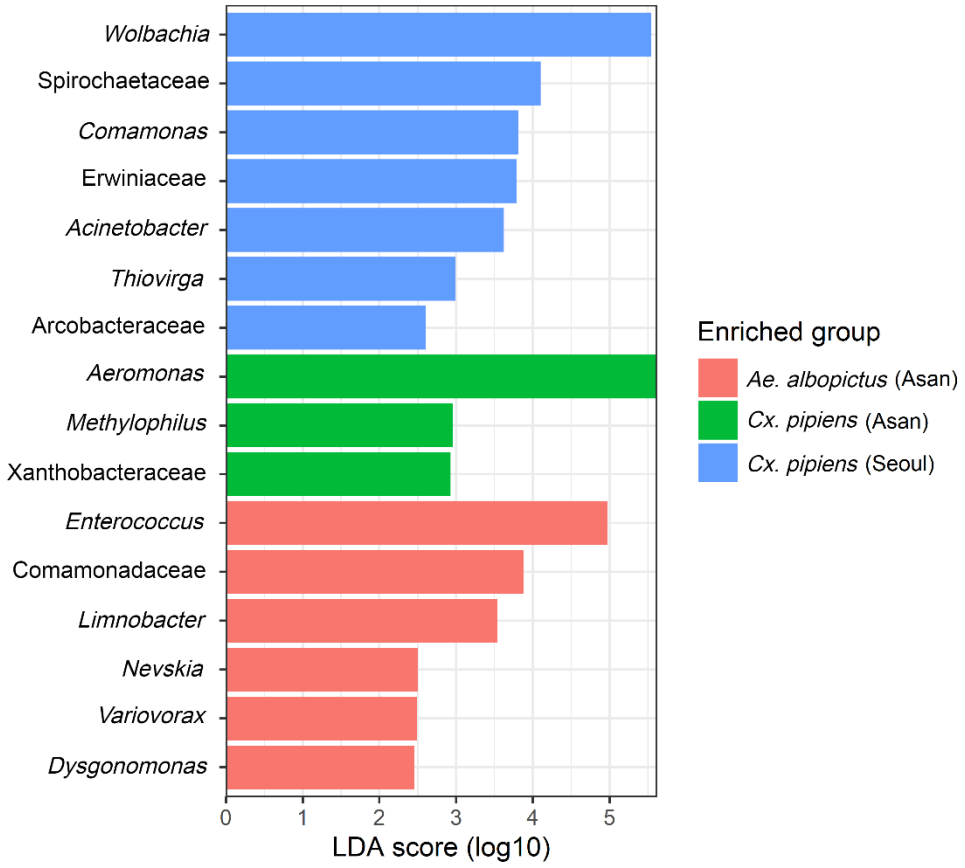

Figure 8. Linear discriminant analysis (LDA) effect size of differentially abundant bacterial taxa of the three adult mosquito populations studied in South Korea. Only comparisons between adult individuals are included.

3.4. ASV co-occurrence analyses

The co-occurrence network showed that Anaplasmataceae co-occurred with Comamonadaceae and Erwiniaceae; Xanthomoadaceae was related to Erwiniaceae, Comamonadaceae, and Anaplamataceae (Figure 9); and Halomonadaceae, Moraxellaceae, and Weeksellaceae were found with Erwiniaceae. At the genus level, *Wolbachia* co-occurred most frequently with *Stenotrophomonas, Pantoea,* and *Comamonas*; *Stenotrophomonas* was associated with *Pantoea* and *Chryseobacterium,* whereas *Erwinia* was associated with *Elizabethkingia*, *Acinetobacter,* and *Chryseobacterium* Finally, *Elizabethkingia* also occurred along with *Chryseobacterium* and *Acinetobacter*.

3.5. Mosquito Eukaryome composition

An average of 37,940 reads and 64 ASVs were identified from the V9 region of the 18S rRNA gene in the 142 mosquitoes analysed (mean frequency per sample: 16,745 reads). Most of the ASVs reads were unassigned or hosts sequences. Two adult mosquitoes from Seoul had a *Crithidia fasciculata* ASV (Table 3). Thirty adult mosquito samples from Asan yielded 18S rRNA ASVs assigned as other than metazoan sequences from the mosquito. Four adult *Cx. pipiens* isolated from Asan contained uncultured eukaryotes (n = 1), *Ganoderma* (n = 1) and an unassigned ASV (n = 2). The remaining 26 mosquitoes were *Ae. albopictus*, one of which had an *Anurofeca* ASV and two had an unresolved eukaryote ASV. However, we did not detect these sequences at a level of at least 10 reads per sample. The parasitic apicomplexan *Ascogregarina taiwanensis* was the most abundant eukaryotic taxon in the microbiome of *Ae. albopictus*, detected in 26 adult mosquitoes. Only four adult mosquitoes did not show reads of *A. taiwanensis* ASV. Positive mosquitoes presented *A. taiwanensis* specific sequences, except for one with poor sequence quality. Uninfected individuals were enriched in *Chryseobacterium*, Rhodobacteraceae, *Enhydrobacter*, *Cloacibacterium*, Acetobacteraceae, *Acinetobacter*, and Roseomonas according to LEfSe analysis (Table 4).

