Strong selection of *Gammaproteobacteria* across *Aedes aegypti* development suggests diagonal microbiome transmission

Aboubakar Sanon[1, 2#], Lander De Coninck[3#], Lanjiao Wang[1], Athanase Badolo[2], Jelle Matthijnssens[3], Katrien Trappeniers[1] and Leen Delang[1*]

[1]KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium

[2]Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo, Burkina Faso

[3]KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Clinical and Epidemiological Virology, Leuven, Belgium

[#]These authors contributed equally to this work.

*Correspondence: Leen Delang, leen.delang@kuleuven.be

Abstract

Aedes aegypti mosquitoes are the main vector of pathogens like dengue virus and chikungunya virus. The immature life stages of mosquitoes share the same habitat with a variety of microorganisms in aquatic environments. To better understand the microbial diversity in field-derived *Ae. aegypti*, we analysed simultaneously collected larvae, pupae, and freshly emerged adults from Burkina Faso together with their breeding water via 16S rRNA gene sequencing. We observed a decrease in bacterial diversity and load across the mosquito life stages. At the phylum level, a strong increase in relative abundance of *Proteobacteria* was found along the mosquito stages. The same 40 amplicon sequence variants were consistently found as most abundant in the adults, regardless of the sample collection site, and all belonged to the *Gammaproteobacteria*. Our data suggests that these bacteria were not randomly derived by chance from the environment in the mosquito but rather deposited by a female mosquito during oviposition, a transmission route recently coined as "diagonal transmission". Indeed, our results indicated that there is a selection of *Gammaproteobacteria* from the breeding water and that these bacterial members are

further maintained from larvae to adult. This study provided new data on the microbiome composition of field-captured *Ae. aegypti*, contributing to an enhanced understanding of the origin and colonization route of the mosquito microbiome, potentially via a diagonal transmission route.

Importance

The past decades the world has experienced a vast increase in epidemics of pathogenic infections transmitted by vectors. These pathogens can cause severe diseases such as haemorrhagic fever, encephalitis, and arthritis. For instance, several arboviruses, such as chikungunya virus, Zika virus, and dengue virus, pose significant global health threats because of their high disease burden, their widespread (re-)emergence and the lack of (good) anti-arboviral strategies. Despite the clear burden, current vector control strategies are clearly insufficient. A promising strategy is the usage of microorganisms to tackle vector-borne transmission. However, a better fundamental understanding of their functioning in the vector is needed. Particularly, how microorganisms in the field develop inside the insect body and are transmitted between different life stages remains intensively debated. We addressed this knowledge gap by studying the mosquito microbiome in African *Aedes aegypti* mosquitoes at different sampling locations, ranging over different mosquito life stages, and including the environmental water.

Key words: *Aedes aegypti*, microbiome, life stages, microbiome origin, bacteria, 16S rRNA gene sequencing, Burkina Faso.

Introduction

Aedes aegypti mosquitoes are the main vector of pathogenic arboviruses like dengue virus, chikungunya virus, and Zika virus in tropical and subtropical areas (1). *Ae. aegypti* thrives mostly in urban environments, where the vector finds suitable conditions to survive and adapt to growing urbanization (2, 3). Humans are the most important host for this mosquito species (2). People in tropical and subtropical regions are the most affected by arbovirus infections compared to other, more temperate regions, although higher latitude areas are not exempt from these infections because of global travel and climate warming (4). Currently used vector control methods are often inefficient, exemplified by *Ae. aegypti* exhibiting a high

level of resistance to insecticides (5). As a result, *Ae. aegypti* is still expanding to new environments due to growing urbanization, deforestation, and global warming (6). New strategies targeting the vector are being explored to fight arbovirus-induced diseases.

The insect microbiota plays an important role in health and fitness, for instance by providing nutritional and defence benefits for the insect host (7–11). Particularly during mosquito development, resident bacterial organisms have an important nutritional role (12). Furthermore, the microbiota also affects vector competence, *i.e.* the intrinsic ability of an arthropod vector to transmit an infectious agent (13–15). This mosquito microbiota has become an interesting target to reduce both mosquito populations as well as vectorial capacity for important mosquito-borne diseases (16). Most notably is the potential of the intracellular *Wolbachia* bacterium to reduce vector competence by manipulating the mosquito reproduction through cytoplasmic incompatibility (14, 17) or through more direct mechanisms such as utilising the same resources (*i.e*. *Wolbachia* can sequester cholesterol from the mosquito environment that the virus also needs during replication) or activation of the mosquito host immunity (16, 18–22). In order to further exploit the mosquito microbiota as a new tool for vector control in the field, a better and more complete understanding of the microbial composition and colonisation process in mosquitoes is needed, particularly for field-caught mosquitoes.

Aedes mosquitoes are holometabolous insects, meaning they proceed to a complete metamorphosis and undergo a developmental process with four distinct stages: egg, larva, pupa, and adult. The egg hatches in aquatic habits, followed by subsequent developmental stages of different instar larval stages and a pupal stage, before emerging as adult. Immature mosquitoes (larvae and pupae) and adult mosquitoes are confined to aquatic and terrestrial habitats respectively. After mating, the female will bite a vertebrate host to acquire a blood meal containing essential amino acids needed for egg maturation (8). Each female mosquito lays multiple egg clutches during its life cycle and each clutch ranges from ten to hundreds of eggs (25). A crucial step in the mosquito life cycle is the deposition of eggs by the gravid female as it determines the fate of the progeny and thus oviposition behaviour is thought to be under strong selection pressure. Mosquito species are known to have a preference for specific breeding habits, depending on certain characteristics such as

sunlight exposition, salinity of the water, stream flow, organic matter, predators, etc. (26). *Ae. aegypti*, for instance, will oviposit in both natural and artificial containers, while some *Culex* species prefer to lay eggs in freshwater pools with high organic matter (26). Urban areas exhibit suitable habitats for *Aedes* mosquitoes as they have diverse sugar and available blood sources, less predators, and multiple water-holding containers needed for egg deposition and larval development (27, 28). Biotic and abiotic factors in the water can drive the selection of breeding sites by the mosquito female (29–32). The aquatic environments of larvae and pupae are often rich in organic matter acquired from the soil, vegetation, animal cadavers, etc. and suited to promote the growth of a diversity of microorganisms (33). Larvae obtain organic compounds from their aquatic habit using different feeding strategies including filtering, suspension feeding, or predation (33). Pupae on the other hand, are a non-feeding stage of the mosquito life cycle and do not ingest any food from their environment.

In the past years, more studies investigated the role of microorganisms colonizing the mosquito habitat. Interestingly, the choice of oviposition site by *Ae. aegypti* was shown to be influenced by the microbial communities in the water (34–36). *Aedes* females find a suitable site to lay their eggs, at least partially, using microbial emitted chemicals (37). However, the exact origin of how microbial communities colonize mosquitoes and which species are maintained across different life stages remains not completely understood. Mosquitoes, like other insects, can acquire their microbiota via vertical transmission, *i.e.* direct transmission from parent to offspring, or horizontal transmission, *i.e.* transfer of bacteria between individuals that are not in a parent-offspring relationship (38). Horizontal transmission often occurs through contact, feeding, mating, or shared environments. Larvae, for instance, acquire their resident microbes by feeding on environmental aquatic organic detritus, unicellular filamentous algae, bacteria, and other microorganisms (14, 39, 40). However, as adult mosquitoes deposit their eggs in aquatic environments, the distinction between horizontal and vertical transmission is not straightforward for mosquitoes. The concept of ‘diagonal transmission’ was proposed for viral transmission by Hamel *et al*. (41). They showed in their study the ability of West Nile virus to infect mosquito larvae through the adult excreta. A similar route could apply for the microbiome as well, where microorganisms of one parent are deposited to the environment and

transmitted to the offspring of the same or another parent. Diagonal transmission differs from vertical transmission as the microbiome of a parent can go to a different offspring, while vertical transmission comprises a direct line from parent to its offspring. Diagonal transmission might be a way for the gravid mosquito to prime the environment, *i.e.* the breeding water, with her own beneficial bacteria. Studies on the microbiome composition in different life stages of *Ae. aegypti* and their breeding sites can therefore provide insights on the origin of the mosquito microbiota and its influence on *Ae. aegypti* fitness and development. Only a limited number of studies have reported on transstadial microbiome analyses for *Ae. aegypti,* including one report studying lab mosquitoes. Most reports on field mosquitoes do not include all mosquito life stages (for instance only water and larvae) (42–46).

In this study, we described (i) the variation of microbial communities of field-collected *Ae. aegypti* mosquitoes from Burkina Faso at different developmental stages (larvae, pupae, adult) and of the breeding water and (ii) determined whether breeding container (in which the mosquitoes breed; plastic or tire) or sampling sites (geographical location; urban or peri-urban) can influence bacterial communities of water and mosquito samples.

Methodology

Mosquito collection

In total, 48 individual samples (12 *Aedes aegypti* individuals from each life stage and 12 water samples) were collected in Ouagadougou, Burkina Faso during the rainy season of 2022 (**Figure 1A**). In brief, samples were collected from two sampling sites (urban and peri-urban) and from two types of breeding material (plastic containers and tires) in each sampling site (**Figure 1B and C**). For each breeding material, mosquito samples from different life stages were collected (larvae, pupae, and freshly emerged adults) as well as a sample of the water present in the breeding material (i.e. three biological replicates per condition). The two sampling sites were located approximately 10 km from each other. Water, larvae, and pupae were taken with a Pasteur pipette from the original water of the breeding site and transferred into Eppendorf tubes. At the same time or the next day, newly emerging adults from collected pupae were aspirated in the lab's insectary with a mouth aspirator, killed by

a cold shock, and stored at -80°C. All samples were shipped on dry ice to Belgium for DNA extraction and 16S rRNA gene sequencing.

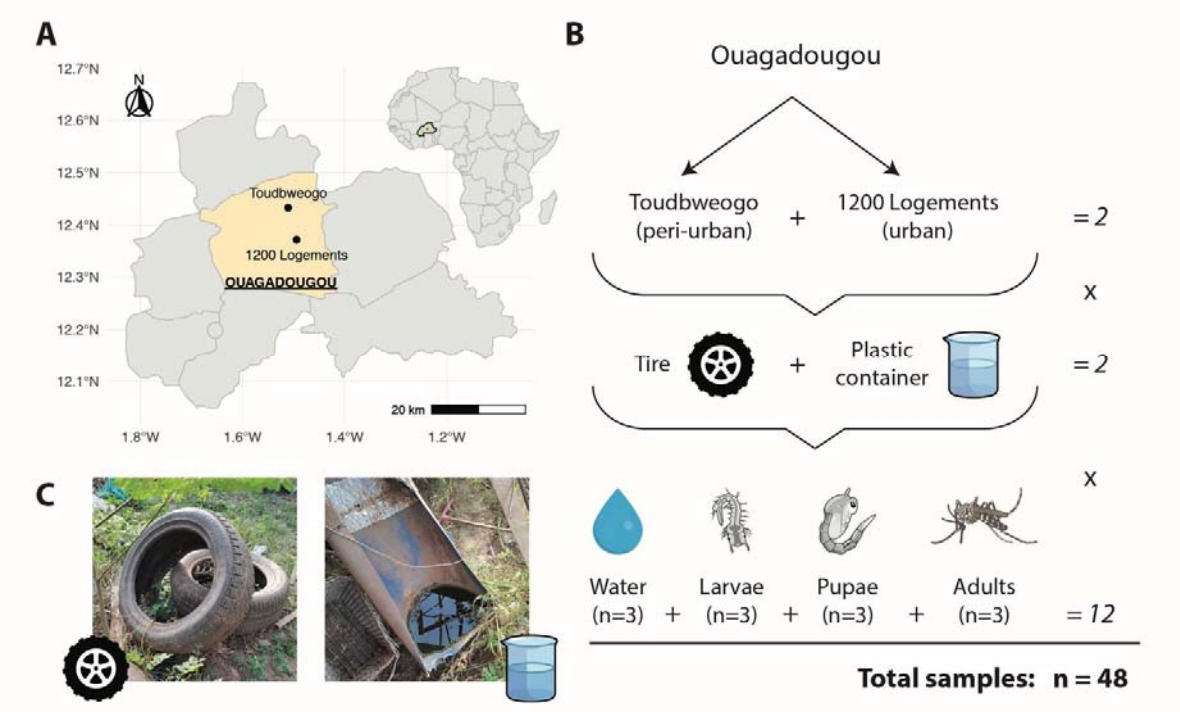


***Figure 1**: **(A)** Map of the Kadiogo region showing the Ouagadougou district in yellow. The two collection sites are indicated with black dots. The inset map shows the African continent with Burkina Faso coloured green and the Kadiogo region red. **(B)** Overview of the sampling collection. Samples were collected from two sampling sites (urban and peri-urban) and from two types of breeding material (plastic containers and tires) in each sampling site. For each breeding material, mosquito samples from different life stages were collected (larvae, pupae, and freshly emerged adults) as well as a sample of the water present in the breeding material, all in triplicate. A total of 48 samples were collected. **(C)** Representative images of the tire and plastic container breeding materials.*

DNA extraction

DNA was extracted from the mosquito samples under a laminar flow in sterile conditions. Larvae, pupae, and adults were rinsed in PBS for 3 minutes, then in 70% ethanol, and finally in sterile water to sterilize their surface and remove any bacteria coming from the external environment. Individual samples, whole larvae, pupae, and female adults (whole bodies) were suspended in tubes with ceramic beads (Precellys) containing 150 µL of PBS, homogenized for 1 min, and centrifuged (12000 rpm for 4 min). The homogenate was treated with lysozyme and proteinase K and incubated at 56°C for 5 min to completely lyse the tissue. Then, the homogenate was transferred in an Eppendorf tube and DNA was extracted using the DNeasy 96 Blood & Tissue DNA extraction kit (Qiagen). An aliquot of the water samples was taken from the original breeding site after pipetting up and down to mix the water

sample and 500 µL of this debris was put into Eppendorf tubes and centrifuged at 5000 g for 30 minutes to pellet microbes in the water. The supernatant was discarded, and the resuspended pellet was used for DNA isolation following the same protocol for mosquito DNA extraction as described above. The final DNA was eluted in 100 µL of sterile elution buffer (100 mM Tris-HCl) and quantified using Nanodrop. The samples were shipped on dry ice to Macrogen Netherlands for 16S rRNA gene sequencing. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was PCR amplified, and the sequencing was carried out on the Illumina MiSeq system with a 300bp paired-end run. Furthermore, 6 negative controls (PBS) which were processed together with the 36 mosquito and 12 water samples following the same DNA extraction protocol were sequenced as blank controls.

16S rRNA gene sequencing data processing and statistical analysis

We used the DADA2 pipeline v1.16 with default parameters specified by the authors to identify amplicon sequence variants (ASVs) (47), assigned each ASV a taxonomic identity using DECIPHER's IDTAXA (48) with the SILVA SSU r138 training data set, and generated count data for each ASV. The code used for sequence processing, bioinformatics, statistical analyses, and the data can be found at https://github.com/absanon/2024_XXX_MICROBIOME_LIFE_STAGE. We imported the ASV data from DADA2 into a phyloseq object for bacterial community analyses and visualization in R. As an additional quality control step, we used the decontam R package (49) with the "prevalence" method and a stringent probability threshold of 0.5 to identify and remove ASVs as contamination. Alpha diversity metrics like the Shannon diversity index and the Simpson index were assessed with the "vegan" package (50) by performing a rarefaction analysis (51) on the original abundance table (1000 iterations sampled to a minimum sequencing depth of 47,006 reads). We used the Wilcoxon test with the Benjamini-Hochberg FDR correction for multiple comparisons to test for differences in the mean alpha diversity between sample types (water *vs* larvae *vs* pupae *vs* adults). Bray-Curtis dissimilarities between samples were calculated using vegan's avgdist function, again sampling the original abundance table to 47,006 reads per sample for 1000 iterations. A principal coordinate analysis (PCoA) was performed on the Bray-Curtis dissimilarities with the "ape" package (52). To identify the impact of the different metadata variables (mosquito life stage, location and breeding material) on the bacterial community

composition of the samples, we used a distance-based redundancy analysis (dbRDA) with functions adapted from https://github.com/raeslab/RLdbRDA.

Analysis of public 16S data from field *Aedes aegypti*

To confirm the alpha diversity and bacterial composition results, we searched through the literature for other studies that did 16S sequencing on field-collected *Aedes aegypti* samples with at least two different life stages. We identified four other studies that had a somewhat similar methodology compared to our study (see **Table 1**) and after downloading the public data, we performed the same data analysis as on our samples to enable comparisons between the datasets (see above).

Table 1: Overview of studies with public 16S data on field Aedes aegypti samples across multiple life stages.

