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Specific primer design for microbiome profiling of the dengue vector *Aedes aegypti* based on 16S ribosomal DNA

Kiky Martha Ariesaka[1*], Lintang Widya Sishartami[1], Anditri Weningtyas[1,2], Moh Mirza Nuryady[3,4], Arif Ladika Wiratama[1], Fannia Yosa Bakhtiar[1]
[1]Department of Medicine, Faculty of Medicine, State University of Malang,
Jl. Semarang no 5, Malang, East Java, 65145, Indonesia
[2]Doctoral Program in Medical Science, Faculty of Medicine, Universitas Brawijaya,
Jl. Veteran, Malang, East Java, 65145, Indonesia
[3]Biology Education Department, Faculty of Teacher and Training Education,
University of Muhammadiyah Malang, Jl Raya Tlogomas no 246, Malang, East Java, 65144, Indonesia
[4]Institute of Parasitology, Biological Science and Pathobiology Department, Veterinary Medicine
University of Vienna, Veterinärplatz 11210 Wien, Austria
*corresponding author: kiky.martha.fk@um.ac.id

ABSTRACT

Identification of the microbiome in the dengue vector *Aedes aegypti* is crucial for developing effective vector control strategies. The 16S ribosomal DNA (rDNA) gene is frequently used as a genetic marker in microbiome analysis. This study aimed to design and validate specific primers to identify *Serratia* and *Wolbachia* in *Aedes aegypti*. The study employed Primer-BLAST using DNA sequence data from NCBI (accession numbers OR801069.1 for *Serratia* and MN046789.1 for *Wolbachia*). The designed primers included: for *Serratia*—Forward 5'-GTCGACTTTGATCCTGGCTCAG-3' and Reverse 5'-TCAGCCTGTTTCCAATGACC-3'; and for *Wolbachia*—Forward 5'-TGATGAAGTTAGCTTGCTAACG-3' and Reverse 5'-ACGGCTGAGTGAACGGGTG-3'. Validation was conducted through PCR, followed by visualization of the amplification products using 1.5% agarose gel electrophoresis. PCR amplification revealed distinct DNA bands of 766 bp for *Serratia* and 556 bp for *Wolbachia*, confirming primer specificity. The Sanger sequencing results were validated using BLAST, showing high alignment with reference strains in the GenBank database. The specific primers based on the 16S rDNA gene are effective for detecting *Serratia* and *Wolbachia* in *Aedes aegypti*.

Keywords: *Aedes aegypti*, microbiome, *Serratia*, *Wolbachia*, 16S rDNA

INTRODUCTION

Dengue fever remains a major public health issue worldwide, especially in tropical and subtropical regions (Khan et al., 2023), where the mosquito *Aedes aegypti* serves as the primary vector (Kularatne & Dalugama, 2022). This disease, caused by the dengue virus, continues to burden countries with high transmission rates, particularly in densely populated and urbanized areas (Tian et al., 2022). Despite various control efforts, traditional vector management strategies, such as the application of chemical insecticides (Gan et al., 2021), face challenges due to the development of insecticide resistance in mosquito populations (Da Silva et al., 2020; Konkon et al., 2023; Silalahi et al., 2022). The resistance cases of *Aedes aegypti* to several insecticide in Indonesia have been reported like to malathion in Bitung, Banyumas, Semarang, Bantul, and Sleman, pyrethroids in Semarang, Klaten, Kudus, Gombong, and Pekanbaru (Sofiana et al., 2023). Therefore, innovative and sustainable control strategies are needed to reduce dengue transmission effectively.

A promising alternative approach involves leveraging the microbiome of *Aedes aegypti*, specifically certain bacterial species that inhabit the mosquito's gut and other tissues, as potential bioinsecticides (Ferreira et al., 2023). Among the diverse microbiome species associated with *A. aegypti*, *Serratia* (Jupatanakul

et al., 2020) and *Wolbachia* have shown significant potential for vector control (Montenegro et al., 2024). *Serratia* species are known for their insecticidal properties, producing compounds that can adversely affect mosquito larvae and adult mosquitoes (Kozlova et al., 2021). They are naturally found in the midgut of *A. aegypti* and have been observed to interfere with pathogen development within the mosquito (Miesen & van Rij, 2019), which can help reduce vectorial capacity (Scolari et al., 2019). *Wolbachia*, on the other hand, has received extensive attention in recent years for its ability to inhibit virus replication within mosquito hosts (Ant et al., 2023; Hussain et al., 2023) and its unique capability to manipulate mosquito reproduction through cytoplasmic incompatibility, which has been leveraged in mosquito release programs to suppress populations over time (Liang et al., 2020).

