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Diversity of symbiotic bacteria in the reproductive tracts and midgut of *Culex pipiens*

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ABSTRACT

Background: *Culex pipiens* is considered a vector for the transmission of infectious, viral, and parasitic diseases. To control this mosquito vector, investigating symbiotic bacteria colonized inside the mosquitoes' organs to impede the transmission of pathogens has been of recent interest to researchers.

Methods: This revision was intended to provide a comprehensive overview of the diversity of endosymbiont bacteria residing in different tissues of *Cx. pipiens* globally by using reports in Google Scholar, PubMed, and Science Direct until May 2024.

Result: The results showed that more than 50 bacterial genera have been reported in *Culex* tissues, in which the bacterial diversity was higher in the midgut than eggs or the whole body in *Cx. pipiens*. More than 65% of samples were insect-reared mosquitoes than wild types. Various methods based on culture-dependent and culture-independent approaches followed by molecular techniques such as 16SrRNA Illumina sequencing, Next Generation Sequencing (NGS), RNA shotgun metagenomic sequencing (RNA-seq), RT-PCR, and MALDI-TOF MS have been used for the recognition of bacteria at genera or species levels. The most favorable gene for molecular analysis via PCR is 16SrRNA; however, *the wsp* gene was another candidate used for identification of the bacterial community.

Conclusion: These results provide a library of defined bacteria associated with *Cx. pipiens*. The characterization of tissue-specific and host-specific bacterial communities sheds light on further studies pursuing the functional role of endosymbiont organisms in control strategies and vector biology.

Kewwors: Mosquito-borne pathogens, Mosquito microbiota, Paratransgenesis, Endosymbionts

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1. Introduction

Culex pipiens L. (Diptera: Culicidae), a widespread mosquito species, is considered a vector for the transmission of infectious diseases such as West Nile disease, Rif Valley fever, Japanese encephalitis, St. Louis encephalitis, Sindbis virus, and Lymphatic Filariasis (1–12) and also a probable vector associated with the Hepatitis C virus (HCV) (5, 13). To control this vector with medical and veterinary importance (14, 15), synthetic insecticides (e.g., organochlorine and organophosphate combinations) are the most favored approach (16). Due to the robust insecticide resistance in mosquito populations (17), resulting in detrimental effects on human health and the environment (18, 19), researchers are interested in inspecting various alternative strategies to control mosquitoes (15, 20–22).

Bacteria, fungi, viruses, and archaea are the microorganisms living inside insects' mid-gut and other organs that directly or indirectly control their reproduction, growth, and other physiological activities like immune system functions (23). Stagnant water sources, such as sewage pools, cesspools, and sewers, are suitable places for *Cx. Pipiens* populations are also associated with some commensal bacterial strains found inside mosquitoes' midguts (24). The microorganisms harbored by the midgut originated from the environment in which the larvae lived (25). As soon as they metamorphose into pupae, their microbiota will disappear, and bacteria from the larval phase are transferred to adult insects (25, 26). The similarity of bacterial communities found in different places facilitates the utilization of alternative strategies to control vectors.

Investigating symbiotic bacteria colonized inside the mosquitoes' organs to impede the transmission of pathogens has been of recent interest to researchers. Lately, investigations revealed the association between residing symbiotic bacteria inside the insect midgut and resistance to insecticides by direct metabolism of chemical insecticides (27) or indirectly growing the activity of some enzymes or augmenting gene expression (28). Due to the interactions between pathogens and other bacterial communities inside the vector's body for the transmission of specific diseases (24, 29), the characterization of their colonies reveals an influence on host biology and vector competence. For instance, bacteria harbored in the midgut have a significant role in the growth and development of mosquitoes, as well as digestion and absorption, nutrient metabolism, and regulation of the immune system (30–32). Thus, any modification in bacterial composition results in altered gene expression patterns and mosquito tissues (33, 34).

Alternatively, the use of antibiotics is common to control microbes inside mosquito guts; however, antibiotic resistance should be considered another challenge, not to omit the natural microbiota (35). Besides, the application of these components may have some influences on host immunity and metabolism in misquoted hosts and modify bacterial functionality (36). This revision was intended to provide a comprehensive overview of the diversity of endosymbiont bacteria residing in different tissues of *Cx. pipiens* globally by using different morphological and molecular detection approaches.