Study	Journal	16S regions	Country	Life stages	BioProject
Bennett et al. (2019)	Scientific reports	V4	Panama	Larvae, adults	PRJNA523634
Hery et al. (2021)	Microbial Ecology	V6-V8	Guadeloupe/French Guiana	Water, larvae	PRJNA600474
Rodpai et al. (2023)	Insects	V3-V4	Thailand	Water, larvae, adults	PRJNA919511
Hernández et al. (2024)	PLoS One	V3-V4	Mexico	Eggs, larvae, adults	PRJNA836318

Absolute quantification of 16S copies with qPCR

A qPCR was performed to obtain the absolute number of 16S copies in each sample. Total bacterial DNA load was quantified using quantitative PCR (qPCR) by amplification of the bacterial 16S rRNA gene. A total reaction volume of 20 µL, containing 5 µL sample and 15 µL master mix, was used. Per reaction, the master mix consisted of 10 µL SYBR green reaction mix, 0.4 µL 10 µM FP (IDT), 0.4 µL 10 µM RP (IDT), and 4.2 µL nuclease-free water. Following 16S primer sequences were used: fwd primer 5'-TCCTACGGGAGGCAGCAGT-'3 and rev primer 5'-GGACTACCAGGGTATCTAATCCTGTT-'3. Reactions were performed in the QuantStudio 5 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). The amplification conditions were 1 cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 15 sec at 55°C. To quantify the bacterial genome copies in each sample, a 1:10 standard curve was made using a 16S DNA gene block (IDT).

Results

Bacterial richness and composition are determined by mosquito life stage

In total, 48 samples were collected in the capital of Burkina Faso consisting of 12 water samples and 36 mosquito samples. Different mosquito life stages were collected (larvae/pupae/newly emerged adult mosquitoes) for two sampling sites (urban/peri-urban), and two types of breeding material (tire/plastic container), all in triplicate. The DNA of the mosquito samples (larvae, pupae, and whole body for the adults) as well as the water samples was extracted and analysed using 16S rRNA gene sequencing. On average, 114,143 sequencing reads were generated for each sample with a minimum of 84,548 and a maximum of 147,901 reads. After removing chimeras and filtering out contamination, a total of 24,141 ASVs were identified across the 48 samples with the DADA2 pipeline.

To evaluate the bacterial community diversity, the alpha diversity was calculated based on the obtained ASVs and compared between the mosquito and water samples. A decreasing bacterial alpha diversity was observed from the water samples over the larvae and pupae towards the adult mosquito samples. Although the alpha diversity between the water and larval stages was not significantly different, a statistically significant decrease was found in the pupal and adult stages ($P<0.05$; **Figure 2A**). Overall, the bacterial community diversity decreased according to the mosquito developmental stages. These results were mostly confirmed by the public data analysis (see **Supplemental Figure 1**), only in the Rodpai *et al.* dataset (46) there was no clear decrease in alpha diversity across the different life stages likely due to the low number of replicates. On the other hand, all other datasets showed a decrease of alpha diversity across the available life stages and these were significant for both the Hery and Hernández studies (44, 45) (see **Supplemental Figure 1**).

To study the impact of the mosquito life stages (larvae, pupae, adults), the sampling location (urban, peri-urban), and the type of breeding material (tire, plastic) on the microbiome composition, a principal coordinate analysis (PCoA) was performed. Again, the PCoA showed that the bacterial communities of the water samples as well as the early mosquito life stages were highly diverse, whereas the microbial composition of the freshly emerged adult mosquitoes was very similar and

irrespective of the sampling site or breeding material (**Figure 2B**). In addition, the water and immature stages of plastic containers from the urban site displayed a large variation among the samples (**Figure 2B**). The impact of the life stages, the type of breeding material, and the sampling site on the microbial communities was investigated using a distance-based redundancy statistical analysis (dbRDA) showing the influence of these three factors on the microbiome composition. Mosquito life stage explained the majority (24%) of the microbial variation while sampling location and the type of breeding material explained respectively 8% and 5% of the microbial variation (**Figure 2C**). In the multivariate analysis, only the mosquito life stage and the location were explanatory with respect to the microbiome composition, without additional information from the breeding material. Next, we further analysed the effect of the sampling site and the type of breeding material on the microbial variation within each mosquito life stages. The redundancy analysis identified that in the early life stages, location and breeding material explained a large amount of the bacterial variation with respectively 18% and 15% in larvae. While the breeding material still explained 18% of the variation in pupae, neither location nor the breeding material showed a major contributing role on the bacterial composition in adults (**Figure 2D**).

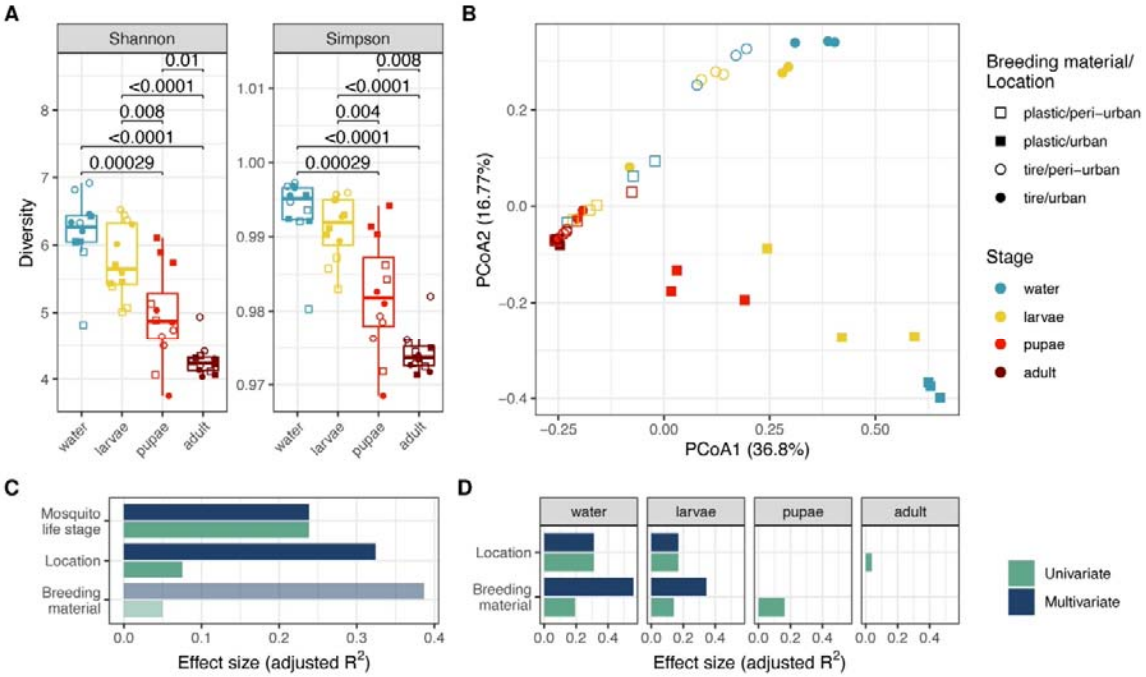


Figure 2: (A) *Alpha diversity measures (Shannon and Simpson) between the water samples and the different mosquito developmental stages. The number on top of the boxplots represents the respective adjusted p-value of the comparisons between life stages. Wilcoxon tests with Benjamini-Hochberg*

FDR correction were used to compare the diversity of the bacteria between the water community and the different mosquito developmental stages. ***(B)*** *PCoA plot showing the clustering in bacterial communities of mosquito developmental stages, sampling site, and breeding material.* ***(C)*** *The effect size of developmental stage, location and breeding material on the bacterial composition calculated using dbRDA. Variables are ordered according to effect size of significant covariates selected by redundancy analysis (navy bars) compared with individual effect sizes assuming independence (green bars). Light coloured bars indicate a non-significant multivariate effect of that variable.* ***(D)*** *Effect size of location and breeding material on the variance in the developmental stage of mosquito microbiota composition. The variables were given a fixed order in this plot with location preceding breeding material. No bars indicate a non-significant effect of the variable on an independent test, making the multivariate test unnecessary.*

Gammaproteobacteria dominate the *Aedes aegypti* microbiome

Next, the bacterial composition was investigated at the family level for the mosquito life stages, types of breeding material, and sampling sites (**Figure 3**). For this, only bacterial phyla with a relative abundance of >1% are shown, otherwise they were grouped in 'Others' together with unclassified ASVs. In addition, only bacterial families with an abundance higher than 5% in at least one sample, or at least the most abundant family per phylum, are shown. Remaining bacterial families were pooled together based on their phylum. In total, we found 9 phyla with a relative abundance higher than 1%, which included 33 bacterial families (**Figure 3**). The water samples showed a large variability of different bacterial phyla with the *Proteobacteria*, *Actinobacteriota*, *Bacteriodota,* and *Firmicutes* as most abundant phyla (**Figure 3**). Across the life stages, the abundance of the *Actinobacteriota*, *Bacteriodota,* and *Firmicutes* decreased gradually along the developmental axis. Eventually, the *Proteobacteria*, more specifically the *Gammaproteobacteria* class, became dominant in all adult mosquitoes, occupying 80% to 90% of the relative abundance in each adult sample, regardless of their sampling location or breeding material (**Figure 3** and **Supplemental Figure 2**).

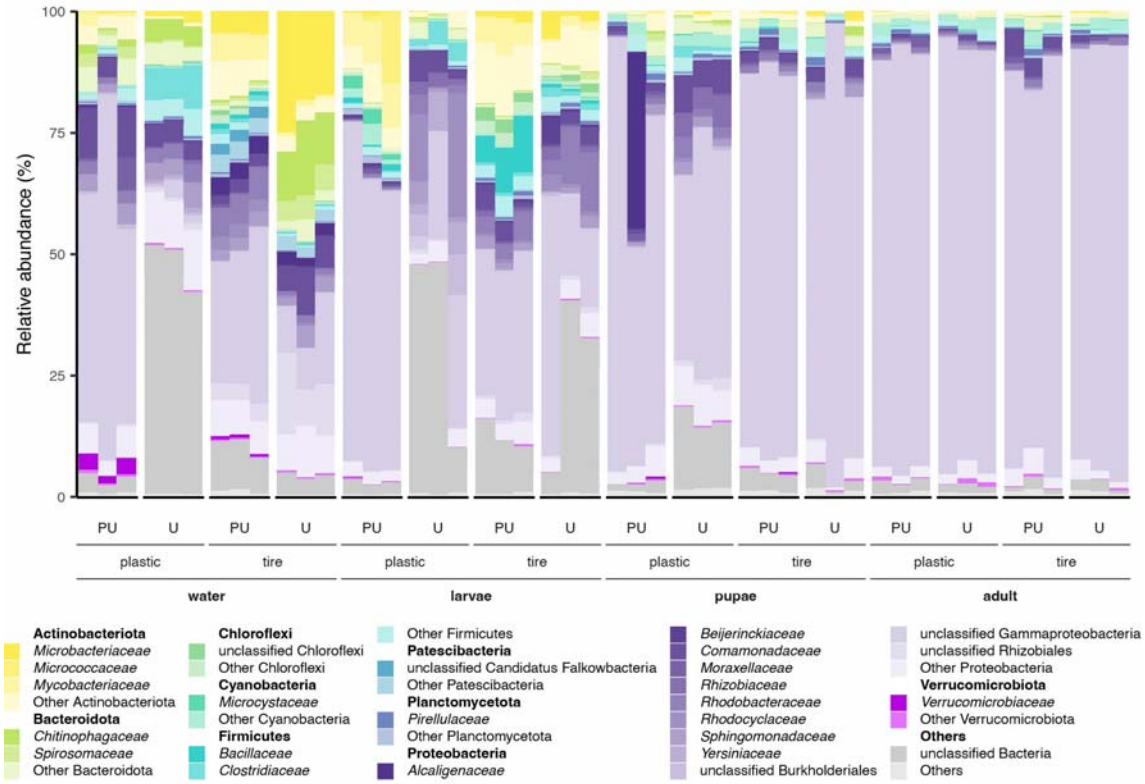


***Figure 3**: Relative abundance of identified bacterial families shown with different shades of the same colour per phylum. Only phyla with a relative abundance >1% are shown, and within each phylum only families with a relative abundance >5% in at least one sample are shown individually. Unclassified bacteria and phyla with a relative abundance <1% are shown as 'Others'. Samples are grouped by biological replicates. U: urban, PU: peri-urban.*

Convergence to low divergent gut microbial composition in adult mosquitoes, irrespective of sampling site and breeding material

Interestingly, from the wide diversity of ASVs in the water, consistently the same set of 40 ASVs were found to be most abundant in the adult mosquitoes, regardless of the sampling site or breeding material (**Figure 4A** and **Supplemental Figure 3**). Next, we zoomed-in on the top 15 most abundant bacterial ASVs per mosquito life stage, sampling site, and breeding material (**Figure 4B and 4C**). In contrast to larvae, pupae are no longer filter feeding from the water, and hence no new environmental bacteria are introduced. Our data suggest that a small set of bacterial ASVs is further selected inside the mosquito during the later stages of the mosquito development. As a consequence, many bacterial ASVs abundant in the water and larval stages completely disappeared in the pupae and adult stages, explaining the decrease in microbial diversity with the subsequent life stage and the increase in relative abundance of the same set of 15 ASVs in later life stages, which represent approximately 60% of the bacterial load in the adult samples (**Figure 4C**).

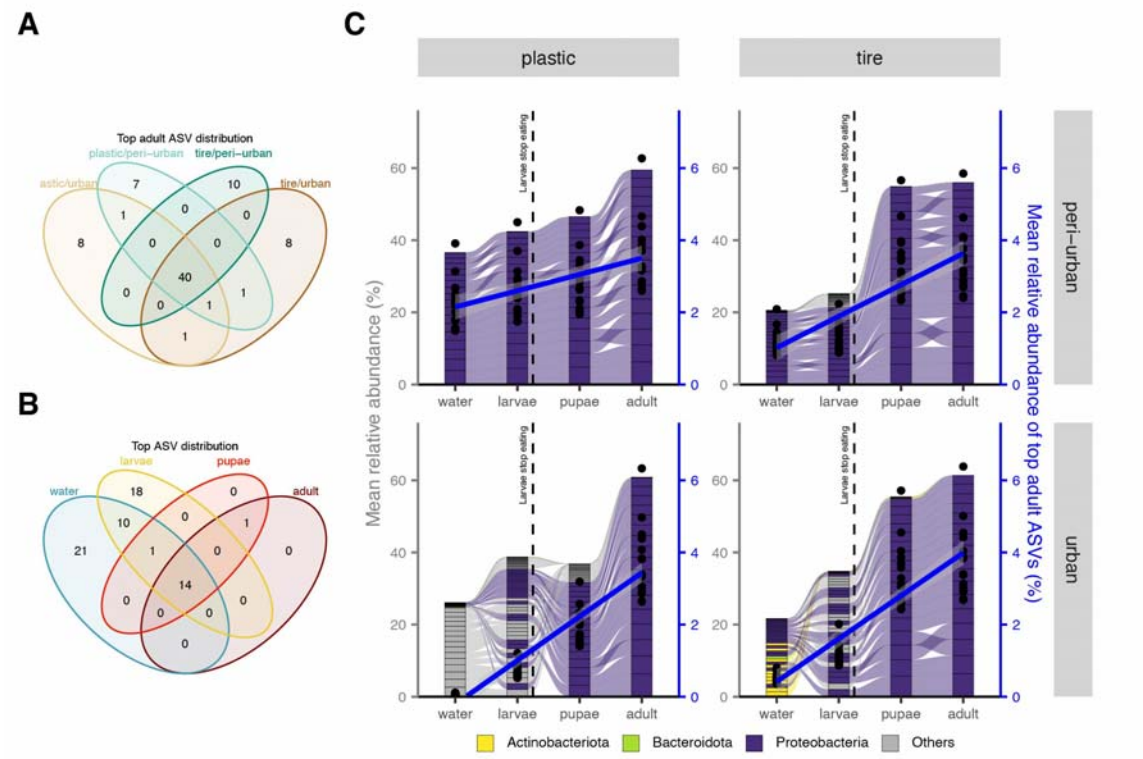


***Figure 4**: **(A)** Venn diagram displaying the distribution of the top 50 ASVs in the adults across the different locations and breeding materials showing that adult mosquitoes share the same most abundant 40 ASVs regardless of their collection site. **(B)** Venn diagram showing the sharing of the top 15 ASVs in the different developmental stages. **(C)** Alluvial plot showing the changes of the mean relative abundance of the top 15 ASVs across developmental stage (x-axis), location (y-axis split) and breeding material (x-axis split). For each location, breeding material and developmental stage the 15 most abundant ASVs were selected, and all are shown in each panel of the plot. The second y-axis (blue) indicates the mean relative abundance of the 15 top ASVs in the adult mosquitoes with a linear regression (blue line and black dots) showing the increase of their relative abundance across mosquito developmental stages.*

The increase in relative abundance of these ASVs can be explained by either an overgrowth of these specific bacteria (most likely resulting in a stable or increasing bacterial load) or otherwise by a die-off of the other bacterial ASVs (most likely resulting in a decreasing bacterial load), with both resulting in an increase of the relative abundance of the remaining ASVs. To further investigate this, we performed a 16S qPCR analysis in the same DNA samples that were used for the 16S rRNA gene sequencing, allowing to evaluate the bacterial load and combine this with the relative 16S data. These results showed that the bacterial load decreased along the different developmental stages with the adult mosquitoes having the lowest total number of bacteria (**Figure 5A** and **Supplemental Figure 4**), corroborating with the decreasing alpha diversity pattern shown in **Figure 2A**. In fact, the absolute copy number of the 15 most abundant ASVs, obtained by combining the qPCR and NGS

data, in the adults is even higher in the water and larval stages (**Figure 5B**), suggesting that the developing mosquito body is a challenging environment for bacteria to thrive and only a few bacteria can survive here.