To accurately profile these bacterial symbionts, this study targets the 16S rDNA gene, a well-established molecular marker widely used in bacterial taxonomy and microbiome research (Bartoš et al., 2024; Johnson et al., 2019). The 16S rDNA gene is composed of conserved regions that are shared across bacterial species (Fuks et al., 2018), interspersed with hypervariable regions unique to specific taxa, which enables reliable identification of bacterial species within complex communities (Hassler et al., 2022). Its extensive database coverage and high specificity make the 16S rDNA gene particularly suitable for microbiome profiling in *Aedes aegypti*, as it allows for the discrimination of bacterial symbionts down to the genus or species level (Ames et al., 2017; Scolari et al., 2019). Despite its advantages, few studies have specifically targeted the 16S rDNA-based molecular identification of bacterial symbionts like *Serratia* and *Wolbachia* within *A. aegypti*, especially in a manner that allows for precise, species-specific microbiome profiling.

This study addresses this gap by designing specific primers for the 16S rDNA gene to identify key microbiome species, *Serratia* and *Wolbachia*, in *Aedes aegypti*. By employing both in silico and in vitro techniques, this study will contribute to the growing body of knowledge on mosquito microbiome diversity and its potential applications in vector control strategies. The findings could serve as a foundational step toward using microbial symbionts in innovative mosquito management programs, ultimately enhancing the effectiveness of dengue control efforts.

METHOD

Study design

This study employed an observational descriptive design with a cross-sectional approach, conducted from April to October 2024 in Malang Raya, Indonesia. The primary aim was to develop and optimize specific primers for the identification of *Serratia* and *Wolbachia* bacteria in *Aedes aegypti* using in silico and in vitro methods. Ethical approval for the study was obtained from the Ethics Committee of Universitas Negeri Malang (Approval No: 22.5.4/UN32.14.2.8/LT/2024).

Sample collection

Larval *Aedes aegypti* were collected by direct collection in house hold water tank, from various districts across Malang Raya, including Junrejo (Batu City), Klojen and Lowokwaru (Malang City), Pakis and Tumpang (Malang Regency). These locations were selected to ensure wide geographic coverage across Malang Raya, capturing a broad representation of mosquito populations. The collected larvae were then transported to the laboratory, where they were reared under controlled environmental conditions to maturity. This rearing process facilitated optimal growth of the mosquitoes until adulthood. The adult mosquitoes obtained through this method were subsequently used for microbiome analysis, enabling the identification of symbiotic microorganisms, such as *Serratia* and *Wolbachia*, within the tissues of adult *A. aegypti*.

Morphological identification of *A. aegypti*

Morphological identification was conducted before DNA extraction to confirm the collected samples as *Aedes aegypti*. Using a 3.6 MP digital microscope, key morphological characteristics (Supriyono et al., 2023), such as the presence of white scales on the legs and a lyre-shaped pattern on the thorax, were observed to ensure accurate species identification.

DNA template selection

The 16S rDNA sequences of *Serratia* and *Wolbachia* were obtained from the NCBI database to serve as the DNA templates for primer design. The criteria of the sequence were full gene of 16s rDNA, isolated from mosquito especially *Aedes sp.,* come from Indonesia or at least south east asia region. These sequences were aligned in FASTA format for consistency across in silico primer design tools, ensuring accurate targeting of the bacterial species within *Aedes aegypti*.

Primer design

Specific primers for the 16S rDNA gene were designed using primer 3 (https://primer3.ut.ee/) with optimized parameters: an annealing temperature (Tm) between 55–60°C, GC content between 40–60% for primer stability and specificity, and a product size of 500–800 bp for clear resolution during gel electrophoresis. A primer pair were selected for each bacterial target based on their compatibility with the sequences of *Serratia* and *Wolbachia*, and in silico confirmation of specificity by using primer blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

DNA extraction

DNA extraction was performed using a modified salting-out extraction method. Samples were homogenized with 100 µL of homogenizing buffer, and 40 µL of 20% SDS and 8 µL of proteinase K were added. The mixture was incubated at 65°C for 2 hours, followed by the addition of 300 µL of 6 M NaCl and centrifugation at 10,000 rpm for 1 minute at 4°C. The supeDNAtant was then mixed with an equal volume of isopropanol, incubated at -20°C, and centrifuged at 13,000 rpm for 3 minutes to obtain DNA pellets. These pellets were subsequently washed with 300 µL of 70% ethanol, dried, and resuspended in 50 µL of sterile distilled water for further analysis.