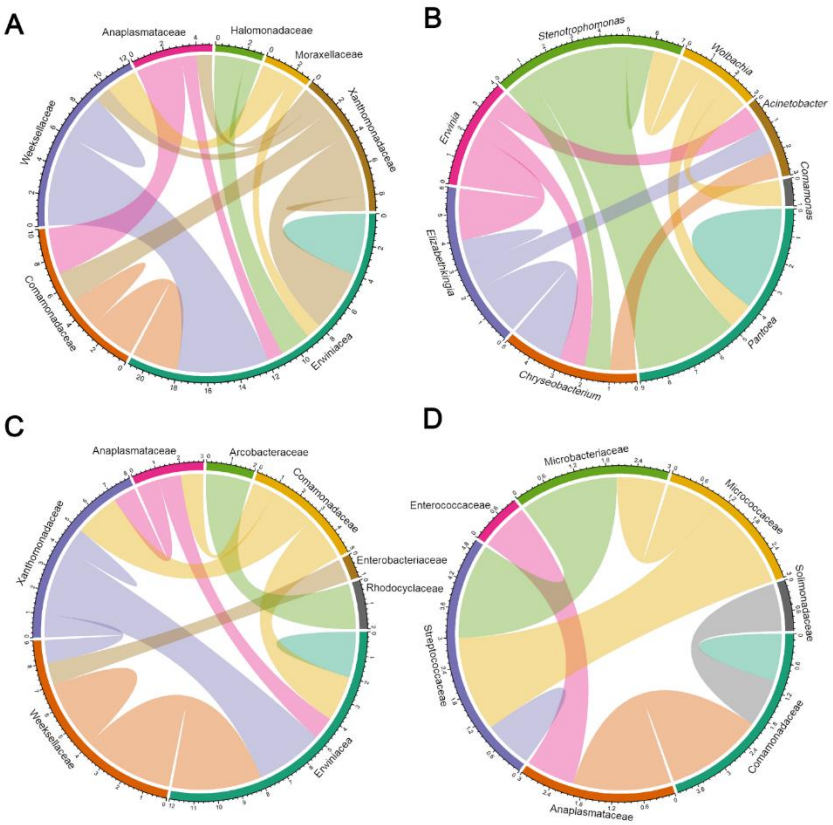

Figure 9. Co-occurrence network of bacterial ASVs in the populations. Co-occurrence network based on Spearman correlation (optimized Spearman correlation, $p < 0.05$). Co-occurrence network of bacterial ASVs at the family level (A). Co-occurrence network of bacterial ASVs at the genus level (B). Co-occurrence network of bacterial ASVs in mosquitoes from Seoul at the family level (C). Co-occurrence network of bacterial ASVs in mosquitoes from Asan at the family level (D).

Table 3. Read counts of eukaryote ASVs microbiome of three adult mosquito populations identified by metabarcoding of the V9 region of the 18S rRNA gene in South Korea

Population	Read count	Mean read count ± SD	Species	Positive individuals
Cx. pipiens (Seoul)	6	3 ± 1	*Crithidia fasciculata*	2
Cx. pipiens (Asan)	2	-	*Ganoderma* sp. (Fungi)	1
Cx. pipiens (Asan)	3	-	*Uncultured eukaryote*	1
Ae. albopictus (Asan)	1399	53.81 ± 59.81	*Ascogregarina taiwanensis*	26
Ae. albopictus (Asan)	2	-	*Anurofeca* sp.	1

Table 4. Linear discriminant analysis (LDA) effect size of enriched bacterial taxa from gregarine-negative adult *Ae. albopictus* mosquitoes in Asan

Feature	Group	LDA	*p*-value	Adjusted *p*-value
Chryseobacterium sp.	Not infected	4.336785	0.0253	0.0253
Rhodobacteraceae	Not infected	3.242327	0.0253	0.0253
Enhydrobacter sp.	Not infected	3.192106	0.0253	0.0253
Cloacibacterium sp.	Not infected	2.838307	0.0253	0.0253
Acetobacteraceae	Not infected	2.833764	0.0253	0.0253
Acinetobacter sp.	Not infected	2.788569	0.0253	0.0253
Roseomonas sp.	Not infected	2.481199	0.0253	0.0253