2. Materials and Methods

This revision was intended to provide a comprehensive overview of the diversity of endosymbiont bacteria residing in different tissues of *Cx. pipiens* globally using reports indexed in Google Scholar, PubMed, and Science Direct until May 2024. Key-words encompassed “mosquitoes and bacteria,” “*Cx. pipiens* and bacteria," “*Cx. pipiens* and microbiota," and “Culicidae and bacteria." These data were thoroughly extracted to provide a library of bacteria detected in *Cx. pipenes* worldwide and with various recognition methods.

3. Results and discussion

In this review, the bacterial communities detected in the mid-gut of *Cx. pipiens,* the main vector of several viral diseases, are summarized. The results showed that more than 50 bacterial genera have been reported in *Culex* including *Acinetobacter, Actinobacillus, Aeromonas, Anaplasma, Aquitalae, Asaia, Azospirillum, Bacillus, Bdellovibrio, Bosea, Bradyrhyzobium, Brevundimonas, Cedecea, Chryseobacterium, Clostridium, Comamonas, Cutibacterium, Dietzia, Dyadobacter, Elizabethkingia, Elstera, Enterobacter, Erwinia, Escherichia, Flavobacterium, Flectobacillus, Gluconobacter, Halomonas, Klebsiella, Lactiplantibacillus, Lactobacillus, Legionella, Leifsonia, Lentilactobacillus, Leucobacter, Methylobacterium, Moraxella, Morganella, Novosphingobium, Pantoea, Providencia, Pseudomonas, Ralstonia, Ralstonia, Ramlibacter, Rhodobacter, Rickettsiella, Salmonella, Serratia, Shigella, Sphingobacterium, Sphingomonas, Staphylococcus, Streptococcus, Thorsella, Variovorax, Wolbachia,* and *Xanthomonas* (Table 1). In addition, in some studies, the bacteria were reported at the family level or as an uncategorized bacterium. Proteobacteria have been recorded as the dominant phyla detected in Culicidae members (37, 38). Different bacterial strains of dissimilar phylums and classes have been recorded in *Culex* species’ residing mid-guts, salivary glands, and reproductive tracts (ovary and testes) in field-collected larvae and laboratory-reared samples (29, 39–43). Though these organs or tissues are recognized to harbor bacterial communities, more concentration is on the mosquito midgut, which has a vital role as the first obstacle that mosquito-borne pathogens encounter (44, 45).

The successful development of pathogens needs to overcome this barrier to be transmitted through the mesquite's next bite. In this regard, bacterial diversity was higher in the midgut than in eggs or the whole body in *Cx. pipiens*. More than 65% of samples were insect-reared mosquitoes than wild types. In my revision, no result was found about bacterial communities in the salivary gland, testis, or ovary. In these reports (Table 1), various methods based on culture-dependent and culture-independent approaches followed by molecular techniques such as 16SrRNA Illumina sequencing, Next Generation Sequencing (NGS), RNA shotgun metagenomic sequencing (RNA-seq), RT-PCR, and MALDI-TOF MS have been used for the recognition of bacteria at genera or species levels (9, 37, 42, 46, 57, 58).

Table 1. Bacterial diversity of *Culex pipiens* is reported at the genus and species level, in addition to more information about their hosts, tissues that they collected, geographical location, and experimental detection methods.