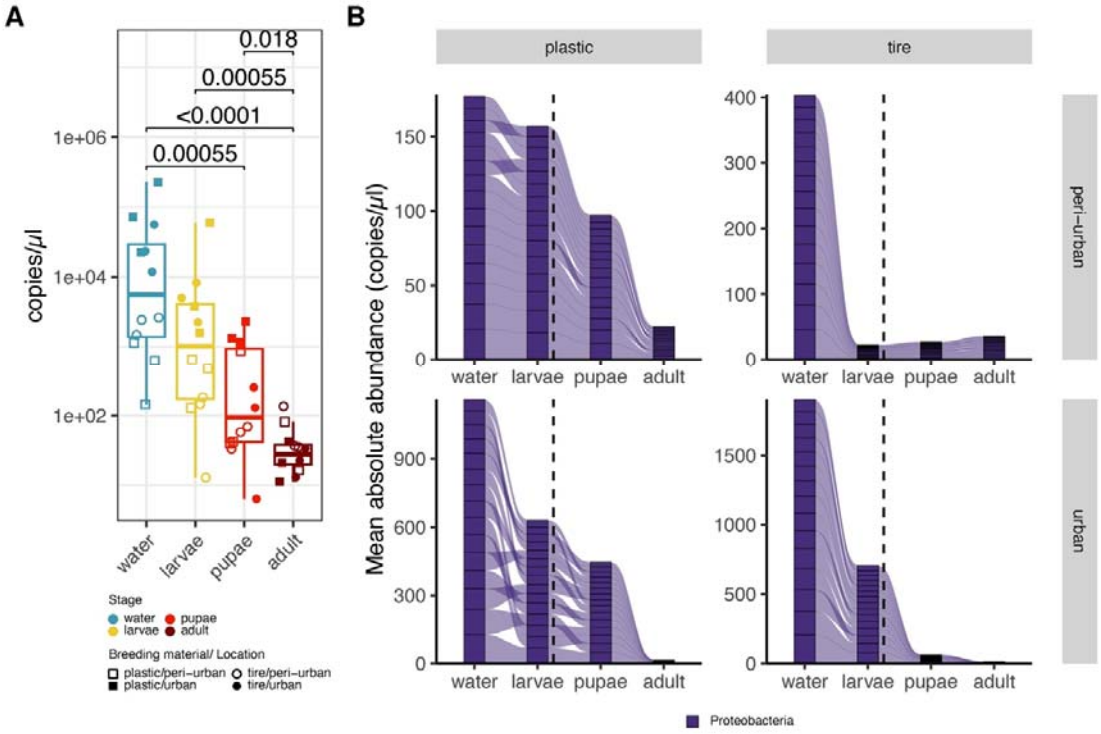


Figure 5: (A) *Box plots showing the 16S copies/µl per sample derived from a SYBR qPCR with universal 16S primers. The number on top of the boxplots represents the respective adjusted p-value of the comparisons between life stages from Wilcoxon tests with a Benjamini-Hochberg FDR correction.* ***(B)*** *Alluvial plot showing the mean absolute abundance of the 15 most abundant ASVs in the adult mosquitoes across the developmental stages, location and breeding material. This was calculated by multiplying the 16S copies/µl from the qPCR with the relative abundance of the NGS data. The dotted line specifies the transition from larvae to pupae indicating the time when environmental bacteria can no longer enter the larvae.*

Discussion

This study described the bacterial communities of *Aedes aegypti* from the capital city of Burkina Faso according to mosquito life stages, breeding material, and sampling sites from urban and peri-urban locations. In general, we observed a high diversity and bacterial load in water samples compared to the different mosquito developmental stages. Bacterial diversity and load were still similar between the water and the larvae, reflecting the abundant inflow of environmental bacteria during the filter feeding activity of the larvae. However, once the pupae stage is reached, this filter feedings stop and a strong selection towards a small number of bacterial

ASVs was observed in the adult stage. This observation can be explained by the process of metamorphosis. As larvae shed their epithelial gut lining with each moulting and at pupation excrete their gut contents, this will lead to a decrease in microbial diversity and abundance (38, 53).

Our data showed significantly distinct bacterial compositions between pupae and adults, both with respect to diversity (**Figure 2A**) and bacterial load (**Figure 5A**), which is in line with previous observations (29, 32), but in contrast to the study of Bennet and colleagues (42), which described few differences between bacterial communities of larvae and adult *Aedes* species despite distinct habitat features like geographical location, type of breeding material, and associated environmental variables of the water. We also showed that the sampling site (urban or peri-urban) or the breeding material (plastic containers or tires) had an impact on the bacterial composition of the water and the larvae, but not on the pupae and the adult mosquitoes (**Figure 2D**). Of interest was the observation that the water, larvae and pupae samples collected in the urban area in the plastic containers showed a greater number of (unclassified) ASVs compared to other collection sites and the adult stages. This might be explained by a greater contamination of this environment due to human activities.

As shown in **Figure 4C**, the most abundant ASVs observed in adult mosquitoes were already present in the breeding water. This suggests that mosquitoes acquire their microbiota from their breeding water, which is in line with previous findings (30, 32, 54). The question remains whether the breeding site shapes the bacterial community in the mosquito or, the other way around, whether female mosquitoes inoculate the breeding sites with relevant bacteria (eg. by excreting them from the gut) before, during or after deposition of their eggs in the aquatic environment as shown by Mosquera *et al.* (55). The fact that across the different locations and the different breeding materials we consistently find the same most abundant ASVs in the adults, strongly suggests the latter. Particularly as ASV classification is very specific (a single nucleotide difference makes it a different ASV), the chance of randomly finding the same set of bacteria from so many different environmental sources should be extremely rare. This means that these bacteria were rather derived from a mosquito female and deposited in the breeding water from which the larvae acquired them via filter feeding. Indeed, our data seem to indicate that diagonal transmission applies

for the mosquito microbiome, although further experimental studies are required to support these observations.

We found that the water of the breeding sites and the *Aedes aegypti* microbiome were dominated by *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, and *Firmicutes.* These phyla include many bacteria generally found in aquatic and soil environments. Other bacteria were present in the samples, but in negligible relative abundances (**Figure 3**). Interestingly, the abundance of the *Actinobacteriota*, *Bacteroidota,* and *Firmicutes* decreased in the later developmental stages while the *Proteobacteria* became completely dominant in the adult samples. This result is in line with previous studies reporting that the microbiome of adult *Ae. aegypti* from other countries is also dominated by *Proteobacteria* (42, 44–46). With this high degree of agreement across these different studies, we hypothesize that the *Proteobacteria* play an important role in the development of the mosquito. This seems to be supported by the fact that the 15 most abundant ASVs of the newly emerged adults analysed in our study all belonged to the *Proteobacteria* (**Figure 4C**), reflecting a consistent profile at adulthood. Moreover, also the absolute abundances of the top 15 ASVs were quite similar across all the adults (**Figure 5A**).

Proteobacteria is a major phylum of gram-negative bacteria. In our study, we identified *Gammaproteobacteria* as the most abundant class in adult mosquitoes within the *Proteobacteria*. Although further distinction into different bacterial species is not possible with our 16S V3-V4 dataset, the retention of *Proteobacteria* as 15 most abundant ASVs in the newly emerged adults across all the different conditions can indicate a selection of these bacteria in the mosquito. On one hand, these bacteria could be more adapted to grow in the ‘hostile’ mosquito gut environment and are therefore more likely to survive after passive selection from the water during larval feeding. In *Anopheles stephensi* mosquitoes, the Malpighian tubules, excretory and osmoregulatory organs that stay intact during metamorphosis, function as a hide-out for *Pseudomonas* bacteria from the continuous regeneration of the gut epithelial lining during mosquito development (56). A similar mechanism could possibly explain the trans-stadial selection of these 15 most abundant ASVs and other *Proteobacteria* in our study. On the other hand, *Proteobacteria* present in the breeding water could be emitting chemical cues which are picked up by *Aedes* females to deposit their eggs at that site. In both cases, the acquired *Proteobacteria* can have an impact on

the development and/or later life adult life stages of the mosquito. For instance, *Proteobacteria* could provide the mosquito with a developmental advantage by providing essential nutrients for the developing mosquito. This is further exemplified by restoring developmental issues of germ-free *Ae. aegypti* after recolonization with *E. coli*, a *Gammaproteobacteria* member (57). Also in other mosquito genera, *i.e. Anopheles*, the *Gammaproteobacteria* (*i.e. Pseudomonas* and *Enterobacter*) were identified as dominant and important members of the mosquito microbiome (53, 56). Furthermore, *Serratia* and *Enterobacter* were demonstrated to aid in blood digestion via hemolysin production in *Aedes* mosquitoes (33). These examples support the hypothesis that female mosquitoes deposit their beneficial *Gammaproteobacteria* in the breeding water to facilitate the immature life stages of the offspring mosquito by providing them with developmental benefits.

Although the samples in all examined public studies were also dominated by *Proteobacteria* (see **Supplemental Figures 5-8**), we did not observe a convergence towards a small set of ASVs in the adult samples of these public datasets. This can be explained by the sampling methods used in the analysed datasets. These were not focused on sampling each life stage from exactly the same habitat (*i.e.* same breeding container on the same date) as was done in our study. In addition, studies with more than two life stages had rather limited samples (44, 46) and the Hery study (45) did not contain any adult samples, while also not having any water and larvae samples from the same source. This highlights the unique advantage of our sampling procedure (**Figure 1**) and the necessity to confirm our results in future studies with similar sampling methods that can track the abundance of ASVs across the maturation of a mosquito.

On the other hand, limitations of our study are the low sample size per condition (three replicates) and the limited sampling locations (two locations) which may not be representative of the totality of the microbiome of *Aedes aegypti* from Burkina Faso. Additional sampling over a wider geographical distribution and over multiple timepoints (more seasons, more years) might allow the evaluation of the consistency of the microbiome in *Aedes* mosquitoes. Furthermore, we did not include mosquito eggs and only included newly emerged adults which does not allow to see what the effect of nectar and blood feeding is on the composition of the microbiome in adult mosquitoes. Finally, we performed 16S sequencing on the V3-V4 regions which limits

the bacterial identification to genus level at best and thus could not give us more information on which bacterial species or strains are present.

Conclusion

This study profiled the bacterial communities over different mosquito life stages in field *Ae. aegypti* females from Burkina Faso. We demonstrated a decrease in both the bacterial diversity and the bacterial load over the different developmental stages, with the highest diversity and load observed in the breeding water and larvae samples whereas adult mosquitoes possessed the lowest diversity and load. Interestingly, a strong selection of *Proteobacteria* (more specific *Gammaproteobacteria*) in the adult females was identified. The presence of *Proteobacteria* was demonstrated to be consistent over all adult samples and was irrespective of environmental variables including breeding containers (tire or plastic container) and geographical sampling site (urban or peri-urban site), suggesting a potential for diagonal transmission.

References

1. Souza-Neto JA, Powell JR, Bonizzoni M. 2019. Aedes aegypti vector competence studies: A review. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 67:191–209.

2. Badolo A, Sombié A, Yaméogo F, Wangrawa DW, Sanon A, Pignatelli PM, Sanon A, Viana M, Kanuka H, Weetman D, McCall PJ. 2022. First comprehensive analysis of Aedes aegypti bionomics during an arbovirus outbreak in west Africa: Dengue in Ouagadougou, Burkina Faso, 2016–2017. PLoS Negl Trop Dis 16:e0010059–e0010059.

3. Rose NH, Sylla M, Badolo A, Lutomiah J, Ayala D, Aribodor OB, Ibe N, Akorli J, Otoo S, Mutebi JP, Kriete AL, Ewing EG, Sang R, Gloria-Soria A, Powell JR, Baker RE, White BJ, Crawford JE, McBride CS. 2020. Climate and Urbanization Drive Mosquito Preference for Humans. Curr Biol CB 30:3570-3579.e6.

4. Shaw WR, Catteruccia F. 2019. Vector biology meets disease control: using basic research to fight vector-borne diseases. Nat Microbiol 4:20–34.

5. Badolo A, Sombié A, Pignatelli PM, Sanon A, Yaméogo F, Wangrawa DW, Sanon A, Kanuka H, McCall PJ, Weetman D. 2019. Insecticide resistance levels and mechanisms in Aedes aegypti populations in and around Ouagadougou, Burkina Faso. PLoS Negl Trop Dis 13:e0007439–e0007439.

6. Accoti A, Quek S, Vulcan J, Cansado-Utrilla C, Anderson ER, Abu AEI, Alsing J, Narra HP, Khanipov K, Hughes GL, Dickson LB. 2023. Variable microbiomes between mosquito lines are maintained across different environments. PLoS Negl Trop Dis 17.

7. Bando H, Okado K, Guelbeogo WM, Badolo A, Aonuma H, Nelson B, Fukumoto S, Xuan X, Sagnon N, Kanuka H. 2013. Intra-specific diversity of Serratia marcescens in Anopheles mosquito midgut defines Plasmodium transmission capacity. Sci Rep 3.

8. Clements AN. 1992. The biology of mosquitoes. Volume 1: development, nutrition and reproduction. Chapman & Hall, London.

9. Dong Y, Manfredini F, Dimopoulos G. 2009. Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites. PLOS Pathog 5:e1000423–e1000423.

10. Merritt RW, Dadd RH, Walker ED. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annu Rev Entomol 37:349–374.

11. Ricci I, Valzano M, Ulissi U, Epis S, Cappelli A, Favia G. 2012. Symbiotic control of mosquito borne disease. Pathog Glob Health 106:380–385.

12. Hyde J, Correa MA, Hughes GL, Steven B, Brackney DE. 2020. Limited influence of the microbiome on the transcriptional profile of female Aedes aegypti mosquitoes. Sci Rep 2020 101 10:1–12.

13. Hegde S, Rasgon JL, Hughes GL. 2015. The microbiome modulates arbovirus transmission in mosquitoes. Curr Opin Virol 15:97–102.

14. Scolari F, Casiraghi M, Bonizzoni M. 2019. Aedes spp. and Their Microbiota: A Review. Front Microbiol 10.

15. Wang J, Gao L, Aksoy S. 2023. Microbiota in disease-transmitting vectors. Nat Rev Microbiol 2023 219 21:604–618.

16. Gao H, Cui C, Wang L, Jacobs-Lorena M, Wang S. 2020. Mosquito Microbiota and Implications for Disease Control. Trends Parasitol 36:98–111.

17. Cansado-Utrilla C, Zhao SY, McCall PJ, Coon KL, Hughes GL. 2021. The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. Microbiome 9:111.

18. Caragata EP, Dutra HLC, Moreira LA. 2016. Inhibition of Zika virus by Wolbachia in Aedes aegypti. Microb Cell Graz Austria 3:293–295.

19. Cirimotich CM, Ramirez JL, Dimopoulos G. 2011. Native microbiota shape insect vector competence for human pathogens. Cell Host Microbe 10:307–310.

20. Indriani C, Tantowijoyo W, Rancès E, Andari B, Prabowo E, Yusdi D, Ansari MR, Wardana DS, Supriyati E, Nurhayati I, Ernesia I, Setyawan S, Fitriana I, Arguni E, Amelia Y, Ahmad RA, Jewell NP, Dufault SM, Ryan PA, Green BR, McAdam TF, O'Neill SL, Tanamas SK, Simmons CP, Anders KL, Utarini A. 2020. Reduced dengue incidence following deployments of Wolbachia-infected Aedes aegypti in Yogyakarta, Indonesia: a quasi-experimental trial using controlled interrupted time series analysis. Gates Open Res 4:50.

21. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA, O'Neill SL. 2009. A Wolbachia Symbiont in Aedes aegypti Limits Infection with Dengue, Chikungunya, and Plasmodium. Cell 139:1268–1278.

22. Pascar J, Middleton H, Dorus S. 2023. Aedes aegypti microbiome composition covaries with the density of Wolbachia infection. Microbiome 11:255.

23. Girard M, Martin E, Vallon L, Raquin V, Bellet C, Rozier Y, Desouhant E, Hay A-E, Luis P, Valiente Moro C, Minard G. 2021. Microorganisms Associated with Mosquito Oviposition Sites: Implications for Habitat Selection and Insect Life Histories. Microorganisms 9:1589.