Polymerase Chain Reaction (PCR)

Conventional polymerase chain reaction (PCR) was performed to amplify the 16S rDNA gene regions of *Serratia* and *Wolbachia* using the designed primers. Each reaction mixture contained 3 µL of template DNA, 12.5 µL of PCR master mix, 1 µL each of forward and reverse primers, and sterile distilled water to a final volume of 25 µL. The amplified products were visualized through 1.5% agarose gel electrophoresis stained with ethidium bromide under UV illumination. The PCR Condition can be seen at Table 1.

Table. 1 PCR condition

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	3 minutes	1
Denaturation	95°C	1 minutes	30
Anealing	50°C	1 minute	30
Extension	72°C	1 minute	30
final extension	72°C	5 minute	1

Sequencing

After PCR amplification, the 16S rDNA gene amplicons for *Serratia* and *Wolbachia* were purified and subjected to Sanger sequencing. The resulting sequences were verified by aligning them with reference sequences in the NCBI database to confirm species identity.

Phylogenetic analysis

The verified sequences of *Serratia* and *Wolbachia* were analyzed for phylogenetic relationships using MEGA (Molecular Evolutionary Genetics Analysis) software, version X. Multiple sequence alignments were performed using the ClustalW algorithm,

followed by phylogenetic tree construction using the Neighbor-Joining (NJ) method with 1,000 bootstrap replicates to ensure robustness of the tree. The evolutionary distances were computed using the Maximum Composite Likelihood model, allowing for a comprehensive comparison of bacterial strains associated with *Aedes aegypti*.

RESULTS AND DISCUSSION

Study area and landing collection

The study area covered multiple districts in Malang Raya, including Junrejo (Batu City), Klojen and Lowokwaru (Malang City), and Pakis and Tumpang (Malang Regency). These districts were chosen to provide a comprehensive geographic representation across the region, capturing diverse habitats that support *Aedes aegypti* populations (Knoblauch et al., 2024; Vulu et al., 2024). Conducting this study in Malang Raya is particularly relevant due to its status as a densely populated metropolitan area with a high incidence of dengue fever (Masluhiya et al., 2022), making it a critical region for vector control studies. Figure 1 presents a map illustrating the distribution of collection sites within the study area.

Figure 1. Map of study area showing *Aedes aegypti* collection sites in Malang Raya

Malang Raya, encompassing Malang City, Batu City, and Malang Regency (Rofiah et al., 2022), offers a unique blend of urban, suburban, and rural landscapes in East Java, Indonesia. This diversity in topography and population density provides ideal conditions for studying the microbiome of *Aedes aegypti* across varied environmental contexts, potentially yielding insights that can be applied to other regions with similar ecological conditions.

Morphological identification of *Aedes aegypti*

The collected larva samples were reared in the laboratory until adulthood, after which morphological identification was conducted. Under a 3.6 MP digital microscope, *Aedes aegypti* was identified based on distinct morphological characteristics. The thorax exhibited a lyre-shaped pattern, and the legs showed white scales, both of which are defining features of *Aedes aegypti* (Miranti et al., 2023). These morphological markers ensured the accurate identification of mosquito species before further microbiome analysis (Facchinelli et al., 2023; Gunara et al., 2023).

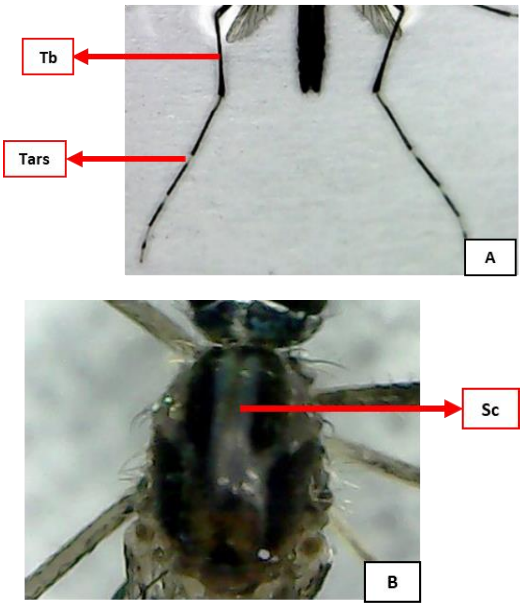

Figure 2. Morphological characteristics of *Aedes aegypti* (personal documentation): (A) Adult *Aedes aegypti* showing the tarsus marked by silvery white bands on a black base (Tb = tibia; Tars = tarsus); (B) Dorsal scutum displaying a transparent white area between two curved lines.