Bacteria phylum	Bacteria class	Bacteria order	Bacteria genus	Bacteria species	Mosquitoes species/ genders	Wild/ insectary reared	Tissue	Country	References	Detection methods
Pseudomonadota	Gammaproteobacteria	Pasteurellales	*Actinobacillus*	-	*Cx. pipiens*	W	H	Florida	(46)	RNA shotgun metagenomic sequncing
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Acinetobacter*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Acinetobacter*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Acinetobacter*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Gammaproteobacteria	Aeromonadales	*Aeromonas*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Gammaproteobacteria	Aeromonadales	*Aeromonas*	-	*Cx. pipiens* ♀♂	I	M, L, P	Argentina	(50)	Morphological methods
Pseudomonadota	Gammaproteobacteria	Aeromonadales	*Aeromonas*	*veronii*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Gammaproteobacteria	Aeromonadales	*Aeromonas*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Alphaproteobacteria	Rickettsiales	*Anaplasma*	-	*Cx. pipiens*	W	H	Florida	(46)	RNA shotgun metagenomic sequncing

Pseudomonadota	Alphaproteobacteria	Rhodospirillales	*Asaia*	*bogorensis, platycodi, siamensis, lannaensis, prunellae, spathodeae , astilbes*	*Cx. pipiens pallens* ♀♂ *Cx. pipiens pipiens* ♀♂ *Cx. pipiens molestus* ♀♂	W I	M, L, P	Hawaii, U.S.A. Kyoto, Japan New Jersey, U.S.A. Burkina Faso	(52)	PCR 16S rRNA, DNA sequencing
Pseudomonadota	Alphaproteobacteria	Rhodospirillales	*Asaia*	*Bogorensis,* sp., *platycodi*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Alphaproteobacteria	Rhodospirillales	*Azospirillum*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Bacillota	Bacilli	Bacillales	*Bacillus*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Bacillota	Bacilli	Bacillales	*Bacillus*	-	*Cx. pipiens*♀♂	W	M	Egypt	(53)	16srRNA genes sequencing
Bacillota	Bacilli	Bacillales	*Bacillus*	-	*Cx. pipiens*	I	M	Egypt	(54)	Culture and morphological methods
Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	*Bdellovibrio*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	*Bosea*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	*Bradyrhyzobium*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing

Pseudomonadota	Alphaproteobacteria	Caulobacterales	*Brevundimonas*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacteroidota	Flavobacteriia	Flavobacteriales	*Chryseobacterium*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Cedecea*	*neteri*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Bacillota	Clostridia	Eubacteriales	*Clostridium*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Comamonas*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Comamonas*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Comamonas*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Actinomycetota	Actinomycetia	Propionibacteriales	*Cutibacterium*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Actinomycetota	Actinomycetia	Mycobacteriales	*Dietzia*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacteroidota	Cytophagia	Cytophagales	*Dyadobacter*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Entrobacter*	-	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing

Pseudomonadota	Alphaproteobacteria	Rhodospirillales	*Elstera*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacteroidota	Flavobacteriia	Flavobacteriales	*Elizabethkingia*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacteroidota	Flavobacteriia	Flavobacteriales	*Elizabethkingia*	*miricola, anophelis*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Erwinia*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Escherichia/ Shigella*	-	*Cx. pipiens*	W	H	Florida	(46)	RNA shotgun metagenomic sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Escherichia*	*coli*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Escherichia*	*coli*	*Cx. pipiens*♀♂	W	M	Egypt	(53)	16srRNA genes sequencing
Bacteroidota	Flavobacteriia	Flavobacteriales	*Flavobacterium*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Bacteroidota	Flavobacteriia	Flavobacteriales	*Flavobacterium*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacteroidota	Flavobacteriia	Flavobacteriales	*Flectobacillus*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Alphaproteobacteria	Rhodospirillales	*Gluconobacter*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing

Pseudomonadota	Gammaproteobacteria	Oceanospirillales	*Halomonas*		*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Klebsiella*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Klebsiella*	-	*Cx. pipiens* ♀♂	I	M, L, P	Argentina	(50)	Morphological methods
Bacillota	Bacilli	Lactobacillales	*Lactiplantibacillus*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacillota	Bacilli	Lactobacillales	*Lactobacillus*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Actinomycetota	Actinomycetia	Micrococcales	*Leifsonia*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Gammaproteobacteria	Legionellales	*Legionella*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Bacillota	Bacilli	Lactobacillales	*Lentilactobacillus*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Actinomycetota	Actinomycetia	Micrococcales	*Leucobacter*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Actinomycetota	Actinomycetia	Micrococcales	*Leucobacter*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	*Methylobacterium*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Moraxella*	-	*Cx. pipiens*	W	H	Florida	(46)	RNA shotgun metagenomic sequencing

Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Morganella*	*morganii*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Alphaproteobacteria	Sphingomonadales	*Novosphingobium*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Pantoea*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Providencia*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Pseudomonas*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Pseudomonas*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Pseudomonas*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Gammaproteobacteria	Pseudomonadales	*Pseudomonas*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Ralstonia*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Ralstonia*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Ramlibacter*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene

Pseudomonadota	Alphaproteobacteria	Rhodobacterales	*Rhodobacter*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing
Pseudomonadota	Gammaproteobacteria	Legionellales	*Rickettsiella*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Salmonella*	-	*Cx. pipiens*♀♂	W	M	Egypt	(53)	16srRNA genes sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Serratia*	*Marcescens, nematodiphila*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Serratia*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Shigella*	-	*Cx. pipiens*	I	M	Egypt	(54)	Culture and morphological methods
Pseudomonadota	Gammaproteobacteria	Enterobacterales	*Shigella*	-	*Cx. pipiens*♀♂	W	M	Egypt	(53)	16srRNA genes sequencing
Bacteroidota	Sphingobacteriia	Sphingobacteriales	*Sphingobacterium*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Alphaproteobacteria	Sphingomonadales	*Sphingomonas*	-	*Cx. pipiens*	W	M	United States	(47)	Culture, biochemical, and morphological methods
Pseudomonadota	Alphaproteobacteria	Sphingomonadales	*Sphingomonas*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Alphaproteobacteria	Sphingomonadales	*Sphingomonas*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing

Bacillota	Bacilli	Bacillales	*Staphylococcus*	-	*Cx. pipiens*	I	M	Egypt	(54)	Culture and morphological methods
Bacillota	Bacilli	Bacillales	*Staphylococcus*	-	*Cx. pipiens*♀♂	W	M	Egypt	(53)	16srRNA genes sequencing
Bacillota	Bacilli	Lactobacillales	*Streptococcus*	-	*Cx. pipiens*	I	M	Egypt	(54)	Culture and morphological methods
Pseudomonadota	Gammaproteobacteria	-	*Thorsella*	*anophelis*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Betaproteobacteria	Burkholderiales	*Variovorax*	-	*Cx. pipiens pallens*♀	I	M	China	(49)	16SrRNA sequencing
Pseudomonadota	Alphaproteobacteria	Rickettsiales	*Wolbachia*	-	*Cx. pipiens/restuans* ♀	I	H	Canada	(55)	16SrRNA Illumina sequencing
Pseudomonadota	Alphaproteobacteria	Rickettsiales	*Wolbachia*	sp., *pipientis*	*Cx. pipiens quinquefasciatus*	I	M	Guangzhou, China	(51)	Metagenomic sequencing
Pseudomonadota	Alphaproteobacteria	Rickettsiales	*Wolbachia*	-	*Cx. pipiens*	W	M, E	United States, Illinois	(48)	MiSeq sequencing of the 16SrRNA gene
Pseudomonadota	Alphaproteobacteria	Rickettsiales	*Wolbachia*	-	*Cx. pipiens*♀	W	H	Sweden	(56)	morphological method and PCR (wsp gene)
Pseudomonadota	Gammaproteobacteria	Xanthomonadales	*Xanthomonas*	-	*Cx. pipiens* ♀	I	L, M	Champaign County, IL,	(43)	16SrRNA Illumina sequencing

Mid-gut (M), whole mosquito (H), larvae (L), pupae (P), egg (E), and wild (W) or insectary reared population (I) are specified.

To delimit bacterial diversity in mid-gut and reproductive tracts by molecular approaches like PCR, different primers for general bacteria and species-specific sets (such as *Asaia*) could be used, followed by DNA sequencing, which reveals their similarity through blast hits in the online database GenBank (NCBI). The most favorable gene for molecular analysis via PCR is 16SrRNA (43, 49, 52, 55); however, the *wsp* gene was another candidate used for the identification of the bacterial community in *Cx. pipiens* from Sweden (56).