24. Day JF. 2016. Mosquito Oviposition Behavior and Vector Control. 4. Insects 7:65.

25. Boissière A, Tchioffo MT, Bachar D, Abate L, Marie A, Nsango SE, Shahbazkia HR, Awono-Ambene PH, Levashina EA, Christen R, Morlais I. 2012. Midgut

Microbiota of the Malaria Mosquito Vector Anopheles gambiae and Interactions with Plasmodium falciparum Infection. PLOS Pathog 8:e1002742.

26. Steven B, Hyde J, LaReau JC, Brackney DE. 2021. The Axenic and Gnotobiotic Mosquito: Emerging Models for Microbiome Host Interactions. Front Microbiol 12.

27. Coon KL, Brown MR, Strand MR. 2016. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. Mol Ecol 25:5806–5826.

28. Dada N, Jumas-Bilak E, Manguin S, Seidu R, Stenström TA, Overgaard HJ. 2014. Comparative assessment of the bacterial communities associated with Aedes aegypti larvae and water from domestic water storage containers. Parasit Vectors 7:1–12.

29. Juma EO, Allan BF, Kim C-H, Stone C, Dunlap C, Muturi EJ. 2021. The larval environment strongly influences the bacterial communities of Aedes triseriatus and Aedes japonicus (Diptera: Culicidae). Sci Rep 11:7910.

30. Scolari F, Sandionigi A, Carlassara M, Bruno A, Casiraghi M, Bonizzoni M. 2021. Exploring Changes in the Microbiota of Aedes albopictus: Comparison Among Breeding Site Water, Larvae, and Adults. Front Microbiol 12:624170–624170.

31. Capinera JL. 2008. Encyclopedia of Entomology. Springer Science & Business Media.

32. Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, Ghozlane A, Bouchier C, Ayala D, Paupy C, Moro CV, Lambrechts L. 2017. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. Sci Adv 3:e1700585.

33. Gaio A de O, Gusmão DS, Santos AV, Berbert-Molina MA, Pimenta PF, Lemos FJ. 2011. Contribution of midgut bacteria to blood digestion and egg production in Aedes aegypti (diptera: culicidae) (L.). Parasit Vectors 4:105.

34. Gusmão DS, Santos AV, Marini DC, Bacci M, Berbert-Molina MA, Lemos FJA. 2010. Culture-dependent and culture-independent characterization of microorganisms associated with *Aedes aegypti* (Diptera: Culicidae) (L.) and dynamics of bacterial colonization in the midgut. Acta Trop 115:275–281.

35. Guégan M, Tran Van V, Martin E, Minard G, Tran F-H, Fel B, Hay A-E, Simon L, Barakat M, Potier P, Haichar F el Z, Valiente Moro C. 2020. Who is eating fructose within the Aedes albopictus gut microbiota? Environ Microbiol 22:1193–1206.

36. Nichols HL, Coon KL. 2025. Leveraging microbial ecology for mosquito-borne disease control. Trends Parasitol https://doi.org/10.1016/j.pt.2025.06.010.

37. Carvajal-Lago L, Ruiz-López MJ, Figuerola J, Martínez-de la Puente J. 2021. Implications of diet on mosquito life history traits and pathogen transmission. Environ Res 195:110893.

38. Wang X, Liu T, Wu Y, Zhong D, Zhou G, Su X, Xu J, Sotero CF, Sadruddin AA, Wu K, Chen X-G, Yan G. 2018. Bacterial microbiota assemblage in Aedes

albopictus mosquitoes and its impacts on larval development. Mol Ecol 27:2972–2985.

39. Hamel R, Narpon Q, Serrato-Pomar I, Gauliard C, Berthomieu A, Wichit S, Missé D, Sofonea MT, Pompon J. 2024. West Nile virus is transmitted within mosquito populations through infectious mosquito excreta. bioRxiv 2024.01.29.577888-2024.01.29.577888.

40. Bennett KL, Gómez-Martínez C, Chin Y, Saltonstall K, McMillan WO, Rovira JR, Loaiza JR. 2019. Dynamics and diversity of bacteria associated with the disease vectors Aedes aegypti and Aedes albopictus. Sci Rep 2019 91 9:1–12.

41. Coon KL, Vogel KJ, Brown MR, Strand MR. 2014. Mosquitoes rely on their gut microbiota for development. Mol Ecol 23:2727–2739.

42. Hernández AM, Alcaraz LD, Hernández-Álvarez C, Romero MF, Jara-Servín A, Barajas H, Ramírez CM, Peimbert M. 2024. Revealing the microbiome diversity and biocontrol potential of field Aedes ssp.: Implications for disease vector management. PLOS ONE 19:e0302328–e0302328.

43. Hery L, Guidez A, Durand AA, Delannay C, Normandeau-Guimond J, Reynaud Y, Issaly J, Goindin D, Legrave G, Gustave J, Raffestin S, Breurec S, Constant P, Dusfour I, Guertin C, Vega-Rúa A. 2021. Natural Variation in Physicochemical Profiles and Bacterial Communities Associated with Aedes aegypti Breeding Sites and Larvae on Guadeloupe and French Guiana. Microb Ecol 81:93–109.

44. Rodpai R, Boonroumkaew P, Sadaow L, Sanpool O, Janwan P, Thanchomnang T, Intapan PM, Maleewong W. 2023. Microbiome Composition and Microbial

Community Structure in Mosquito Vectors Aedes aegypti and Aedes albopictus in Northeastern Thailand, a Dengue-Endemic Area. Insects 14.

45. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–581.

46. Murali A, Bhargava A, Wright ES. 2018. IDTAXA: A novel approach for accurate taxonomic classification of microbiome sequences. Microbiome 6:1–14.

47. Davis NM, Proctor DiM, Holmes SP, Relman DA, Callahan BJ. 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:1–14.

48. Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette M-H, Ribeiro Cunha E, Smith T, Stier A, Ter Braak CJF, Weedon J. 2024. vegan: Community Ecology Package.

49. Schloss PD. 2024. Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. mSphere 9.

50. Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528.

51. Galeano-Castañeda Y, Bascuñán P, Serre D, Correa MM. 2020. Trans-stadial fate of the gut bacterial microbiota in *Anopheles albimanus*. Acta Trop 201:105204.

52. Zouache K, Martin E, Rahola N, Gangue MF, Minard G, Dubost A, Van VT, Dickson L, Ayala D, Lambrechts L, Moro CV. 2022. Larval habitat determines the bacterial and fungal microbiota of the mosquito vector Aedes aegypti. FEMS Microbiol Ecol 98.

53. Mosquera KD, Martínez Villegas LE, Rocha Fernandes G, Rocha David M, Maciel-de-Freitas R, A. Moreira L, Lorenzo MG. 2023. Egg-laying by female Aedes aegypti shapes the bacterial communities of breeding sites. BMC Biol 21:1–15.

54. Chavshin AR, Oshaghi MA, Vatandoost H, Yakhchali B, Zarenejad F, Terenius O. 2015. Malpighian tubules are important determinants of Pseudomonas transstadial transmission and longtime persistence in Anopheles stephensi. Parasit Vectors 8.

55. Correa MA, Matusovsky B, Brackney DE, Steven B. 2018. Generation of axenic Aedes aegypti demonstrate live bacteria are not required for mosquito development. Nat Commun 9:4464.

Acknowledgements

We thank Honoré Badolo for his assistance for the mosquito sampling in the different sites**.** We also thank the population from the sampling sites who allowed us to collect mosquito samples.

The geographic shapefile for Figure 1A was provided by the Institut Géographique du Burkina (https://data.humdata.org/dataset/cod-ab-bfa) and mosquito vector illustrations for Figure 1B were used from NIAID NIH BIOART Source.

Author contributions

A.S, L.W, A.B and L.D conceived and organized the study. A.S conducted the experiments, performed basic analysis and wrote the manuscript. L.D.C generated the ASVs table, cleaned the datasets, performed statistical analyses, generated the plots and wrote the manuscript. K.T wrote the manuscript. J.M and L.D reviewed the manuscript. All authors read and approved the manuscript.

Funding

This work was supported by the International Office of KU Leuven through a Global Minds Scholarship to Aboubakar Sanon, a North-South cooperation between KU Leuven and Université Joseph Ki-Zerbo, and by a C1 grant from KU Leuven to Leen Delang and Jelle Matthijnssens (C14/20/108). Lander De Coninck was supported by the Research Foundation Flanders (11L1325N).

Data availability

Raw sequence data is accessible on SRA under BioProject PRJNA1301862. Scripts used to analyse the data are available at github.com/absanon/2024_XXX_MICROBIOME_LIFE_STAGE.

Supplementary files

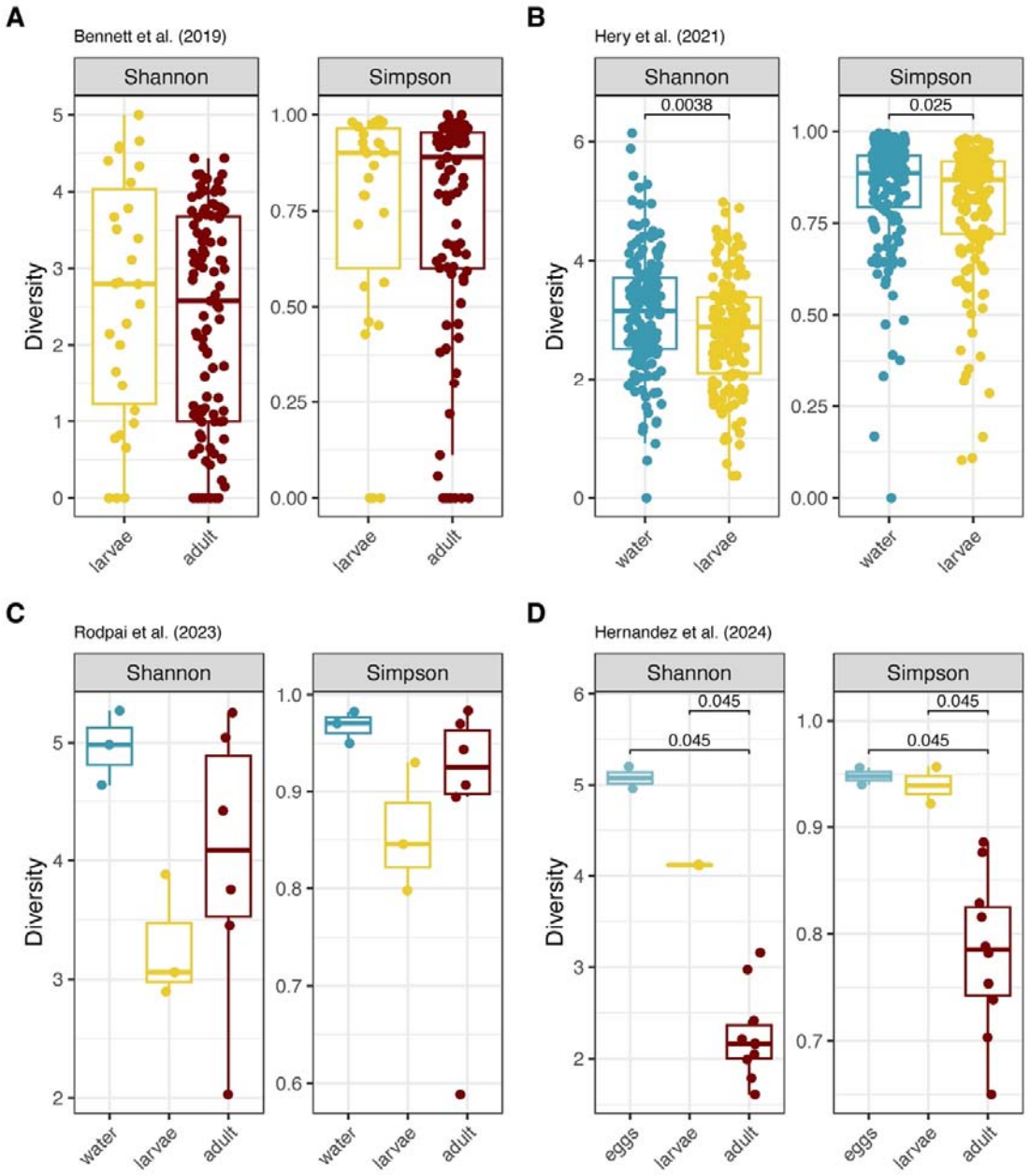


Figure S1: Shannon and Simpson diversity across the different life stages in each study. Differences between mean alpha diversity were tested with Wilcoxon tests and a Benjamini-Hochberg FDR for multiple testing. Only significant differences between groups are shown on top of the boxplots.

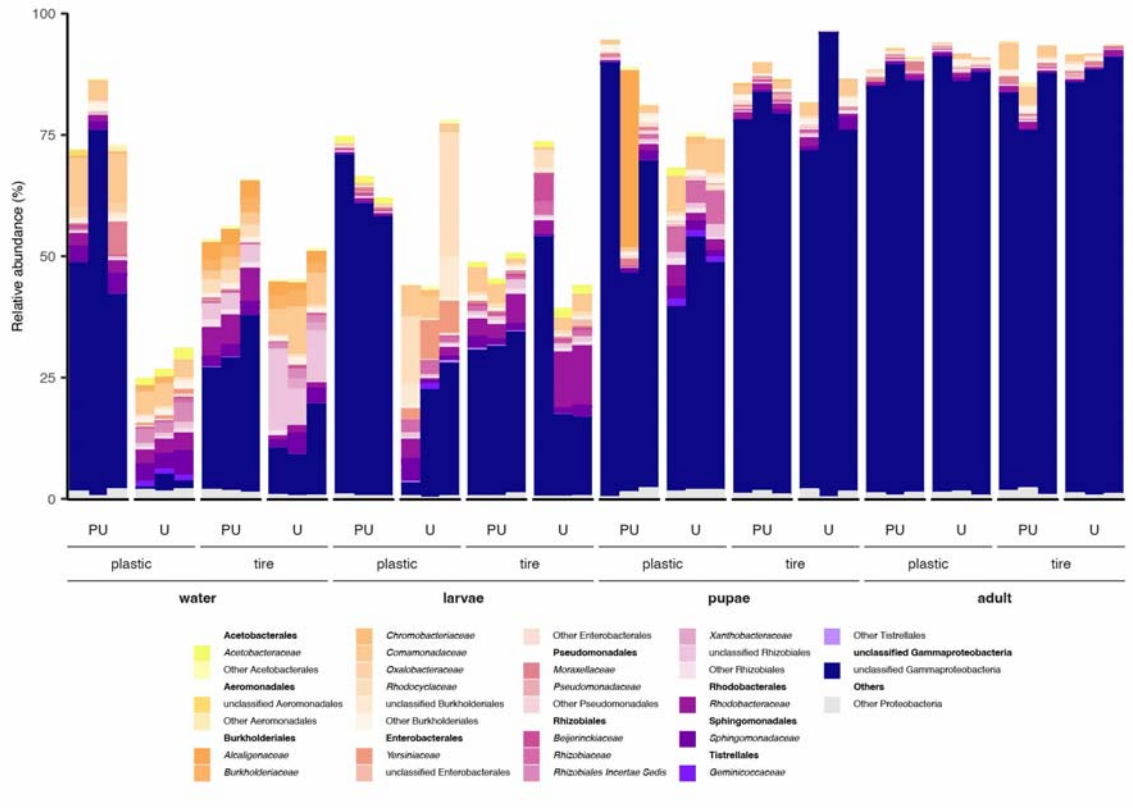


Figure S2: Relative abundance of the *Proteobacteria* phylum per breeding material, sampling sites, and developmental stage. Families have different shades of the same colour if they belong to the same order.