Primer design and in silico analysis

Using Primer-BLAST, specific primers targeting the 16S rDNA gene of *Serratia* and

Wolbachia were designed (Kozyreva et al., 2021). The *Serratia* sequence with GenBank accession number OR801069.1 and the *Wolbachia* sequence with accession number MN046789.1 were used to confirm primer binding sites and optimize specificity. The primers met criteria for annealing temperature and GC content, with in silico analysis predicting a product size of 766 bp for *Serratia* and 556 bp for *Wolbachia*. Table 2 lists the primer sequences along with their respective annealing temperatures and GC content.

PCR amplification and visualization

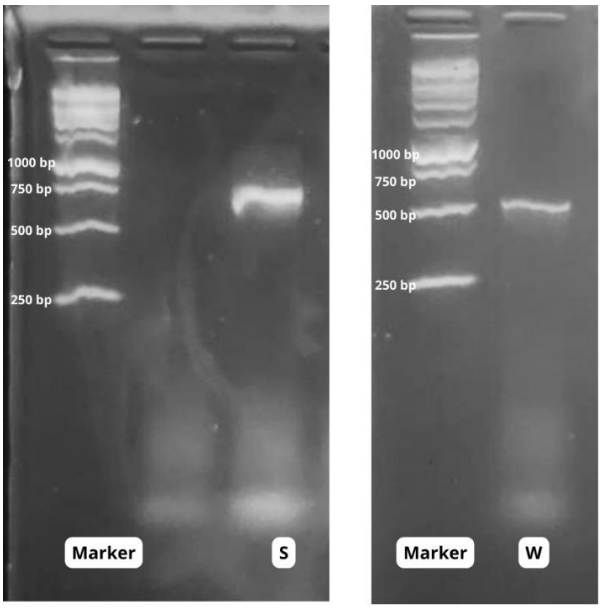

Figure 3. Gel electrophoresis of PCR products for *Serratia* and *Wolbachia*. *Lane S = Serratia, Lane W = Wolbachia.*

PCR amplification confirmed the primer specificity, with distinct DNA bands visible on a 1.5% agarose gel. The bands corresponding to *Serratia* (766 bp) and *Wolbachia* (556 bp) confirmed successful amplification of the target genes, as shown in Figure 3. This verification supports the primers' utility in microbiome profiling for these symbiotic bacteria within *Aedes aegypti*.

Phylogenetic analysis

Phylogenetic analysis of the Sanger sequencing data was conducted using MEGA X software (Hall, 2013; Kumar et al., 2018). The sequences obtained from the study, including *Serratia* and *Wolbachia* isolates, were aligned with reference sequences from the NCBI database to assess their evolutionary relationships. The Neighbor-Joining method was used to construct the phylogenetic tree, as shown in Figure 4 (Zou et al., 2024). The evolutionary history was inferred using the Neighbor-Joining method (Fernández et al., 2023), and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches.

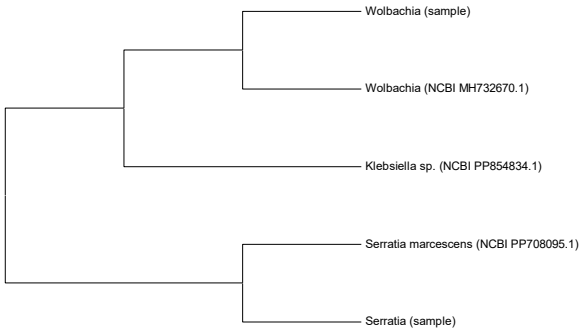

Figure 4. Phylogenetic tree of *Serratia* and *Wolbachia* strains constructed using MEGA X software.

Table 2. Primer sequences and predicted product sizes for *Serratia* and *Wolbachia*

Target Species	Primer Sequence (5' - 3')	Tm (°C)	%GC Content	Predicted Product Size (bp)
Serratia	Forward: [GCCATCAGATGTGCCCAGAT]	[60.18]	[55.00]	766
	Reverse: [GGTAAGGTTCTTCGCGTTGC]	[59.83]	[55.00]	
Wolbachia	Forward: [TACGGAGAGGGCTAGCGTTA]	[59.82]	[55.00]	556
	Reverse: [CCCAACATCTCACGACACGA]	[60.04]	[55.00]	

The tree shows the evolutionary relationships among the sample isolates and reference strains from the NCBI database: *Wolbachia* (NCBI MH732670.1), *Serratia marcescens* (NCBI PP708095.1), and *Klebsiella* sp. (NCBI PP854834.1).