4. DISCUSION

This study revealed differences in the microbiome composition and diversity across three mosquito populations: Asan *Ae. Albopictus,* Asan *Cx. pipiens*, and Seoul *Cx. pipiens*. *Ae. albopictus* was found only in Asan, consistent with prior findings that report higher presence in peripheral areas with abundant abandoned artificial habitats such as tires and flower pots [12]. In contrast, *Cx. pipiens,* which exhibits greater habitat versatility and is commonly found in urban environments [19], was prevalent in Seoul. The Seoul location presented fewer of the artificial environments found in Asan, but more polluted environments such as street ponds, ditches and gutters. Microbial diversity was higher in Asan *Ae. albopictus* than in both *Cx. pipiens* populations. However, richness was not different between Asan *Ae. albopictus* and Seoul *Cx. pipien*s. This difference is likely due to the higher evenness observed in the *Ae. albopictus* microbiome, where microbial taxa were more evenly distributed than in Seoul *Cx. pipiens*. In contrast, the microbiome of *Cx. pipiens* from Seoul was completely dominated by *Wolbachia* (Anaplasmataceae), while *Aeromonas* (Aeromonadaceae) was notably abundant in *Cx. pipiens* from Asan.

These variations in the microbiome composition between *Cx. pipiens* populations in Seoul and Asan align with findings that microbial diversity in mosquitoes is not only species-specific but can also vary significantly with environmental factors unique to each collection site [21]. In this study, *Wolbachia* was found to be dominant in all populations, a frequent occurrence in *Cx. pipiens* and *Ae. albopictus* [1]. This genus is a maternally inherited obligate endosymbiont found in mosquitoes with the ability to promote or antagonize the dissemination of pathogens [8]. Furthermore, certain strains of this bacterium can cause cytoplasmic incompatibility, making it an important bacterium for vector control [8]. Recent studies in the south eastern regions of Korea also found *Ae. albopictus* and *Cx. pipiens* to be heavily colonised by *Wolbachia* [32,33].

Cx. pipiens from Seoul were associated with the families Spirochaetaceae, Erwiniaceae, Arcobacteraceae, and the genera *Comamonas* (Comamonadaceae), *Thiovirga* (Halothiobacillaceae) and *Acinetobacter* (Moraxellaceae). The species *Spironema culicis*, was also prevalent in the *Cx. pipiens* from the Seoul location. This species belongs to the family Spirochaetaceae, which was an enriched taxon in this population. Spirochaetaceae bacteria and have been found in previous studies along with other commensals, such as *the families Acetobacteraceae and Yersiniaceae, and the genera Acinetobacter, Xanthomonas (Xanthomonadaceae), Wolbachia, and Erwinia (Erwiniaceae)*

[34]. Although the genus *Erwinia* was not identified to be enriched by the LEfSe analysis, the family Erwiniaceae was enriched in the *Cx. pipiens* Seoul population. *This family* includes phytopathogens carried by insects that may elicit antimicrobial gene expression in dipterans [35]. *Acinetobacte*r was enriched in this population (n = 27) but was also detected in Asan *Cx. pipiens* (n = 1) and Asan *Ae. albopictus* (n = 4). *Acinetobacte*r has been associated with the reproductive organs of *Aedes* mosquitoes [36] and the salivary glands of *Anopheles* mosquitoes in malaria-endemic areas in Korea [37]. The last two enriched taxa from the Seoul *Cx. pipiens* population were the sulfur-oxidizing bacterium *Thiovirga* and the family Arcobacteraceae, that harbours human foodborne pathogens [38].

In contrast, *Cx. pipiens* from Asan were associated with a high prevalence of the genera *Aeromonas* and *Methylophilus* (Methylophilaceae), and the family Xanthobacteraceae. *Aeromonas* was one of the dominating taxa of *Cx. pipiens* in this location (n = 52). *Aeromonas* is a commensal bacterium found in the female *Aedes* spp. gut and increases susceptibility to DENV-2 [39]. This species was also reported from the guts of *Cx. pipiens* and *An. sinensis* in the south eastern regions of Korea [32]. This and other Gammaproteobacteria like the family Enterobacteriaceae, found in this study are known to populate mosquitoes in high abundance [5].