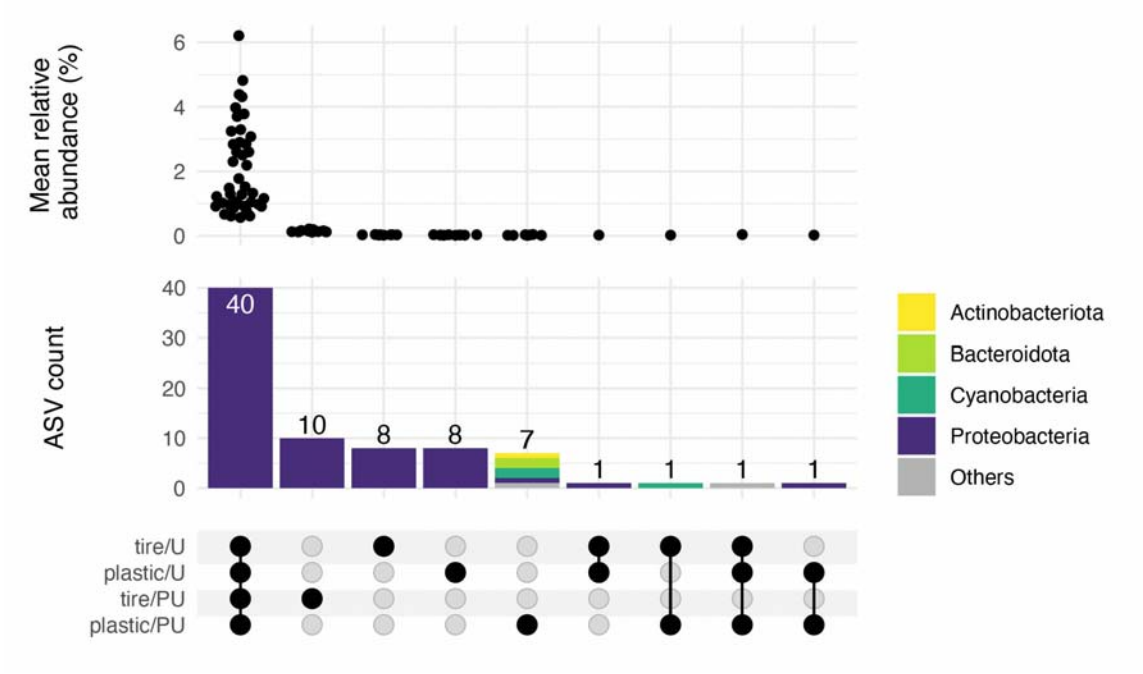


Figure S3: *Upset plot showing the distribution of the 50 most abundant ASVs in the adults from each sampling combinatio*n (plastic material in urban area, tire in peri-urban area, etc.). On top of the plot the mean relative abundance of each ASV across all considered sampling combinations is given.

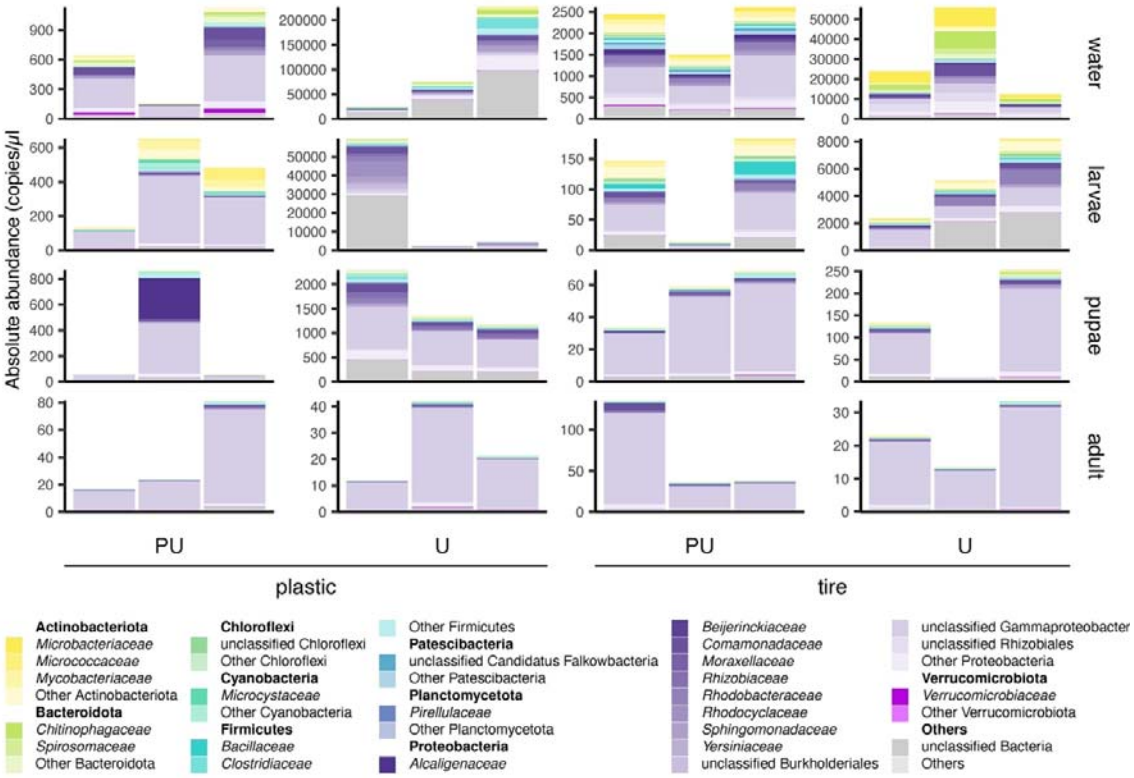


Figure S4: Absolute abundances of the bacterial families based on 16S qPCR. The y-axis shows the number of 16S copies/µl and is scaled to the highest number of copies for each developmental stage with each combination of breeding material and location.

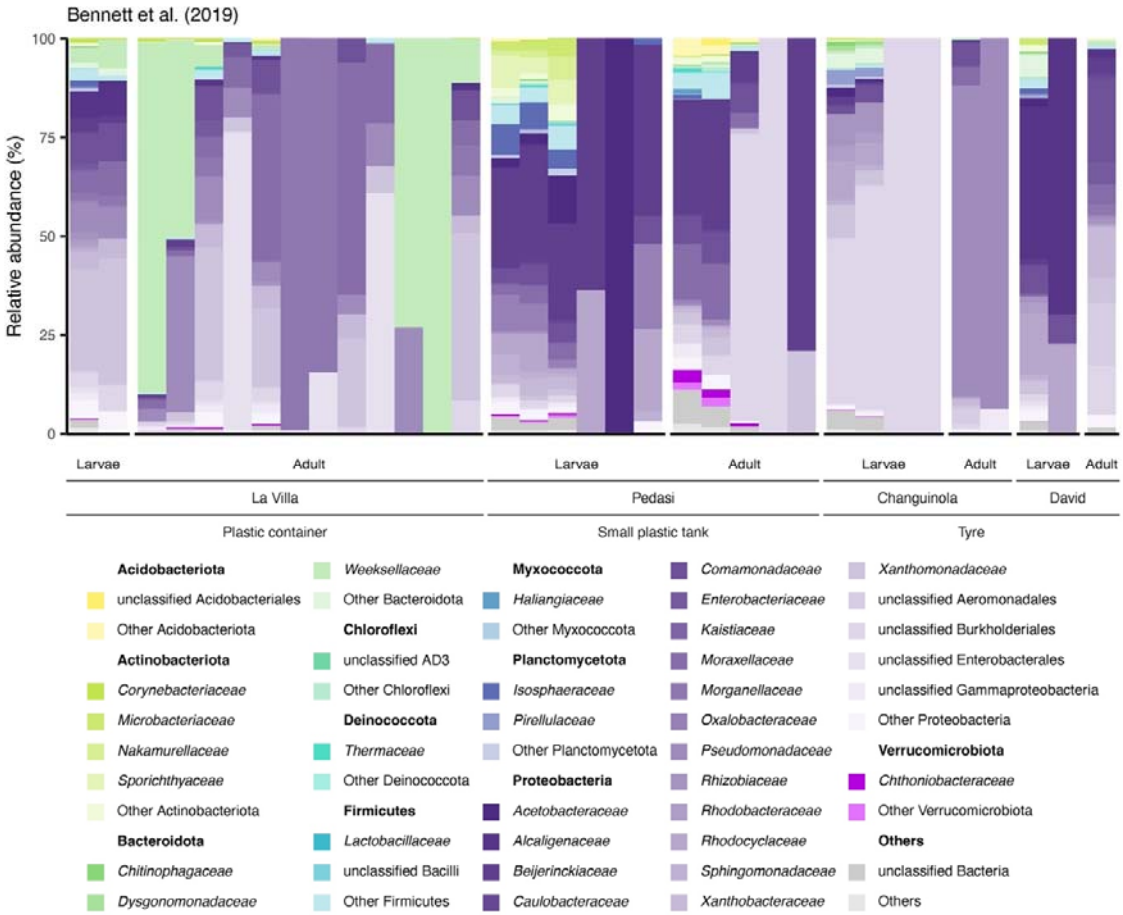


Figure S5: Relative abundance of bacterial families in mosquito samples from Bennett *et al.* (2019).

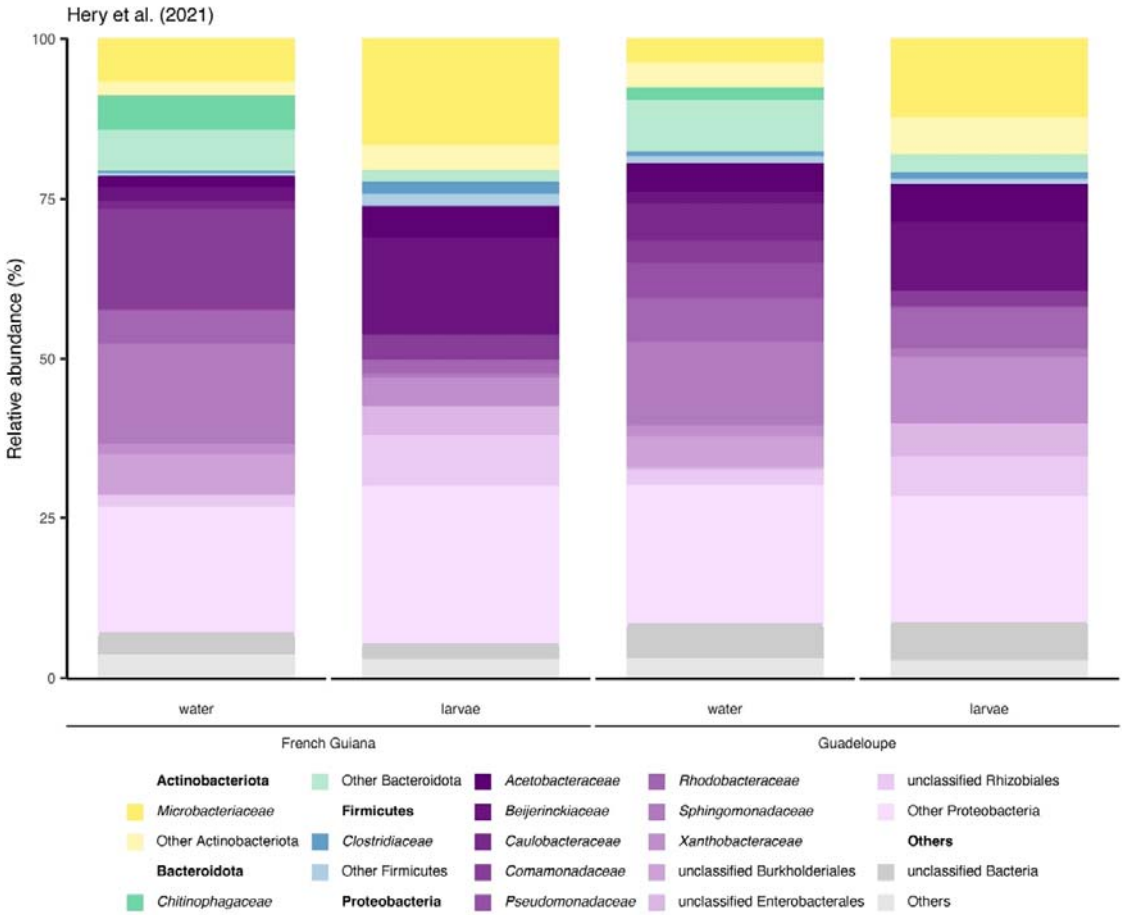


Figure S6: Relative abundance of bacterial families in mosquito samples from Hery *et al.* (2021).

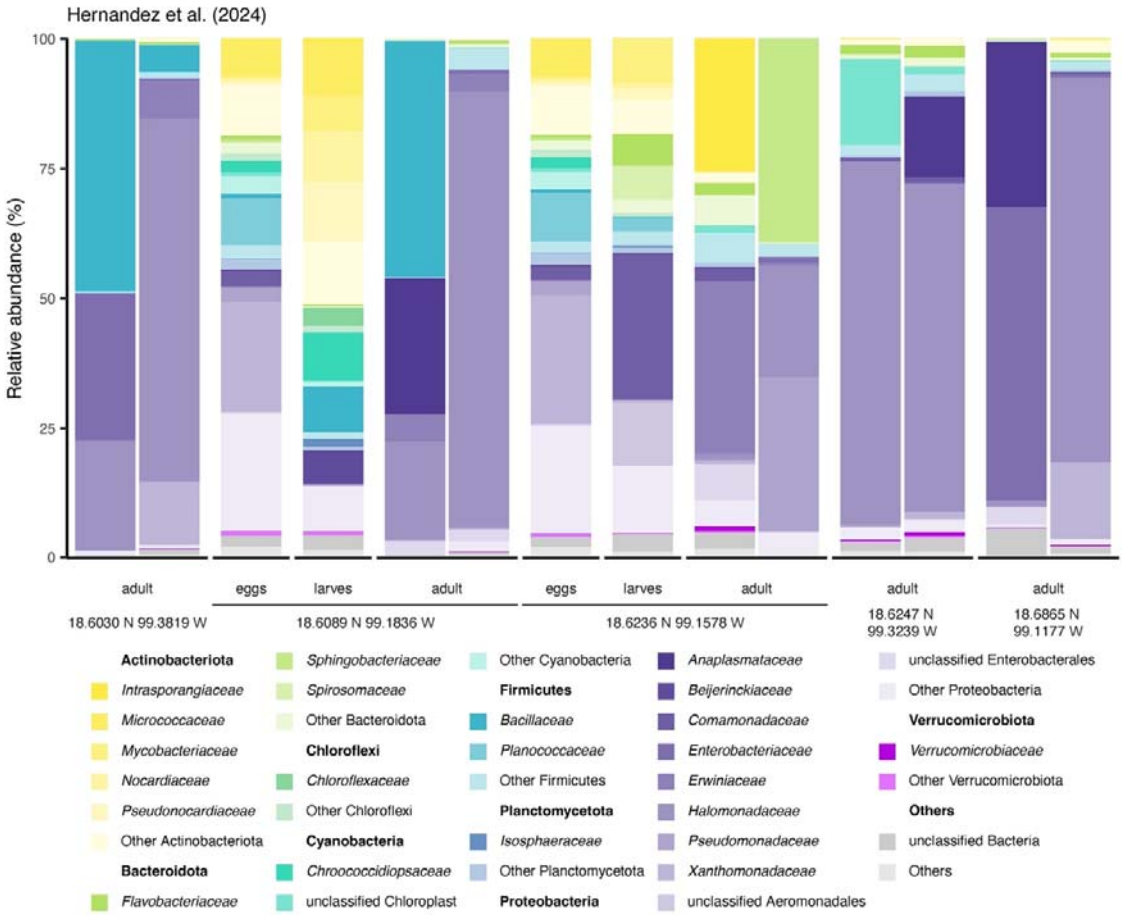


Figure S7: Relative abundance of bacterial familes in mosquito samples from Hernandez *et al.* (2024).