The evolutionary distances were computed using the Maximum Composite Likelihood method and are expressed as the number of base substitutions per site (Al-Atiyat & Aljumaah, 2014). The rate variation among sites was modeled with a gamma distribution (shape parameter = 5). Codon positions included were 1st, 2nd, 3rd, and non-coding. All ambiguous positions were removed for each sequence pair using the pairwise deletion option (Mohd Salleh et al., 2023). A total of 741 positions were analyzed in the final dataset.

The genetic distances between taxa provide insights into the evolutionary relatedness of the analyzed sequences. The *Wolbachia* sample isolate showed a close genetic distance (4.9912) to the *Wolbachia* reference strain (NCBI MH732670.1) (Table 3), suggesting a high level of similarity and potential evolutionary conservation. The genetic distance between the *Serratia* sample and *Serratia marcescens* (NCBI PP708095.1) was 7.0582, indicating a related yet distinct relationship between the two.

Klebsiella sp. (NCBI PP854834.1) was used as an outgroup and showed a genetic distance of 6.6844 from *Wolbachia* (NCBI MH732670.1) and 6.8185 from the *Serratia* sample, underscoring the distinct evolutionary pathway compared to the other taxa. These findings highlight the genetic variation among the symbiotic bacteria associated with *Aedes aegypti*, contributing to our understanding of local adaptations and strain diversity. The strain diversity of microbiome is supported by the host-associated functions of its microbiome, as the composition of microbiomes can change over time, with hosts within a population exhibiting significant turnover in microbiome composition among individuals (Medeiros et al., 2022).

The phylogenetic analysis involved five nucleotide sequences and demonstrated that the sample isolates of *Serratia* and *Wolbachia* share evolutionary relationships with known reference strains, supporting their stable presence in the *Aedes aegypti* microbiome.

This study successfully demonstrated the in silico and in vitro design of specific primers for the 16S rDNA gene to identify key microbiome species, *Serratia* and *Wolbachia*, within *Aedes aegypti*. The study was conducted across Malang Raya, an area with diverse environmental contexts that provided a comprehensive sampling ground for *Aedes aegypti* populations. Morphological identification confirmed the accurate selection of the mosquito species, which was essential for ensuring reliable microbiome analysis.

Table 3. Genetic distance matrix among *Wolbachia*, *Serratia*, and reference strains

	***Wolbachia* NCBI**	***Wolbachia* sample**	***Serratia* NCBI**	***Serratia* sample**	***Klebsiella* NCBI**
Wolbachia NCBI					
Wolbachia sample	4.9912				
Serratia NCBI	16.6279	12.893			
Serratia sample	10.4884	6.4349	7.0582		
Klebsiella NCBI	6.6844	1.5103	8.0581	6.8185	

The primers designed for *Serratia* and *Wolbachia* showed high specificity, as evidenced by successful PCR amplification and subsequent Sanger sequencing. The sequences obtained aligned closely with reference strains from the NCBI database, specifically *Serratia marcescens* (NCBI PP708095.1) and *Wolbachia* (NCBI MH732670.1), indicating a stable association within *Aedes aegypti* populations. The genetic distance matrix further underscored

the evolutionary relationships, with the *Wolbachia* sample showing a close genetic distance to its reference strain (4.9912), suggesting high similarity and conservation. The *Serratia* sample, while related to *Serratia marcescens*, showed a genetic distance of 7.0582, indicating some degree of genetic diversity that may reflect local adaptations.

The results align with prior studies that have demonstrated the importance of *Serratia* and *Wolbachia* in modulating vector competence (Lee et al., 2023). *Serratia* has been noted for its insecticidal properties, which can impact mosquito survival and vectorial capacity, while *Wolbachia* is recognized for its ability to inhibit pathogen transmission and manipulate mosquito reproduction through cytoplasmic incompatibility (Liang et al., 2020). These findings contribute to a deeper understanding of the microbiome in *Aedes aegypti* and its potential applications in sustainable vector control strategies.