Asan *Ae. albopictus* presented *Enterococcus* (Enterococcaceae), *Limnobacter* (Rhodobacteraceae), *Nevskia* (Xanthomonadaceae), *Variovorax* (Comamonadaceae) and *Dysgomonas* (Dysgonomonadaceae). The genus *Enterococcus* (n = 23) and the family Comamonadaceae (n = 42) were the most representative of this population. *Enterococcus* is abundant in the midgut of *Ae. albopictus* and *Cx. pipiens* [4], while some species exhibit larvicidal toxicity [40]. Comamonadaceae populates mosquito larvae and adults [5]. Unresolved Acetobacteraceae were detected in several *Ae. albopictus* individuals. From this family, some subordinate genera were found in Asan, such as the insect symbionts *Acetobacter*, *Roseomonas*, and *Gluconobacter*. These genera usually populate the guts of the mosquitoes studied here, for example, *Roseomonas* is a commensal in *Aedes* [21], and *Gluconobacter* was found in *Cx. pipiens* and *Cx. restuans* [1]. Interestingly, in this location, the genus *Thorsellia* was found in high abundance in *Ae. albopictus* larvae but was absent adults. The application of this bacteria for the symbiotic control of mosquito-transmitted parasites has been proposed because environmental *Thorsellia* colonise the gut of numerous species and seems to be a consistent symbiont of *Culex* and *Anopheles* [41].

Previously, *Wolbachia* has been found to co-occur with *Comamonas*, *Acinetobacter*, and *Stenotrophomonas* in *Ae. albopictus* [42]. In the present study, *Stenotrophomonas* co-occurred with

Pantoea and *Chryseobacterium.* The genus *Erwinia* co-occurred with the ASVs of *Acinetobacter* and *Chryseobacterium*. Notably, the same profile was previously observed in *An. sinensis* in the hyperendemic malarial areas of Korea [37]. Additionally, commonly co-occurring genera like *Elizabethkingia* and *Chryseobacterium*, known to be involved in metabolizing sugars after nectar and blood feeding [5] were also present in this study. *Erwinia* has also been reported to co-occur with the genus *Elizabethkingia* [43]*, a pairing that was detected here*. Interestingly, *Erwinia can provide antimicrobial traits to the host* [43]*,* and *Elizabethkingia is a mosquito commensal that also elicits antimicrobial activity* [44]*. These antimicrobial properties are notable because Culex are potential vectors of* lymphatic filariasis that was endemic in Korea until recently [45]. *Both Elizabethkingia and Erwinia also co-occur with their closely related variants, something that can be overlooked in metagenomics by ASV clustering algorithms* [43]. *This suggests that both genera might serve as important commensals providing metabolic and antimicrobial contributions to the host that have the potential to be exploited.* Nonetheless, *Elizabethkingia is a known opportunistic pathogen known to produce hospital-related infections that are difficult to eliminate* [46].

Interestingly, other bacteria of interest for vector and mosquito-borne pathogen control such as *Asaia* and *Serratia* were not found in this study. Their absence in our analysis could potentially point to microbial exclusion by commensals like *Wolbachia*. *Serratia* colonisation is antagonized by certain commensals such as Enterobacteriaceae bacteria [47]. Simmilarly, *Wolbachia* and *Asaia* are known to exclude each other in the mosquito gut [6].

The ASV reads of *A. taiwaneneis* were present in 26 *Ae. albopictus* adults. To our knowledge the genus *Ascogregarina* is the only gregarine that infects mosquitoes and is a parasite of the genus *Aedes* [48]. Similar to other gregarine endoparasites, *A. taiwanensis* displays host specificity [48]. Neither adult, larva, nor pupae of *Culex* contained this ASV. The same ASV was observed for nine *Ae. albopictus* larvae and two pupae. The relationship between *A. taiwanensis* infection and the composition of the gut microbiome of *Ae*. *albopictus has been previously documented* [9]. In this study, uninfected mosquitoes were enriched with the genera *Chryseobacterium* (Flavobacteriaceae), *Enhydrobacter* (Moraxellaceae)*, Acinetobacter* (Moraxellaceae), *Cloacibacterium* (Weeksellaceae) *and Roseomonas* (Acetobacteraceae), and the families Rhodobacteraceae and Acetobacteraceae. *Chryseobacterium* is a commensal of *Aedes* and *Culex* mosquitoes [5] and was found to dominate the gut microbiome of several *Cx. pipiens* populations found in Korea [32]. *Chryseobacterium* decreases the susceptibility of colonisation by *Plasmodium* by modulating basal immunity [49].