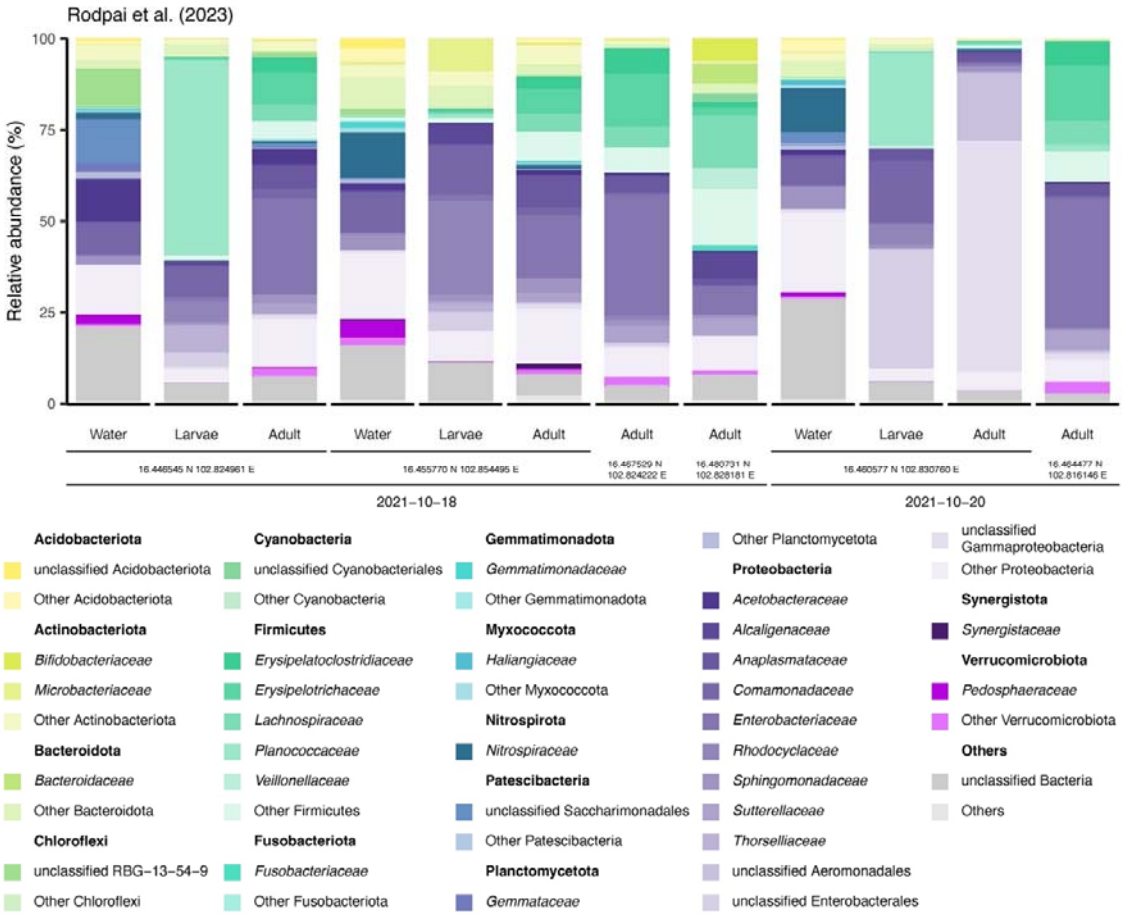
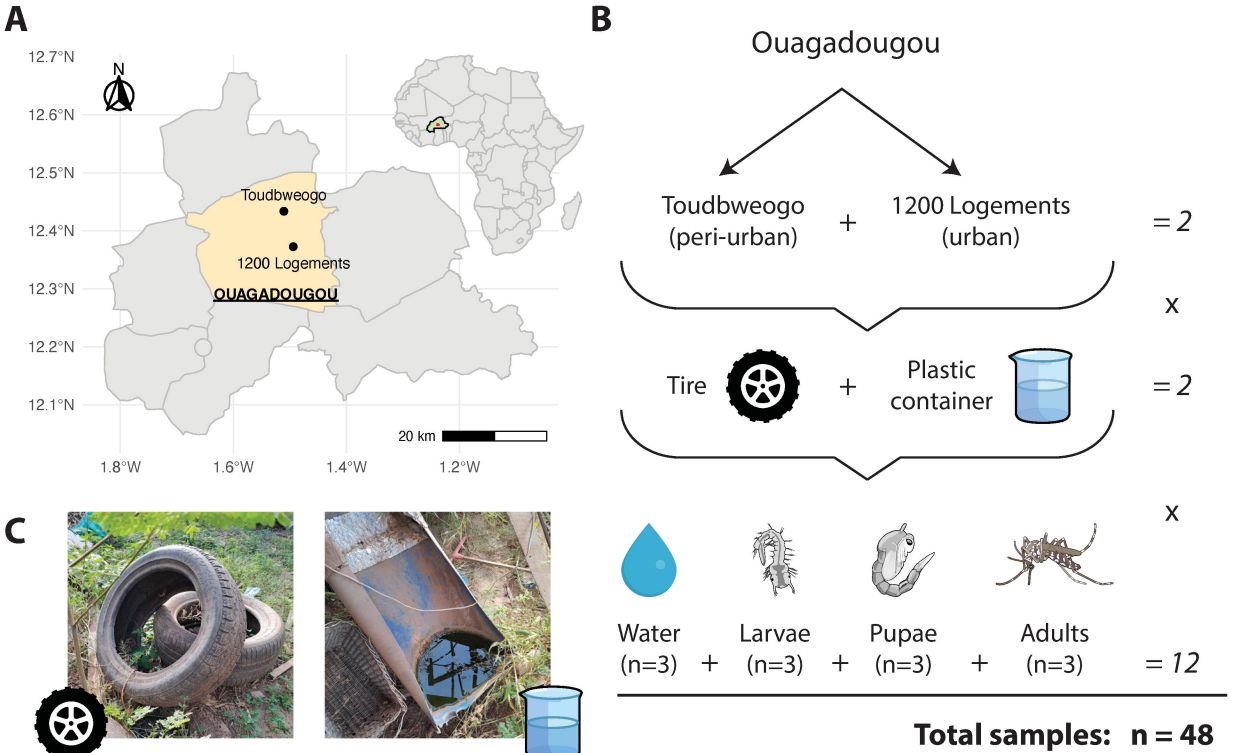
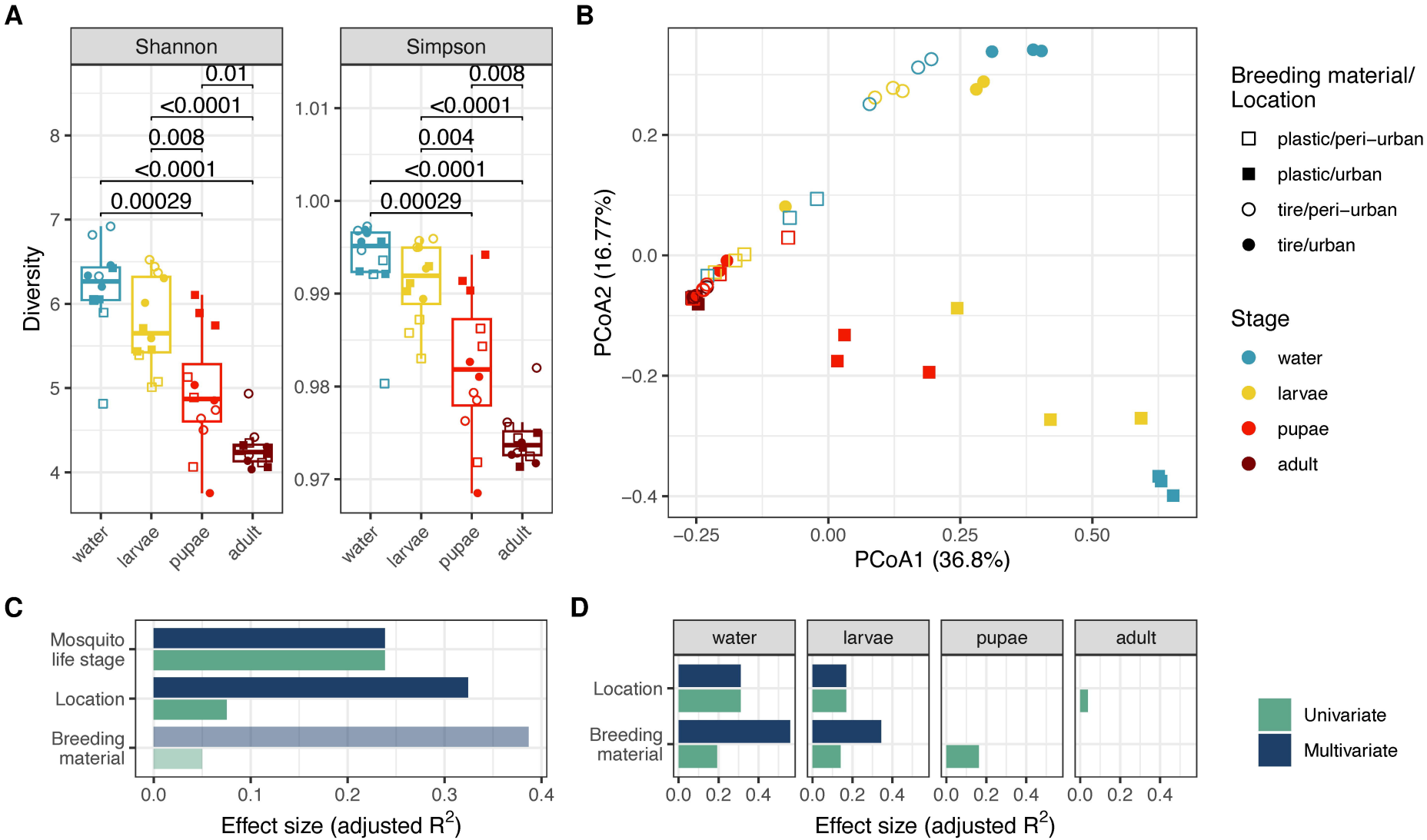
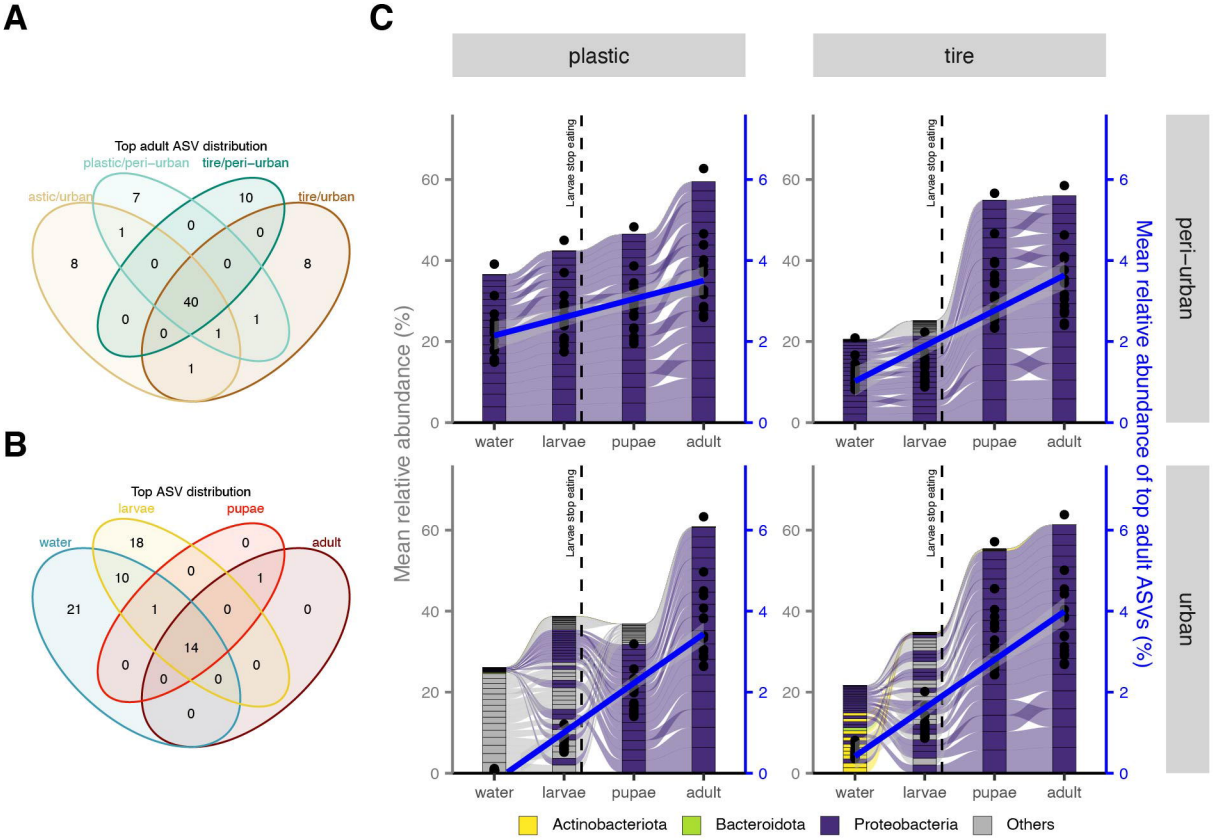


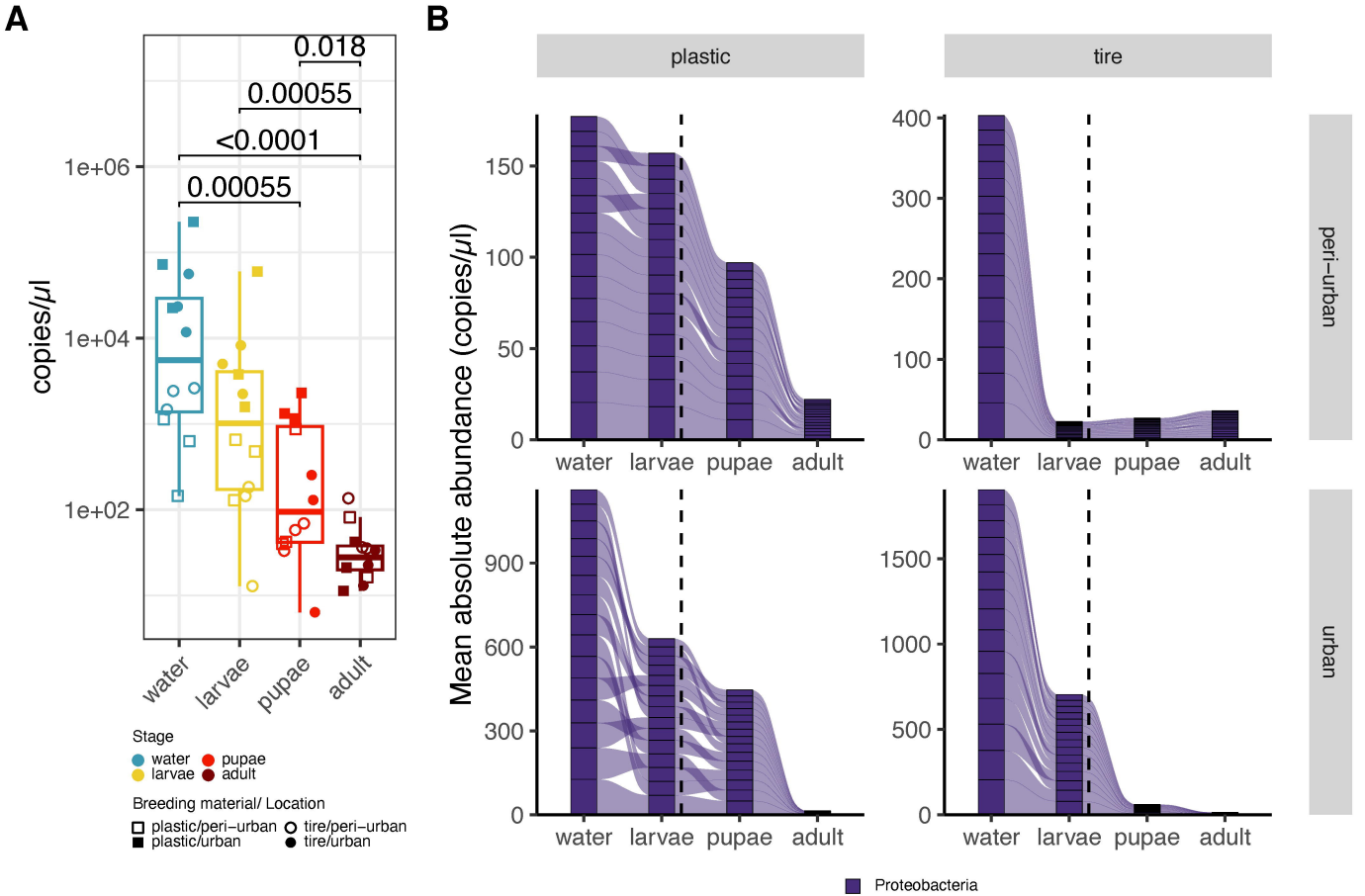
Figure S8: Relative abundance of bacterial families in mosquito samples from Rodpai *et al.* (2023).