However, this study has some limitations. The sample size was restricted to specific districts in Malang Raya, which may limit the generalizability of the findings to other geographic regions. Additionally, the use of conventional PCR and Sanger sequencing, while effective, may not capture the full diversity of the microbiome compared to next-generation sequencing methods. Future studies should consider larger sample sizes and advanced sequencing technologies to provide a more comprehensive view of the mosquito microbiome and its functional implications

CONCLUSION

This study designed and validated specific primers for the 16S rDNA gene to detect *Serratia* and *Wolbachia* in *Aedes aegypti* through in silico and in vitro approaches. The findings highlight the presence and genetic relationships of these symbiotic bacteria, supporting their potential role in vector control strategies. Further research should expand the geographic scope and employ more comprehensive sequencing methods to better understand the microbiome's impact on mosquito biology and vectorial capacity.

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REFERENCES

Al-Atiyat, R. M., & Aljumaah, R. S. (2014). Genetic distances and phylogenetic trees of different Awassi sheep populations based on DNA sequencing. *Genetics and Molecular Research*, *13*(3), 6557–6568. https://doi.org/10.4238/2014.August.26.6

Ames, N. J., Ranucci, A., Moriyama, B., & Wallen, G. R. (2017). The Human Microbiome and Understanding the 16S rDNA Gene in Translational Nursing Science. *Nursing Research*, *66*(2), 184–197. https://doi.org/10.1097/NNR.0000000000000212

Ant, T. H., Mancini, M. V., McNamara, C. J., Rainey, S. M., & Sinkins, S. P. (2023). *Wolbachia* - Virus interactions and arbovirus control through population replacement in mosquitoes. *Pathogens and Global Health*, *117*(3), 245–258. https://doi.org/10.1080/20477724.2022.2117939

Bartoš, O., Chmel, M., & Swierczková, I. (2024). The overlooked evolutionary dynamics of 16S rDNA revises its role as the “gold standard” for bacterial species identification. *Scientific Reports*, *14*(1), 9067. https://doi.org/10.1038/s41598-024-59667-3

Da Silva, W. J., Pilz-Júnior, H. L., Heermann, R., & Da Silva, O. S. (2020). The great potential

of entomopathogenic bacteria Xenorhabdus and Photorhabdus for mosquito control: a review. *Parasites and Vectors*, *13*(1), 1–14. https://doi.org/10.1186/s13071-020-04236-6

Facchinelli, L., Badolo, A., & McCall, P. J. (2023). Biology and Behaviour of *Aedes aegypti* in the Human Environment: Opportunities for Vector Control of Arbovirus Transmission. *Viruses*, *15*(3), 636. https://doi.org/10.3390/v15030636

Fernández, A., Segura-Alabart, N., & Serratosa, F. (2023). The MultiFurcating Neighbor-Joining Algorithm for Reconstructing Polytomic Phylogenetic Trees. *Journal of Molecular Evolution*, *91*(6), 773–779. https://doi.org/10.1007/s00239-023-10134-z

Ferreira, Q. R., Lemos, F. F. B., Moura, M. N., Nascimento, J. O. de S., Novaes, A. F., Barcelos, I. S., FeDNAndes, L. A., Amaral, L. S. de B., Barreto, F. K., & Melo, F. F. de. (2023). Role of the Microbiome in Aedes spp. Vector Competence: What Do We Know? *Viruses*, *15*(3), 779. https://doi.org/10.3390/v15030779

Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P. J., Soen, Y., & Shental, N. (2018). Combining 16S rDNA gene variable regions enables high-resolution microbial community profiling. *Microbiome*, *6*(1), 17. https://doi.org/10.1186/s40168-017-0396-x

Gan, S. J., Leong, Y. Q., bin Barhanuddin, M. F. H., Wong, S. T., Wong, S. F., Mak, J. W., & Ahmad, R. B. (2021). Dengue fever and insecticide resistance in Aedes mosquitoes in Southeast Asia: a review. *Parasites & Vectors*, *14*(1), 315. https://doi.org/10.1186/s13071-021-04785-4

Gunara, N. P., Joelianto, E., & Ahmad, I. (2023). Identification of *Aedes aegypti* and Aedes albopictus eggs based on image processing and elliptic fourier analysis. *Scientific Reports*, *13*(1), 17395. https://doi.org/10.1038/s41598-023-28510-6

Hall, B. G. (2013). Building Phylogenetic Trees from Molecular Data with MEGA. *Molecular Biology and Evolution*, *30*(5), 1229–1235. https://doi.org/10.1093/molbev/mst012