Similarly, the genus *Acinetobacter* (Moraxellaceae) along with other taxa can inhibit *P. falciparum* development in *Anopheles* mosquitoes by stimulating the mosquito Imd signalling pathway, an innate immune system defence mechanism that protects against bacteria and malaria parasites [50]. The potential for exploitation of similar anti-microbial interactions in different mosquito species suggests a need to study similar mosquito parasite-microbiome interactions. Investigating these microbe-endoparasite-host interactions is important, as gregarine parasitism has well-documented adverse effects on the fitness and microbiome of mosquito hosts. These adverse effects include lengthened larval development and mortality, reduced fecundity and egg hatching, and poor competition [7]. Additionally, their host specificity and impact on host biology suggest that gregarine parasites could be viable candidates for biocontrol strategies. Specific bacterial or eukaryotic mosquito pathogens that influence the susceptibility of mosquitoes to commensals or human pathogens will have implications during the development of targeted strategies to manipulate the mosquito microbiome.

Overall, the description of vector microbiomes provides relevant information that can be used to understand host–microbe interactions and develop vector control strategies. The study of the microbiome associated with clinically important vectors helps mitigate the risk that mosquito-borne diseases may recur or extend to Korea because of the effects of climate change and vector movement [12]. In the future it may be useful to monitor the emergence of pathogens and the presence of desirable bacteria taxa for biocontrol reasons in vector populations [1]. However, monitoring of those microbiome-based biocontrol strategies will need to extend beyond the detection of specific microbes of interest to encompass a comprehensive understanding of their effects on the entire microbiome because these approaches might not focus solely on controlling mosquito populations but also on their susceptibility to harbor and transmit pathogens [3]. Metabarcoding offers the capability to simultaneously identify a broader range of microorganisms, providing an insight into the interactions within the microbiome and the overall impact of the biocontrol agents [4]. As sequencing technologies such as iSeq100 evolve and become more cost-effective, we anticipate metabarcoding will complement traditional qPCR assays, enabling a wider approach of microbiome surveillance. Furthermore, the use of technologies such as bead-bound, target-captured libraries as primers offers the potential to expand metagenomic surveillance to include resistome profiling (i.e., the composition of antibiotic resistance genes, or ARGs, in the microbiome) and viral monitoring through targeted metagenomic sequencing [51]. Mosquito viral metagenomic surveillance, for

example, can be used to predict the emergence of arboviruses and help prevent the spread of these pathogens across regions [52].

Future studies should take into account the relationships of the microbiome with biotic and abiotic factors of the habitat over a broader time period. Variations in the microbiota between individuals, populations, species and regions, can provide insights into the factors driving microbiome diversity [21]. Microbiome variations among populations may be influenced by abiotic factors from the habitat. Differing urbanization levels, climate, and available breeding sites, between the two locations may play a role in shaping the microbiomes of mosquito populations in these areas. In turn, individual differences in gut microbiota contribute to variations in vector competence [1]. As those differences are influenced by the environment, feeding patterns, and ecology, distinct populations may as well exhibit different responses to biocontrol strategies [37].

5. CONCLUSION

The present study expands the knowledge of the bacterial and eukaryotic communities associated with these two mosquito species in Korea. Similar studies will become necessary as microbial-based vector control strategies become implemented worldwide for the monitoring of desired bacterial taxa or emerging vector-borne pathogens.

REFERENCES

[1] Muturi EJ, Ramirez JL, Rooney AP, Kim C-H. Comparative analysis of gut microbiota of mosquito communities in central Illinois. PLOS Neglected Tropical Diseases. 2017;11(2):e0005377.

[2] Minard G, Mavingui P, Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. Parasites & Vectors. 2013;6(1):146.

[3] Ramirez JL, Souza-Neto J, Cosme RT, Rovira J, Ortiz A, Pascale JM, et al. Reciprocal Tripartite Interactions between the Aedes aegypti Midgut Microbiota, Innate Immune System and Dengue Virus Influences Vector Competence. PLOS Neglected Tropical Diseases. 2012;6(3):e1561.

[4] Kang X, Wang Y, Li S, Sun X, Lu X, Rajaofera MJN, et al. Comparative Analysis of the Gut Microbiota of Adult Mosquitoes From Eight Locations in Hainan, China. Front Cell Infect Microbiol. 2020;10:596750.

[5] Balaji S, Deepthi KNG, Prabagaran SR. Native Wolbachia influence bacterial composition in the major vector mosquito Aedes aegypti. Arch Microbiol. 2021;203(8):5225–5240.

[6] Flores GAM, Lopez RP, Cerrudo CS, Perotti MA, Consolo VF, Berón CM. Wolbachia dominance influences the Culex quinquefasciatus microbiota. Sci Rep. 2023;13(1):18980.