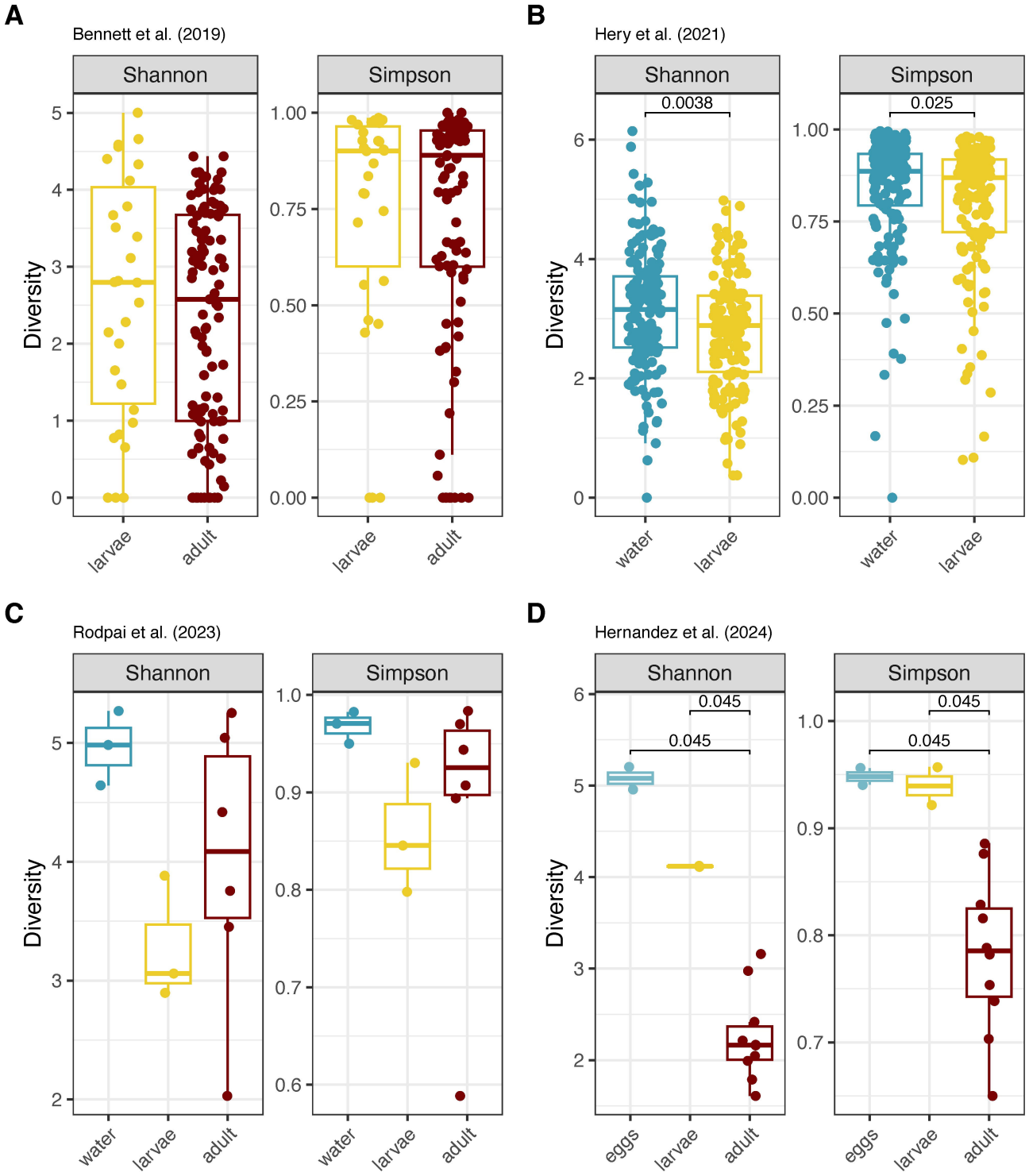

A
12.7°N
12.6°N
12.5°N
12.4°N
12.3°N
12.2°N
12.1°N
N
Toudbweogo
1200 Logements
OUAGADOUGOU
20 km
1.8°W
1.6°W
1.4°W
1.2°W
B
Ouagadougou
Toudbweogo (peri-urban)
+
1200 Logements (urban)
= 2
x
Tire
+
Plastic container
= 2
x
Water (n=3)
+
Larvae (n=3)
+
Pupae (n=3)
+
Adults (n=3)
= 12
Total samples: n = 48
C

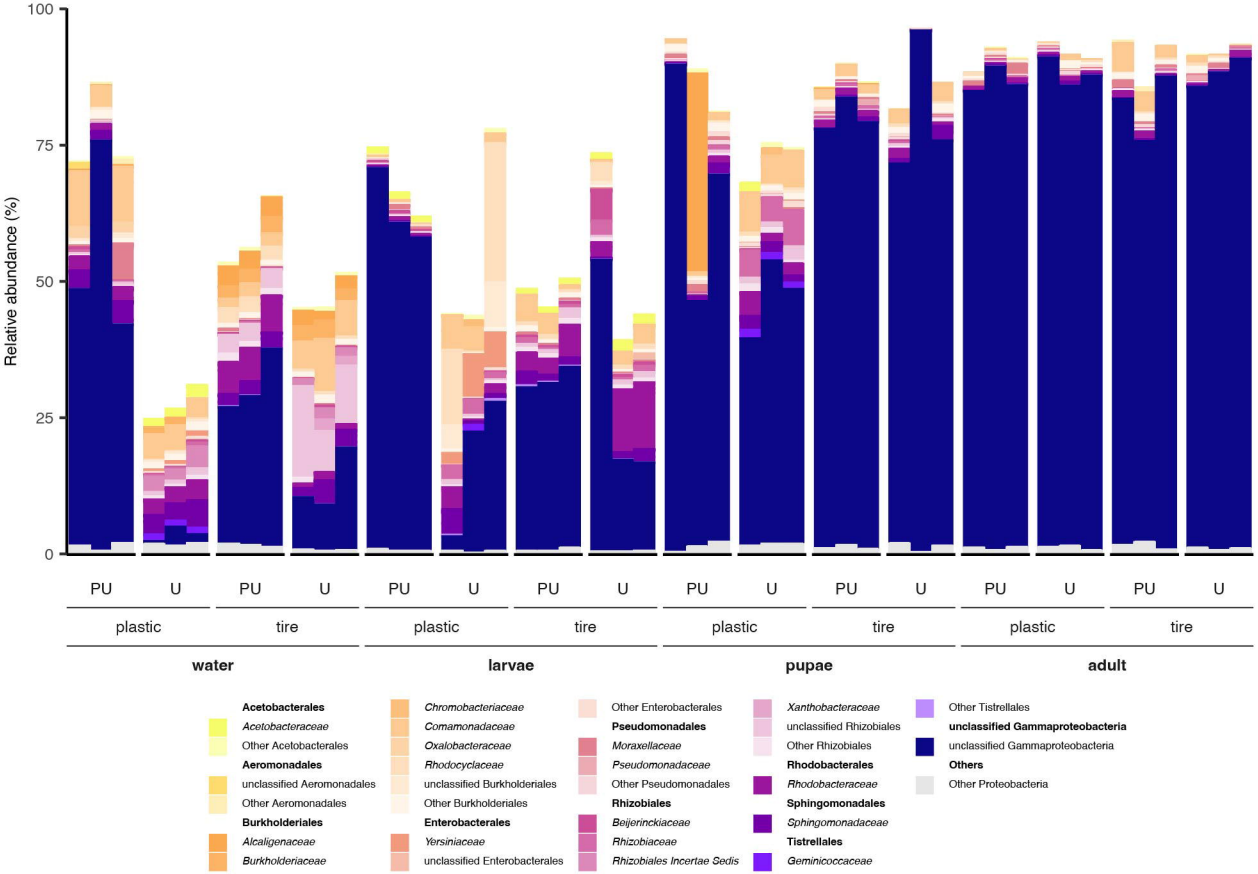

A
Shannon
Simpson
Diversity
0.01
<0.0001
0.008
<0.0001
0.00029
0.008
<0.0001
0.004
<0.0001
0.00029
water
larvae
pupae
adult
B
PCoA2 (16.77%)
PCoA1 (36.8%)
Breeding material/ Location
plastic/peri–urban
plastic/urban
tire/peri–urban
tire/urban
Stage
water
larvae
pupae
adult
C
Mosquito life stage
Location
Breeding material
Effect size (adjusted R2)
D
water
larvae
pupae
adult
Location
Breeding material
Univariate
Multivariate
Effect size (adjusted R2)

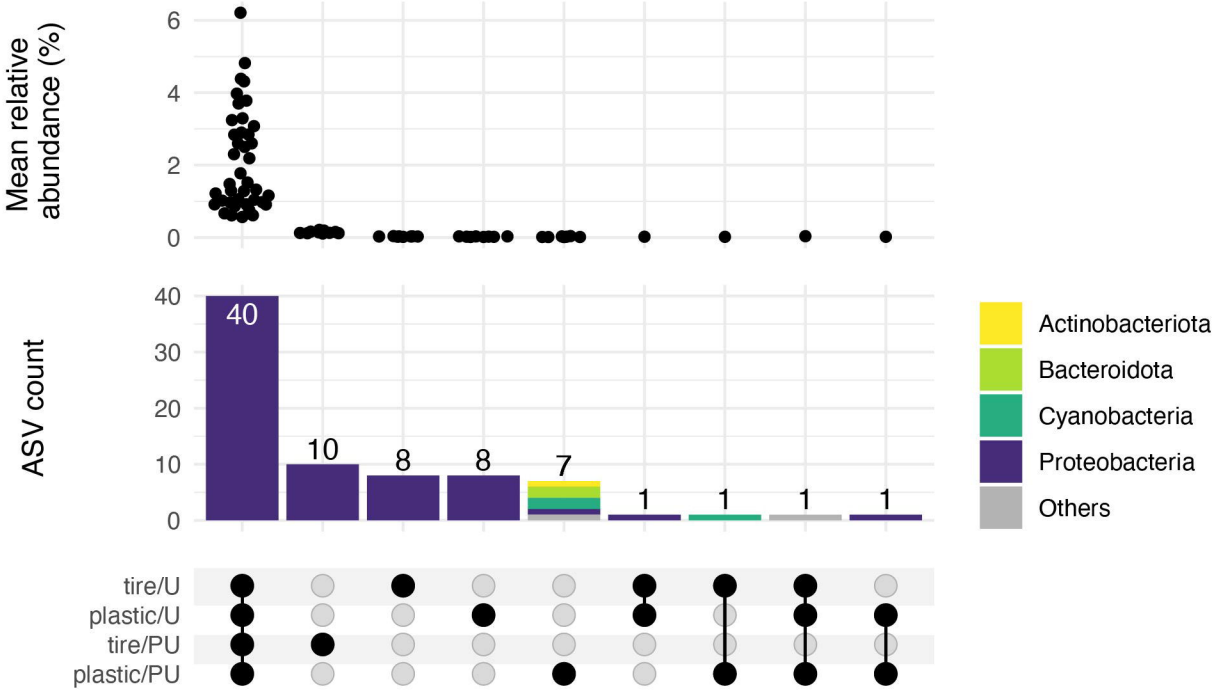
Relative abundance (%)
100
75
50
25
0
PU
U
plastic
tire
water
larvae
pupae
adult
Actinobacteriota
Microbacteriaceae
Micrococcaceae
Mycobacteriaceae
Other Actinobacteriota
Bacteroidota
Chitinophagaceae
Spirosomaceae
Other Bacteroidota
Chloroflexi
unclassified Chloroflexi
Other Chloroflexi
Cyanobacteria
Microcystaceae
Other Cyanobacteria
Firmicutes
Bacillaceae
Clostridiaceae
Other Firmicutes
Patescibacteria
unclassified Candidatus Falkowbacteria
Other Patescibacteria
Planctomycetota
Pirellulaceae
Other Planctomycetota
Proteobacteria
Alcaligenaceae
Beijerinckiaceae
Comamonadaceae
Moraxellaceae
Rhizobiaceae
Rhodobacteraceae
Rhodocyclaceae
Sphingomonadaceae
Yersiniaceae
unclassified Burkholderiales
unclassified Gammaproteobacteria
unclassified Rhizobiales
Other Proteobacteria
Verrucomicrobiota
Verrucomicrobiaceae
Other Verrucomicrobiota
Others
unclassified Bacteria
Others

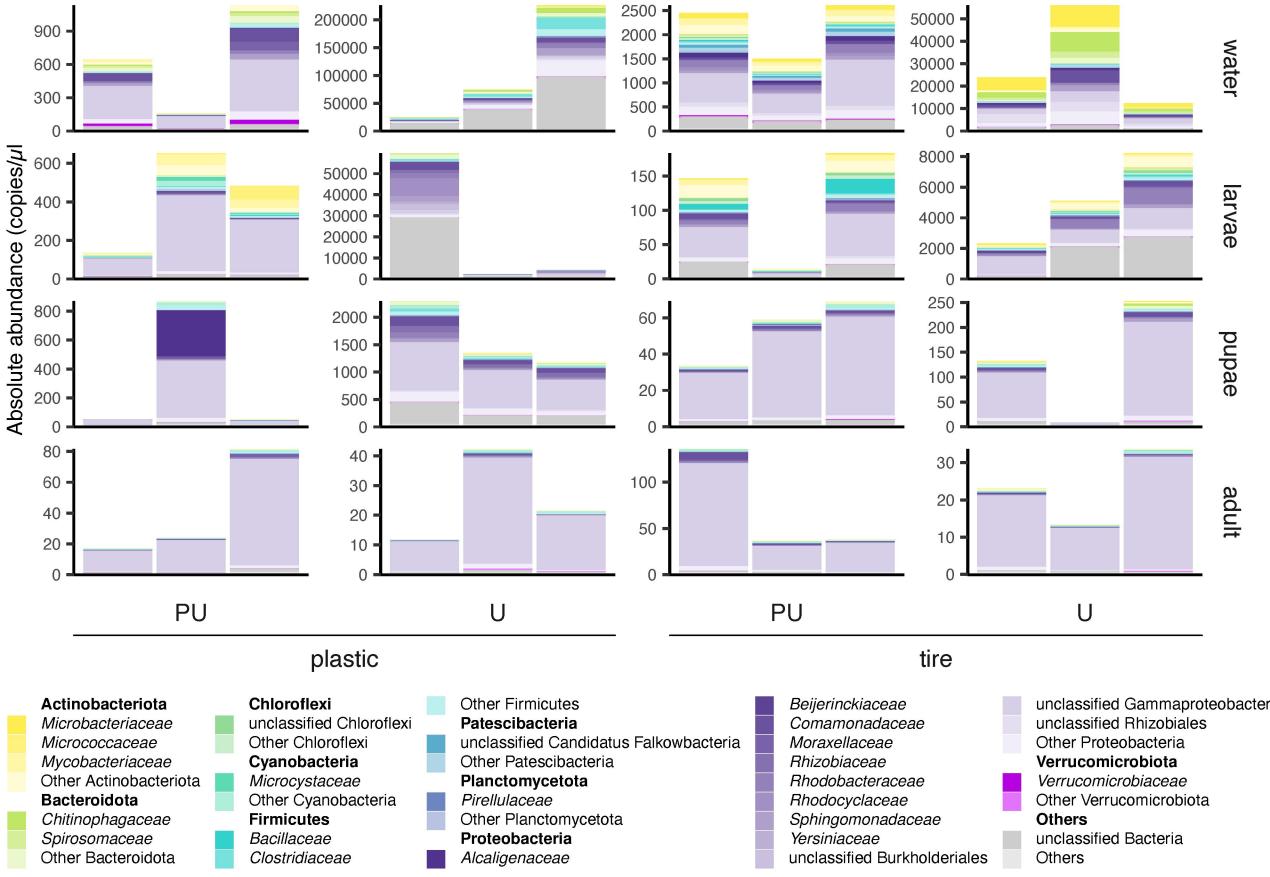

A
Top adult ASV distribution
plastic/peri−urban
tire/peri−urban
astic/urban
tire/urban
7
10
1
0
0
8
0
0
8
40
0
1
0
1
1
B
Top ASV distribution
water
larvae
pupae
adult
18
0
10
0
1
21
1
0
0
14
0
0
0
0
0
C
plastic
tire
peri−urban
urban
Larvae stop eating
Mean relative abundance (%)
Mean relative abundance of top adult ASVs (%)
water
larvae
pupae
adult
Actinobacteriota
Bacteroidota
Proteobacteria
Others

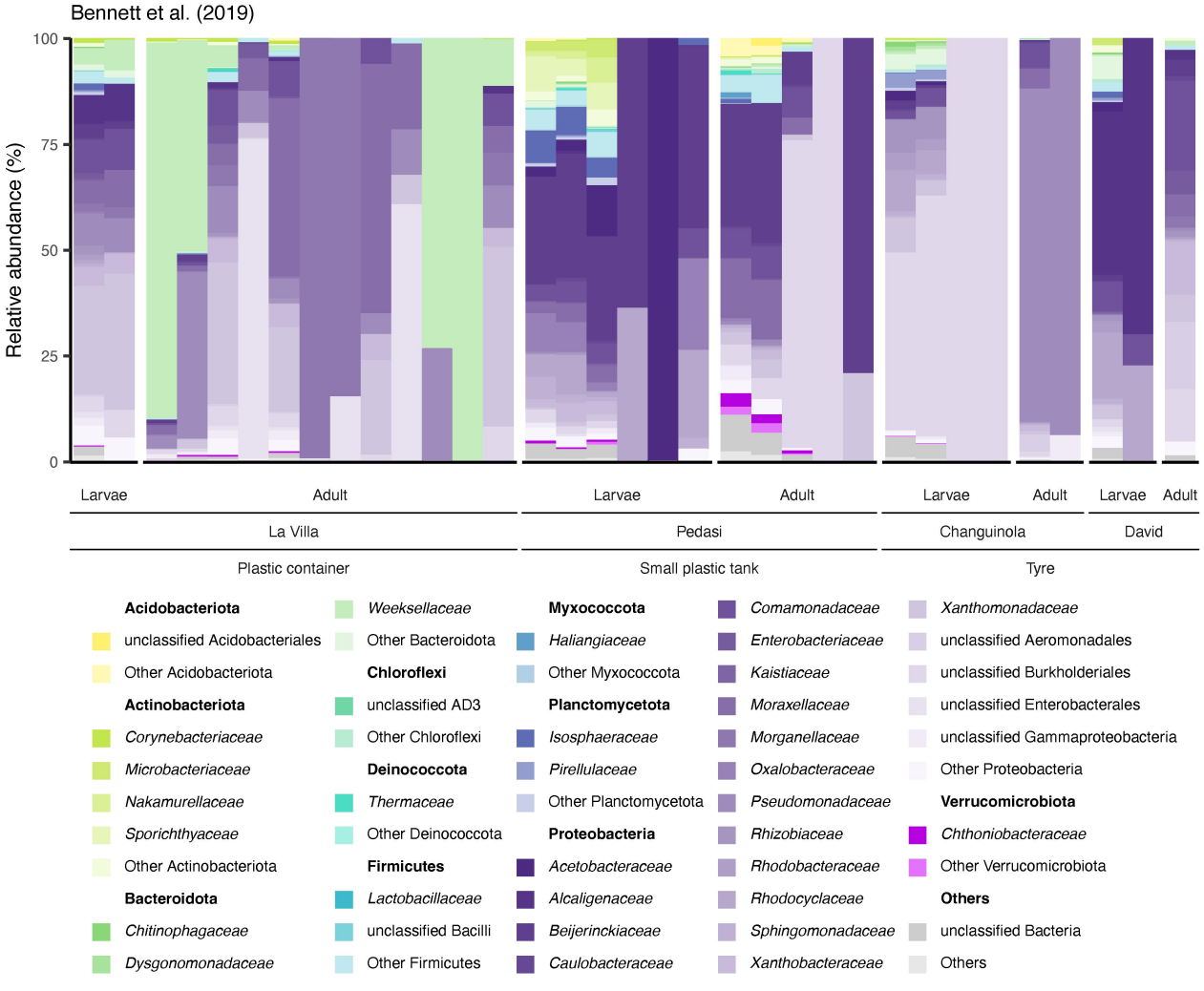

A
0.018
0.00055
<0.0001
0.00055
1e+06
1e+04
1e+02
copies/µl
water
larvae
pupae
adult
Stage
water
pupae
larvae
adult
Breeding material/ Location
plastic/peri−urban
tire/peri−urban
plastic/urban
tire/urban
B
plastic
tire
peri−urban
urban
Mean absolute abundance (copies/µl)
150
100
50
0
400
300
200
100
0
900
600
300
0
1500
1000
500
0
water
larvae
pupae
adult
Proteobacteria