Conclusion

These results provide a library of defined bacteria associated with *Cx. pipiens*. The diversity of bacteria potentially has implications for their differences in vector competence. The characterization of tissue-specific and host-specific bacterial communities sheds light on further studies pursuing the functional role of endosymbiont organisms in control and vector biology.

References

1. Van den Hurk AF, Ritchie SA, Mackenzie JS. Ecology and geographical expansion of the Japanese encephalitis virus. Annual review of entomology. 2009;54:17–35.
2. Fros JJ, Miesen P, Vogels CB, Gaibani P, Sambri V, Martina BE, et al. Comparative Usutu and West Nile virus transmission potential by local Culex pipiens mosquitoes in north-western Europe. One Health. 2015;1:31–6.
3. Meegan JM, Khalil GM, Hoogstraal H, and Adham FK. Experimental transmission and field isolation studies implicate Culex pipiens as a vector of the Rift Valley fever virus in Egypt. The American journal of tropical medicine and hygiene, 1980;29(6):1405–10.
4. Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, and Walker ED. Culex pipiens (Diptera: Culicidae) is a bridge vector of the West Nile virus to humans. Journal of medical entomology. 2008;45(1):125–8.
5. Turell MJ. Members of the Culex pipiens Complex as Vectors of Viruses. Journal of the American Mosquito Control Association. 2012;28(4s):123-6.
6. Diaz LA, Flores FS, Beranek M, Rivarola ME, Almirón WR, and Contigiani MS. Transmission of endemic St. Louis encephalitis virus strains by local Culex quinquefasciatus populations in Cordoba, Argentina. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2013;107(5):332-4.
7. Samy AM, Elaagip AH, Kenawy MA, Ayres CF, Peterson AT, and Soliman DE. Climate change influences the global potential distribution of the mosquito Culex quinquefasciatus, a vector of West Nile virus and lymphatic filariasis. PloS one. 2016;11(10):e0163863.
8. Bakhshi H, Mousson L, Vazeille M, Zakeri S, Raz A, de Lamballerie X, et al. High Transmission Potential of West Nile Virus Lineage 1 for Cx. pipiens sl. of Iran. Viruses. 2020;12(4):397.
9. Bakhshi H, Beck C, Lecollinet S, Monier M, Mousson L, Zakeri S, et al. Serological evidence of West Nile virus infection among birds and horses in some geographical locations of Iran. Veterinary medicine and science, 2021, 7(1):204–9.
10. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z, Zhou G, et al. Competence of Aedes aegypti, Ae. albopictus, and Culex quinquefasciatus mosquitoes as Zika virus vectors in China. Emerging infectious diseases. 2017;23(7):1085.