[7] Stump E, Childs LM, Walker M. Parasitism of Aedes albopictus by Ascogregarina taiwanensis lowers its competitive ability against Aedes triseriatus. Parasites & Vectors. 2021;14(1):79.

[8] Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, Rasgon JL. Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis. PLOS Neglected Tropical Diseases. 2014;8(7):e2965.

[9] Seabourn P, Spafford H, Yoneishi N, Medeiros M. The Aedes albopictus (Diptera: Culicidae) microbiome varies spatially and with Ascogregarine infection. PLOS Neglected Tropical Diseases. 2020;14(8):e0008615.

[10] Bahk YY, Lee H-W, Na B-K, Kim J, Jin K, Hong YS, et al. Epidemiological Characteristics of Re-emerging Vivax Malaria in the Republic of Korea (1993–2017). Korean J Parasitol. 2018;56(6):531–543.

[11] Cha GW, Cho JE, Lee EJ, Ju YR, Han MG, Park C, et al. Travel-Associated Chikungunya Cases in South Korea during 2009–2010. Osong Public Health and Research Perspectives. 2013;4(3):170–175.

[12] Lee SH, Nam KW, Jeong JY, Yoo SJ, Koh Y-S, Lee S, et al. The Effects of Climate Change and Globalization on Mosquito Vectors: Evidence from Jeju Island, South Korea on the Potential for Asian Tiger Mosquito (Aedes albopictus) Influxes and Survival from Vietnam Rather Than Japan. PLOS ONE. 2013;8(7):e68512.

[13] Brass DP, Cobbold CA, Purse BV, Ewing DA, Callaghan A, White SM. Role of vector phenotypic plasticity in disease transmission as illustrated by the spread of dengue virus by Aedes albopictus. Nat Commun. 2024;15(1):7823.

[14] Zhang Y, Guo X, Jiang S, Li C, Xing D, Zhang H, et al. The Potential Vector Competence and Overwintering of West Nile Virus in Vector Aedes Albopictus in China. Front Microbiol [Internet]. 2022 [cited 2024 Nov 6];13.

[15] Wispelaere M de, Després P, Choumet V. European Aedes albopictus and Culex pipiens Are Competent Vectors for Japanese Encephalitis Virus. PLOS Neglected Tropical Diseases. 2017;11(1):e0005294.

[16] Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the Tiger: Global Risk of Invasion by the Mosquito Aedes albopictus. Vector Borne Zoonotic Dis. 2007;7(1):76–85.

[17] Haba Y, McBride L. Origin and status of Culex pipiens mosquito ecotypes. Current Biology. 2022;32(5):R237–R246.

[18] Turell MJ, Mores CN, Dohm DJ, Lee W-J, Kim H-C, Klein TA. Laboratory Transmission of Japanese Encephalitis, West Nile, and Getah Viruses by Mosquitoes (Diptera: Culicidae) Collected near Camp Greaves, Gyeonggi Province, Republic of Korea, 2003. Journal of Medical Entomology. 2006;43(5):1076–1081.

[19] Kim H, Cha G-W, Jeong YE, Lee W-G, Chang KS, Roh JY, et al. Detection of Japanese Encephalitis Virus Genotype V in Culex orientalis and Culex pipiens (Diptera: Culicidae) in Korea. PLOS ONE. 2015;10(2):e0116547.

[20] Ryu J, Choi KS. Species diversity of the Culex pipiens complex in the Republic of Korea. Entomological Research. 2022;52(8):376–381.

[21] Tchouassi DP, Muturi EJ, Arum SO, Kim C-H, Fields CJ, Torto B. Host species and site of collection shape the microbiota of Rift Valley fever vectors in Kenya. PLOS Neglected Tropical Diseases. 2019;13(6):e0007361.

[22] Kim SL, Choi JH, Yi M, Lee S, Kim M, Oh S, et al. Metabarcoding of bacteria and parasites in the gut of Apodemus agrarius. Parasites & Vectors. 2022;15(1):486.

[23] Yi M-H, Choi JH, Kim M, Chavarria X, Yun S, Oh S, et al. Microbiome of lovebug (Plecia longiforceps) in Seoul, South Korea. Microbiol Spectr. 2024;12(7):e0380923.

[24] Bugenyi AW, Cho H-S, Heo J. Association between oropharyngeal microbiome and weight

gain in piglets during pre and post weaning life. J Anim Sci Technol. 2020;62(2):247–262.