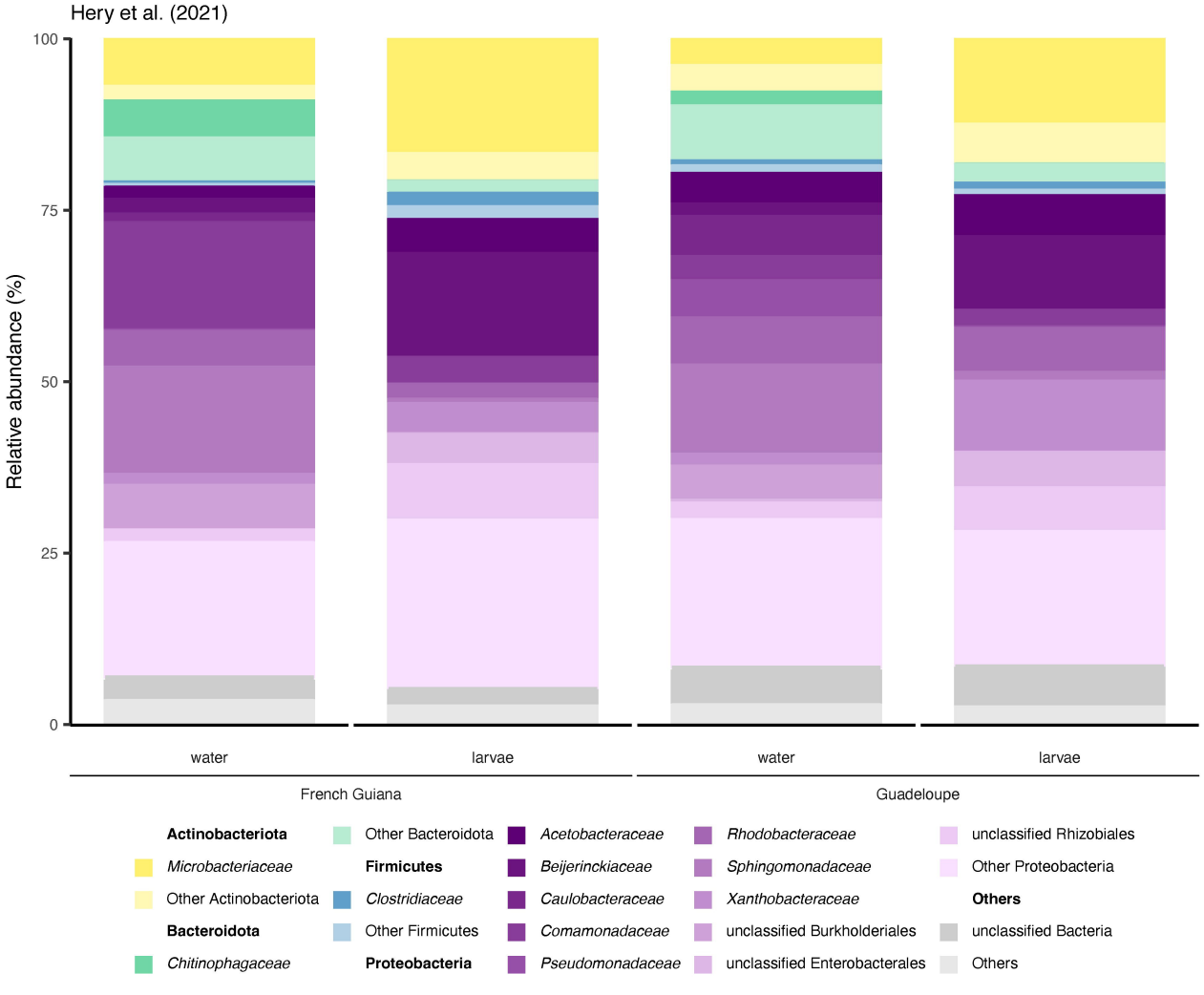

A
Bennett et al. (2019)
Shannon
Simpson
Diversity
larvae
adult
B
Hery et al. (2021)
0.0038
0.025
water
larvae
C
Rodpai et al. (2023)
water
larvae
adult
D
Hernandez et al. (2024)
0.045
0.045
0.045
0.045
eggs
larvae
adult


Relative abundance (%)
100
75
50
25
0
PU
U
plastic
tire
water
larvae
pupae
adult
Acetobacteraceae
Other Acetobacterales
Aeromonadales
unclassified Aeromonadales
Other Aeromonadales
Burkholderiales
Alcaligenaceae
Burkholderiaceae
Chromobacteriaceae
Comamonadaceae
Oxalobacteraceae
Rhodocyclaceae
unclassified Burkholderiales
Other Burkholderiales
Enterobacterales
Yersiniaceae
unclassified Enterobacterales
Other Enterobacterales
Pseudomonadales
Moraxellaceae
Pseudomonadaceae
Other Pseudomonadales
Rhizobiales
Beijerinckiaceae
Rhizobiaceae
Rhizobiales Incertae Sedis
Xanthobacteraceae
unclassified Rhizobiales
Other Rhizobiales
Rhodobacterales
Rhodobacteraceae
Sphingomonadales
Sphingomonadaceae
Tistrellales
Geminicoccaceae
Other Tistrellales
unclassified Gammaproteobacteria
unclassified Gammaproteobacteria
Others
Other Proteobacteria

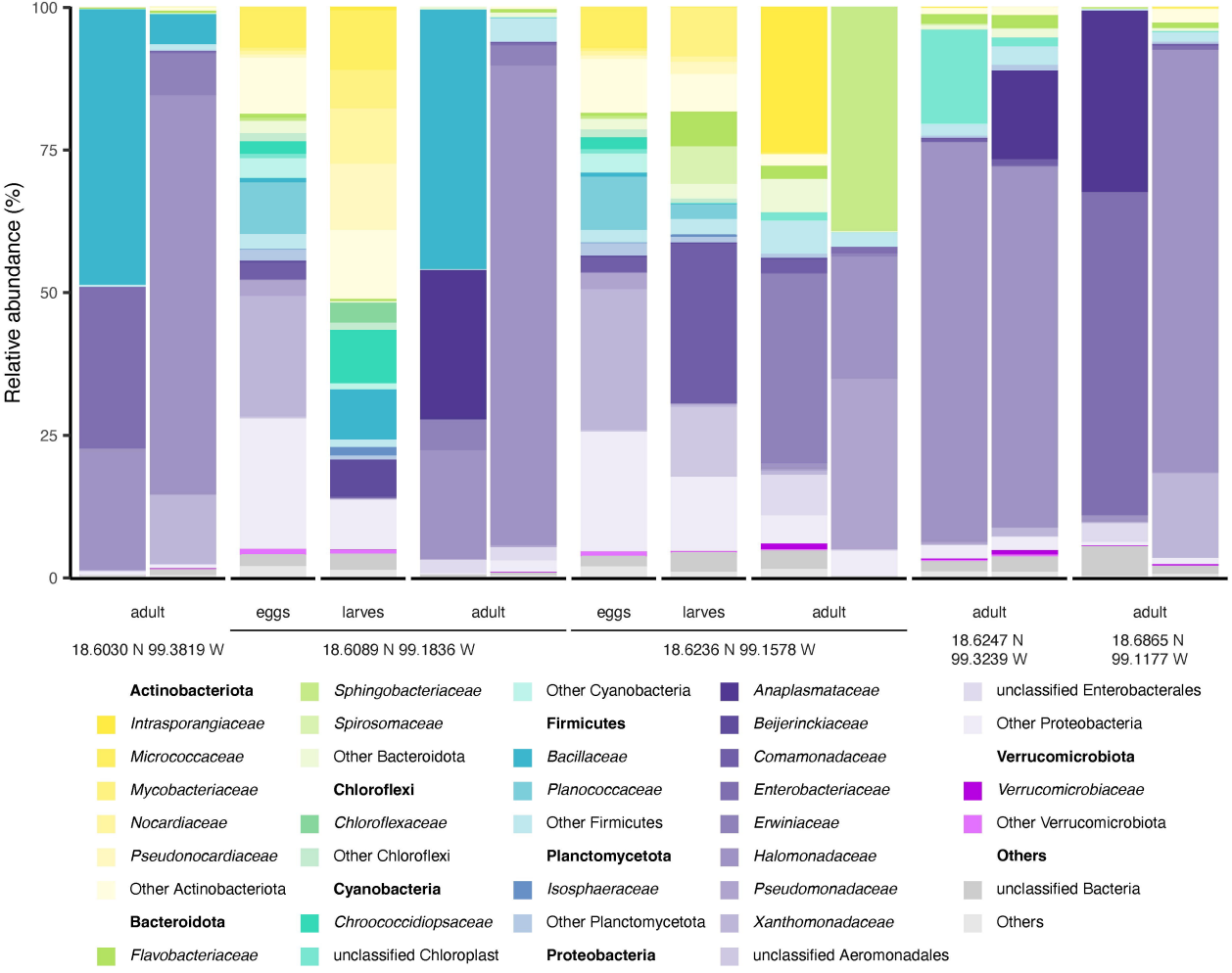

Mean relative abundance (%)
6
4
2
0
ASV count
40
30
20
10
0
40
10
8
8
7
1
1
1
1
tire/U
plastic/U
tire/PU
plastic/PU
Actinobacteriota
Bacteroidota
Cyanobacteria
Proteobacteria
Others


Absolute abundance (copies/μl)
water
larvae
pupae
adult
PU
U
PU
U
plastic
tire
Actinobacteriota
Microbacteriaceae
Micrococcaceae
Mycobacteriaceae
Other Actinobacteriota
Bacteroidota
Chitinophagaceae
Spirosomaceae
Other Bacteroidota
Chloroflexi
unclassified Chloroflexi
Other Chloroflexi
Cyanobacteria
Microcystaceae
Other Cyanobacteria
Firmicutes
Bacillaceae
Clostridiaceae
Other Firmicutes
Patescibacteria
unclassified Candidatus Falkowbacteria
Other Patescibacteria
Planctomycetota
Pirellulaceae
Other Planctomycetota
Proteobacteria
Alcaligenaceae
Beijerinckiaceae
Comamonadaceae
Moraxellaceae
Rhizobiaceae
Rhodobacteraceae
Rhodocyclaceae
Sphingomonadaceae
Yersiniaceae
unclassified Burkholderiales
unclassified Gammaproteobacter
unclassified Rhizobiales
Other Proteobacteria
Verrucomicrobiota
Verrucomicrobiaceae
Other Verrucomicrobiota
Others
unclassified Bacteria
Others


Bennett et al. (2019)
Relative abundance (%)
100
75
50
25
0
Larvae
Adult
Larvae
Adult
Larvae
Adult
Larvae
Adult
La Villa
Pedasi
Changuinola
David
Plastic container
Small plastic tank
Tyre
Acidobacteriota
unclassified Acidobacteriales
Other Acidobacteriota
Actinobacteriota
Corynebacteriaceae
Microbacteriaceae
Nakamurellaceae
Sporichthyaceae
Other Actinobacteriota
Bacteroidota
Chitinophagaceae
Dysgonomonadaceae
Weeksellaceae
Other Bacteroidota
Chloroflexi
unclassified AD3
Other Chloroflexi
Deinococcota
Thermaceae
Other Deinococcota
Firmicutes
Lactobacillaceae
unclassified Bacilli
Other Firmicutes
Myxococcota
Haliangiaceae
Other Myxococcota
Planctomycetota
Isosphaeraceae
Pirellulaceae
Other Planctomycetota
Proteobacteria
Acetobacteraceae
Alcaligenaceae
Beijerinckiaceae
Caulobacteraceae
Comamonadaceae
Enterobacteriaceae
Kaistiaceae
Moraxellaceae
Morganellaceae
Oxalobacteraceae
Pseudomonadaceae
Rhizobiaceae
Rhodobacteraceae
Rhodocyclaceae
Sphingomonadaceae
Xanthobacteraceae
Xanthomonadaceae
unclassified Aeromonadales
unclassified Burkholderiales
unclassified Enterobacterales
unclassified Gammaproteobacteria
Other Proteobacteria
Verrucomicrobiota
Chthoniobacteraceae
Other Verrucomicrobiota
Others
unclassified Bacteria
Others


Hery et al. (2021)
Relative abundance (%)
100
75
50
25
0
water
larvae
water
larvae
French Guiana
Guadeloupe
Actinobacteriota
Microbacteriaceae
Other Actinobacteriota
Bacteroidota
Chitinophagaceae
Other Bacteroidota
Firmicutes
Clostridiaceae
Other Firmicutes
Proteobacteria
Acetobacteraceae
Beijerinckiaceae
Caulobacteraceae
Comamonadaceae
Pseudomonadaceae
Rhodobacteraceae
Sphingomonadaceae
Xanthobacteraceae
unclassified Burkholderiales
unclassified Enterobacterales
unclassified Rhizobiales
Other Proteobacteria
Others
unclassified Bacteria
Others

Hernandez et al. (2024)



Rodpai et al. (2023)
Relative abundance (%)
100
75
50
25
0
Water
Larvae
Adult
Water
Larvae
Adult
Adult
Adult
Water
Larvae
Adult
Adult
16.446545 N 102.824961 E
16.455770 N 102.854495 E
16.467529 N 102.824222 E
16.480731 N 102.828181 E
16.460577 N 102.830760 E
16.464477 N 102.816146 E
2021−10−18
2021−10−20
Acidobacteriota
unclassified Acidobacteriota
Other Acidobacteriota
Actinobacteriota
Bifidobacteriaceae
Microbacteriaceae
Other Actinobacteriota
Bacteroidota
Bacteroidaceae
Other Bacteroidota
Chloroflexi
unclassified RBG−13−54−9
Other Chloroflexi
Cyanobacteria
unclassified Cyanobacteriales
Other Cyanobacteria
Firmicutes
Erysipelatoclostridiaceae
Erysipelotrichaceae
Lachnospiraceae
Planococcaceae
Veillonellaceae
Other Firmicutes
Fusobacteriota
Fusobacteriaceae
Other Fusobacteriota
Gemmatimonadota
Gemmatimonadaceae
Other Gemmatimonadota
Myxococcota
Haliangiaceae
Other Myxococcota
Nitrospirota
Nitrospiraceae
Patescibacteria
unclassified Saccharimonadales
Other Patescibacteria
Planctomycetota
Gemmataceae
Other Planctomycetota
Proteobacteria
Acetobacteraceae
Alcaligenaceae
Anaplasmataceae
Comamonadaceae
Enterobacteriaceae
Rhodocyclaceae
Sphingomonadaceae
Sutterellaceae
Thorselliaceae
unclassified Aeromonadales
unclassified Enterobacterales
unclassified Gammaproteobacteria
Other Proteobacteria
Synergistota
Synergistaceae
Verrucomicrobiota
Pedosphaeraceae
Other Verrucomicrobiota
Others
unclassified Bacteria
Others