Hassler, H. B., Probert, B., Moore, C., Lawson, E., Jackson, R. W., Russell, B. T., & Richards, V. P. (2022). Phylogenies of the 16S rDNA gene and its hypervariable regions lack concordance with core genome phylogenies. *Microbiome*, *10*(1), 104. https://doi.org/10.1186/s40168-022-01295-y

Hussain, M., Etebari, K., & Asgari, S. (2023). Analysing inhibition of dengue virus in *Wolbachia*-infected mosquito cells following the removal of *Wolbachia*. *Virology*, *581*, 48–55. https://doi.org/10.1016/j.virol.2023.02.017

Johnson, J. S., Spakowicz, D. J., Hong, B.-Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rDNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1

Jupatanakul, N., Pengon, J., Selisana, S. M. G., Choksawangkarn, W., Jaito, N., Saeung, A., Bunyong, R., Posayapisit, N., Thammatinna, K., Kalpongnukul, N., Aupalee, K., Pisitkun, T., & Kamchonwongpaisan, S. (2020). *Serratia marcescens* secretes proteases and chitinases with larvicidal activity against Anopheles dirus. *Acta Tropica*, *212*, 105686. https://doi.org/10.1016/j.actatropica.2020.105686

Khan, M. B., Yang, Z.-S., Lin, C.-Y., Hsu, M.-C., Urbina, A. N., Assavalapsakul, W., Wang,

W.-H., Chen, Y.-H., & Wang, S.-F. (2023). Dengue overview: An updated systemic review. *JouDNAl of Infection and Public Health*, *16*(10), 1625–1642. https://doi.org/10.1016/j.jiph.2023.08.001

Knoblauch, S., Su Yin, M., Chatrinan, K., de Aragão Rocha, A. A., Haddawy, P., Biljecki, F., Lautenbach, S., Resch, B., Arifi, D., Jänisch, T., Morales, I., & Zipf, A. (2024). High-resolution mapping of urban *Aedes aegypti* immature abundance through breeding site detection based on satellite and street view imagery. *Scientific Reports*, *14*(1), 18227. https://doi.org/10.1038/s41598-024-67914-w

Konkon, A. K., Padonou, G. G., Osse, R., Salako, A. S., Zoungbédji, D. M., Sina, H., Sovi, A., Tokponnon, F., Aïkpon, R., Noukpo, H., Baba-Moussa, L., & Akogbéto, M. C. (2023). Insecticide resistance status of *Aedes aegypti* and Aedes albopictus mosquitoes in southern Benin, West Africa. *Tropical Medicine and Health*, *51*(1), 22. https://doi.org/10.1186/s41182-023-00514-y

Kozlova, E. V, Hegde, S., Roundy, C. M., Golovko, G., Saldaña, M. A., Hart, C. E., Anderson, E. R., Hornett, E. A., Khanipov, K., Popov, V. L., Pimenova, M., Zhou, Y., Fovanov, Y., Weaver, S. C., Routh, A. L., Heinz, E., & Hughes, G. L. (2021). Microbial interactions in the mosquito gut determine *Serratia* colonization and blood-feeding propensity. *The ISME Journal*, *15*(1), 93–108. https://doi.org/10.1038/s41396-020-00763-3

Kozyreva, A. A., Zlotina, A. M., Golovkin, A. S., Kalinina, O. V., & Kostareva, A. A. (2021). Primer designing in Primer-BLAST. *Translational Medicine*, *8*(3), 37–52. https://doi.org/10.18705/2311-4495-2021-8-3-37-52

Kularatne, S. A., & Dalugama, C. (2022). Dengue infection: Global importance, immunopathology and management. *Clinical Medicine*, *22*(1), 9–13. https://doi.org/10.7861/clinmed.2021-0791

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lee, J. H., Kim, H. W., Mustafa, B., Lee, H. Il, & Kwon, H. W. (2023). The relationships between microbiome diversity and epidemiology in domestic species of malaria-mediated mosquitoes of Korea. *Scientific Reports*, *13*(1), 1–12. https://doi.org/10.1038/s41598-023-35641-3

Liang, X., Liu, J., Bian, G., & Xi, Z. (2020). *Wolbachia* Inter-Strain Competition and Inhibition of Expression of Cytoplasmic Incompatibility in Mosquito. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01638