[25] Lappan R, Classon C, Kumar S, Singh OP, Almeida RV de, Chakravarty J, et al. Meta-taxonomic analysis of prokaryotic and eukaryotic gut flora in stool samples from visceral leishmaniasis cases and endemic controls in Bihar State India. PLOS Neglected Tropical Diseases. 2019;13(9):e0007444.

[26] Arias-Giraldo LM, Muñoz M, Hernández C, Herrera G, Velásquez-Ortiz N, Cantillo-Barraza O, et al. Identification of blood-feeding sources in Panstrongylus, Psammolestes, Rhodnius and Triatoma using amplicon-based next-generation sequencing. Parasites & Vectors. 2020;13(1):434.

[27] Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 2011;17(1):10–12.

[28] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–583.

[29] Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018;6(1):90.

[30] Cao Y, Dong Q, Wang D, Zhang P, Liu Y, Niu C. microbiomeMarker: an R/Bioconductor package for microbiome marker identification and visualization. Bioinformatics. 2022;38(16):4027–4029.

[31] Liu C, Cui Y, Li X, Yao M. microeco: an R package for data mining in microbial community ecology. FEMS Microbiology Ecology. 2021;97(2):fiaa255.

[32] Akintola AA, Hwang UW. Microbiome profile of South Korean vector mosquitoes. Acta Tropica. 2024;255:107213.

[33] Akintola AA, Kim G, Adeniyi KA, Hwang UW. Prevalence of Wolbachia endosymbiont in mosquitoes from South Korea. Entomological Research. 2024;54(1):e12696.

[34] Ramos-Nino ME, Fitzpatrick DM, Eckstrom KM, Tighe S, Hattaway LM, Hsueh AN, et al. Metagenomic analysis of Aedes aegypti and Culex quinquefasciatus mosquitoes from Grenada, West Indies. PLOS ONE. 2020;15(4):e0231047.

[35] Basset A, Khush RS, Braun A, Gardan L, Boccard F, Hoffmann JA, et al. The phytopathogenic bacteria Erwinia carotovora infects Drosophila and activates an immune response. Proc Natl Acad Sci U S A. 2000;97(7):3376–3381.

[36] Gómez M, Martinez D, Muñoz M, Ramírez JD. Aedes aegypti and Ae. albopictus microbiome/virome: new strategies for controlling arboviral transmission? Parasites &

Vectors. 2022;15(1):287.

[37] Lee JH, Kim H-W, Mustafa B, Lee HI, Kwon HW. The relationships between microbiome diversity and epidemiology in domestic species of malaria-mediated mosquitoes of Korea. Sci Rep. 2023;13(1):9081.

[38] Buzzanca D, Kerkhof P-J, Alessandria V, Rantsiou K, Houf K. Arcobacteraceae comparative genome analysis demonstrates genome heterogeneity and reduction in species isolated from animals and associated with human illness. Heliyon. 2023;9(7):e17652.

[39] Apte-Deshpande A, Paingankar M, Gokhale MD, Deobagkar DN. Serratia odorifera a Midgut Inhabitant of Aedes aegypti Mosquito Enhances Its Susceptibility to Dengue-2 Virus. PLOS ONE. 2012;7(7):e40401.

[40] Colvin D, Dhuri V, Verma H, Lokhande R, Kale A. Enterococcus durans with mosquito larvicidal toxicity against Culex quinquefasciatus, elucidated using a Proteomic and Metabolomic approach. Sci Rep. 2020;10(1):4774.

[41] Duguma D, Rugman-Jones P, Kaufman MG, Hall MW, Neufeld JD, Stouthamer R, et al. Bacterial Communities Associated with Culex Mosquito Larvae and Two Emergent Aquatic Plants of Bioremediation Importance. PLOS ONE. 2013;8(8):e72522.

[42] Zouache K, Voronin D, Tran-Van V, Mousson L, Failloux A-B, Mavingui P. Persistent Wolbachia and Cultivable Bacteria Infection in the Reproductive and Somatic Tissues of the Mosquito Vector Aedes albopictus. PLOS ONE. 2009;4(7):e6388.

[43] Schrieke H, Maignien L, Constancias F, Trigodet F, Chakloute S, Rakotoarivony I, et al. The mosquito microbiome includes habitat-specific but rare symbionts. Computational and Structural Biotechnology Journal. 2022;20:410–420.

[44] Ngwa CJ, Glöckner V, Abdelmohsen UR, Scheuermayer M, Fischer R, Hentschel U, et al. 16S rRNA Gene-Based Identification of Elizabethkingia meningoseptica (Flavobacteriales: Flavobacteriaceae) as a Dominant Midgut Bacterium of the Asian Malaria Vector Anopheles stephensi (Dipteria: Culicidae) With Antimicrobial Activities. Journal of Medical Entomology. 2013;50(2):404–414.