Masluhiya AF, S., Wibowo, R. C. A., & Luthfin, A. (2022). Eksplorasi Sebaran Penyakit Demam Berdarah Dengue (DBD) dan Pneumonia di Kota Malang. *JUMANTIK (Jurnal Ilmiah Penelitian Kesehatan)*, *7*(2), 134. https://doi.org/10.30829/jumantik.v7i2.10402

Miesen, P., & van Rij, R. P. (2019). Crossing the Mucosal Barrier: A Commensal Bacterium Gives Dengue Virus a Leg-Up in the Mosquito Midgut. *Cell Host & Microbe*, *25*(1), 1–2. https://doi.org/10.1016/j.chom.2018.12.009

Miranti, K. A., Wahyuni, S., Permana, T. I., Fatmawati, D., & Nuryady, M. M. (2023). Desain Primer Spesifik Vektor Dengue *Aedes aegypti* Berdasarkan DNA Pengkode ITS-1, 5.8S Ribosomal DNA, dan ITS-2. *Jurnal Veteriner*, *24*(1), 76–82. https://doi.org/10.19087/jveteriner.202

3.24.1.76

Medeiros, M. C., Seabourn, P. S., Rollins, R. L., & Yoneishi, N. M. (2022). Mosquito microbiome diversity varies along a landscape-scale moisture gradient. *Microbial ecology*, 1-8. https://doi.org/10.1007/s00248-021-01865-x

Mohd Salleh, M. H., Esa, Y., & Gan, H. M. (2023). Characterization of the complete mitochondrial genome of the critically endangered Southern River Terrapin, Batagur affinis affinis (Reptilia: Geoemydidae). *Mitochondrial DNA Part B*, *8*(7), 719–722. https://doi.org/10.1080/23802359.2023.2222851

Montenegro, D., Cortés-Cortés, G., Balbuena-Alonso, M. G., Warner, C., & Camps, M. (2024). *Wolbachia*-based emerging strategies for control of vector-transmitted disease. *Acta Tropica*, *260*, 107410. https://doi.org/10.1016/j.actatropica.2024.107410

Rofiah, S. K., Nasikh Nasikh, & Grisvia Agustin. (2022). Rural Tourism As an Economic Potential for People in Malang Raya. *InteDNAtional JouDNAl of Economics and Management Research*, *1*(3), 130–143. https://doi.org/10.55606/ijemr.v1i3.50

Sofiana, L., Rokhmayanti, R., Martini, M., & Wulandari, D. A. (2023). Insecticide resistance of *Aedes aegypti* in Indonesia: a systematic review. *International Journal of Public Health Science*, *12*(3), 950-964. https://doi.org/10.11591/ijphs.v12i3.22843

Scolari, F., Casiraghi, M., & Bonizzoni, M. (2019). Aedes spp. and Their Microbiota: A Review. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02036

Supriyono, S., Soviana, S., Musyaffa, M. F., Novianto, D., & Hadi, U. K. (2023). Morphological characteristic of dengue vectors Aedes aegypti and Ae. albopictus (Family: Culicidae) using advanced light and scanning electron microscope. Biodiversitas Journal of Biological Diversity, *24*(2). https://doi.org/10.13057/biodiv/d240227

Silalahi, C. N., Tu, W.-C., Chang, N.-T., Singham, G. V., Ahmad, I., & Neoh, K.-B. (2022). Insecticide Resistance Profiles and Synergism of Field *Aedes aegypti* from Indonesia. *PLOS Neglected Tropical Diseases*, *16*(6), e0010501. https://doi.org/10.1371/journal.pntd.0010501

Tian, N., Zheng, J.-X., Guo, Z.-Y., Li, L.-H., Xia, S., Lv, S., & Zhou, X.-N. (2022). Dengue Incidence Trends and Its Burden in Major Endemic Regions from 1990 to 2019. *Tropical Medicine and Infectious Disease*, *7*(8), 180. https://doi.org/10.3390/tropicalmed7080180

Vulu, F., Futami, K., Sunahara, T., Mampuya, P., Bobanga, T. L., Mumba Ngoyi, D., & Minakawa, N. (2024). Geographic expansion of the introduced Aedes albopictus and other native Aedes species in the Democratic Republic of the Congo. *Parasites & Vectors*, *17*(1), 35. https://doi.org/10.1186/s13071-024-06137-4

Zou, Y., Zhang, Z., Zeng, Y., Hu, H., Hao, Y., Huang, S., & Li, B. (2024). Common Methods for Phylogenetic Tree Construction and Their Implementation in R. *Bioengineering*, *11*(5), 480. https://doi.org/10.3390/bioengineering11050480