[45] Samy AM, Elaagip AH, Kenawy MA, Ayres CFJ, Peterson AT, Soliman DE. Climate Change Influences on the Global Potential Distribution of the Mosquito Culex quinquefasciatus, Vector of West Nile Virus and Lymphatic Filariasis. PLOS ONE. 2016;11(10):e0163863.

[46] Choi MH, Kim M, Jeong SJ, Choi JY, Lee I-Y, Yong T-S, et al. Risk Factors for Elizabethkingia Acquisition and Clinical Characteristics of Patients, South Korea. Emerg Infect Dis. 2019;25(1):42–51.

[47] Kozlova EV, Hegde S, Roundy CM, Golovko G, Saldaña MA, Hart CE, et al. Microbial interactions in the mosquito gut determine Serratia colonization and blood-feeding

propensity. The ISME Journal. 2021;15(1):93–108.

[48] Martin E, Vallon L, Da Silva Carvalho C, Girard M, Minard G. Gregarine parasites are adapted to mosquito winter diapause. Parasites & Vectors. 2022;15(1):249.

[49] Dong Y, Manfredini F, Dimopoulos G. Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites. PLOS Pathogens. 2009;5(5):e1000423.

[50] Bahia AC, Dong Y, Blumberg BJ, Mlambo G, Tripathi A, BenMarzouk-Hidalgo OJ, et al. Exploring Anopheles gut bacteria for Plasmodium blocking activity. Environmental Microbiology. 2014;16(9):2980–2994.

[51] Shay JA, Haniford LSE, Cooper A, Carrillo CD, Blais BW, Lau CH-F. Exploiting a targeted resistome sequencing approach in assessing antimicrobial resistance in retail foods. Environmental Microbiome. 2023;18(1):25.

[52] Park KB, Patnaik HH, Kim T-Y, Jo YH, Kim N-Y, Yang S-C, et al. Current trends in large-scale viral surveillance methods in mosquitoes. Entomological Research. 2020;50(6):292–308.

ABSTRACT IN KOREAN

대한민국 도시 및 교외 지역의 *Culex pipiens* 및 *Aedes albopictus*의 미생물군 및 기생체군 감시

모기의 마이크로바이옴은 적합도와 병원체 전달 능력에 영향을 미친다. 향후 모기 생물학적 방제 전략은 이러한 상호작용에 대한 포괄적인 마이크로바이옴 감시가 요구된다. iSeq100 시퀀싱을 통해 16S 및 18S rRNA 유전자 메타바코딩을 수행하여, 대한민국 내 세 개 집단의 흰줄숲모기 (*Aedes albopictus*) 및 빨간집모기 (*Culex pipiens*)에 존재하는 원핵생물 및 진핵생물 군집을 분석하였다. *Ae. albopictus*와 *Cx. pipiens*는 대한민국 내에서도 의학적으로 중요한 세계적 분포의 모기 매개체이다. 박테리아의 알파 및 베타 다양성은 집단 간에 유의하게 차이를 보여, 미생물 군집 구성이 개체군에 따라 달라짐을 시사하였다. 두 종 모두에서 Pseudomonadota가 우점하였으며, *Cx. pipiens*에서는 *Wolbachia*와 *Aeromonas*가, *Ae. albopictus*에서는 *Enterococcus*가 주요 우점균으로 나타났다. *Ae. albopictus*에 흔히 기생하는 원생생물인 *Ascogregarina taiwanensis*는 해당 종에서 매우 높은 비율로 존재하였으나, *Cx. pipiens*에서는 검출되지 않았다. 또한, *A. taiwanensis*에 감염된 *Ae. albopictus*와 비감염 개체의 마이크로바이옴을 비교하기 위해 선형 판별 분석 효과 크기(LEfSe)를 적용한 결과, 감염이 없는 개체에서 7개의 박테리아 분류군과의 관련성이 나타났다.

핵심되는 말 :마이크로바이옴, 기생충, 메타바코딩, *Aedes albopictus*, *Culex pipiens*, *Ascogregarina Taiwanensis*

PUBLICATION LIST

1. **Chavarria X**, Choi JH, Oh S, Kim M, Kang D, Lee IY, Jang YS, Yi MH, Yong TS, Kim JY. Metabarcoding for the Monitoring of the Microbiome and Parasitome of Medically Important Mosquito Species in Two Urban and Semi-urban Areas of South Korea. *Current Microbiology*. 2025 Mar;82(3):102.