11. Pruck-Ngern M, Pattaradilokrat S, Chumpolbanchorn K, Pimnon S, Narkpinit S, Harnyuttanakorn P, et al. Effects of artesunate treatment on Plasmodium gallinaceum transmission in the vectors Aedes aegypti and Culex quinquefasciatus. Veterinary Parasitology. 2015;207(1-2):161–5.
12. Nourani L., Zakeri S., and Djadid ND. Dynamics of prevalence and distribution pattern of avian Plasmodium species and its vectors in diverse zoogeographical areas—a review. Infection, Genetics, and Evolution. 2020:104244.
13. EL-KHOLY S, EL-HUSSEINY I, Meshrif W, EL-AZM AA, Salem M. Does the mosquito Culex pipiens represent a potential vector of the hepatitis C virus? Medical and veterinary entomology. 2018;32(2):155–61.
14. Pettersson JH-O, Shi M, Eden J-S, Holmes EC, and Hesson JC. Meta-transcriptomic comparison of the RNA viromes of the mosquito vectors Culex pipiens and Culex torrentium in Northern Europe. Viruses. 2019;11(11):1033.
15. Yüksel E, Yıldırım A, İmren M, Canhilal R, Dababat AA. Xenorhabdus and photorhabdus bacteria as potential candidates for the control of Culex pipiens L. (Diptera: Culicidae), the principal vector of West Nile virus and lymphatic filariasis. Pathogens. 2023;12(9):1095.
16. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian journal of medical research. 2012;135(5):581–98.
17. Weiss B., Aksoy S. Microbiome influences on insect host vector competence. Trends in parasitology. 2011;27(11):514–22.
18. Stancu IG, Prioteasa FL, Tiron GV, Cotar AI, Fălcuță E, Porea D, et al. Distribution of Insecticide Resistance Genetic Markers in the West Nile Virus Vector Culex pipiens from South-Eastern Romania. Insects. 2022;13(11):1062.
19. Mastrantonio V, Porretta D, Lucchesi V, Güz N, Çağatay NS, Bellini R, et al. Evolution of adaptive variation in the mosquito Culex pipiens: multiple independent origins of insecticide resistance mutations. Insects. 2021;12(8):676.
20. Wilke ABB, Marrelli MT. Paratransgenesis is a promising new strategy for mosquito vector control. Parasites & vectors. 2015;8(1):1-9.
21. Djadid ND, Jazayeri H, Raz A, Favia G, Ricci I, and Zakeri S. Identification of the midgut microbiota of An. stephensi and An. maculipennis for their application as a paratransgenic tool against malaria. PloS one. 2011;6(12):e28484.
22. Koosha M, Vatandoost H, Karimian F, Choubdar N, Abai MR, and Oshaghi MA. Effect of serratia AS1 (Enterobacteriaceae: Enterobacteriales) on the fitness of Culex pipiens (Diptera: Culicidae) for paratransgenic and RNAi approaches. Journal of medical entomology. 2019;56(2):553–9.
23. Crotti E, Balloi A, Hamdi C, Sansonno L, Marzorati M, Gonella E, et al. Microbial symbionts are a resource for the management of insect-related problems. Microbial Biotechnology. 2012;5(3):307–17.
24. Minard G, Mavingui P, and Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. Parasites and vectors. 2013;6(1):1-12.
25. Guégan M, Zouache K, Démichel C, Minard G, Potier P, Mavingui P, and Moro CV. The mosquito holobiont: fresh insight into mosquito-microbiota interactions. Microbiome. 2018;6(1):1-17.
26. Gimonneau G, Tchioffo MT, Abate L, Boissière A, Awono-Ambéné PH, Nsango SE, et al. Composition of

Anopheles coluzzii and Anopheles gambiae microbiota from larval to adult stages. Infection, Genetics, and Evolution. 2014;28:715–24.

27. Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, and Fukatsu T. Symbiont-mediated insecticide resistance. Proceedings of the National Academy of Sciences. 2012;109(22):8618-22.
28. Pang R, Chen M, Yue L, Xing K, Li T, Kang K, et al. A distinct strain of Arsenophonus symbiont decreases insecticide resistance in its insect host. PLoS Genetics. 2018;14(10):e1007725.
29. Chandel K, Mendki MJ, Parikh RY, Kulkarni G, Tikar SN, Sukumaran D, et al. Midgut microbial community of Culex quinquefasciatus mosquito populations from India. PloS one. 2013;8(11):e80453.
30. Jing T-Z, Qi F-H, and Wang Z-Y. What are the most dominant roles of insect gut bacteria: digestion, detoxification, or essential nutrient provision? Microbiome. 2020;8(1):1-20.
31. Krams IA, Kecko S, Jõers P, Trakimas G, Elferts D, Krams R, et al. Microbiome symbionts and diet diversity incur costs on the immune system of insect larvae. Journal of Experimental Biology. 2017;220(22):4204–12.
32. Guégan M, Tran Van V, Martin E, Minard G, Tran FH, Fel B, et al. Who is eating fructose within the Aedes albopictus gut microbiota? Environmental microbiology. 2020;22(4):1193-206.
33. Lee JB, Park K-E, Lee SA, Jang SH, Eo HJ, Am Jang H, et al. Gut symbiotic bacteria stimulate insect growth and egg production by modulating hexamerin and vitellogenin gene expression. Developmental and Comparative Immunology. 2017;69:12–22.
34. Marra A, Hanson M, Kondo S, Erkosar B, and Lemaitre B. Drosophila antimicrobial peptides and lysozymes regulate gut microbiota composition and abundance. MBio. 2021;12(4):10.1128/mbio. 00824-21.
35. Steven B., Hyde J., LaReau JC, and Brackney DE. The axenic and gnotobiotic mosquito: emerging models for microbiome-host interactions. Frontiers in Microbiology. 2021;12:714222.
36. Moullan N, Mouchiroud L, Wang X, Ryu D, Williams EG, Mottis A, et al. Tetracyclines disturb mitochondrial function across eukaryotic models: a call for caution in biomedical research. Cell reports. 2015;10(10):1681–91.
37. Nourani L, Raz A, and Djadid ND. Isolation and identification of the microbiota of Culex quinquefasciatus for their application as paratransgenic tools in vector control. Iranian Journal of Microbiology, 2023, 15(2).
38. Rani A, Sharma A, Rajagopal R, Adak T, and Bhatnagar RK. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected Anopheles stephensi is an Asian malarial vector. BMC microbiology. 2009;9(1):96.
39. Pidiyar V, Kaznowski A, Narayan NB, Patole M, and Shouche YS. Aeromonas culicicola sp. nov., from the midgut of Culex quinquefasciatus. International Journal of Systematic and Evolutionary Microbiology. 2002;52(5):1723–8.
40. Pidiyar VJ, Jangid K, Patole MS, and Shouche YS. Studies on the cultured and uncultured microbiota of the wild Culex quinquefasciatus mosquito midgut based on 16S ribosomal RNA gene analysis. The American journal of tropical medicine and hygiene, 2004;70(6):597–633.
41. Chandel K, Parikh RY, Mendki MJ, Shouche YS, and Veer V. Isolation and characterization of Vagococcus sp. from the midgut of the Culex quinquefasciatus

(Say) mosquito. Journal of Vector Borne Diseases. 2015;52(1):52.

42. Mancini M, Damiani C, Accoti A, Tallarita M, Nunzi E, Cappelli A, et al. Estimating bacterial diversity in different organs of nine species of mosquitoes by next-generation sequencing. BMC microbiology. 2018;18(1):1–10.
43. Juma EO, Allan BF, Kim C-H, Stone C, Dunlap C, and Muturi EJ. Effect of life stage and pesticide exposure on the gut microbiota of Aedes albopictus and Culex pipiens L. Scientific Reports. 2020;10(1):1–12.
44. Dong Y., Manfredini F., Dimopoulos G. Implications of the mosquito midgut microbiota in the defense against malaria parasites. PLoS pathogens. 2009;5(5):e1000423.
45. Cirimotich CM, Dong Y, Clayton AM, Sandiford SL, Souza-Neto JA, Mulenga M, Dimopoulos G. Natural microbe-mediated refractoriness to Plasmodium infection in Anopheles gambiae. Science. 2011;332(6031):855-8.
46. Chandler JA, Liu RM, and Bennett SN. RNA shotgun metagenomic sequencing of northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi. Frontiers in Microbiology, 2015, 6:185.
47. Demaio J., Pumpuni CB., Kent M., and Beier JC. The midgut bacterial flora of wild Aedes triseriatus, Culex pipiens, and Psorophora columbiae mosquitoes. The American journal of tropical medicine and hygiene. 1996;54(2):219–23.
48. Juma EO, Kim C-H, Dunlap C, Allan BF, and Stone CM. Culex pipiens and Culex restuans egg rafts harbor diverse bacterial communities compared to their midgut tissues. Parasites & Vectors. 2020;13:1–12.
49. Lv W-X, Cheng P, Lei J-J, Peng H, Zang C-H, Lou Z-W, et al. Interactions between the gut micro-community and transcriptome of Culex pipiens pallens under low-temperature stress. Parasites & Vectors, 2023, 16(1):12.
50. Diaz-Nieto LM, D'Alessio C, Perotti MA, Beron CM. Culex pipiens development is greatly influenced by native bacteria and exogenous yeast. PLoS One. 2016;11(4):e0153133.
51. Wang Y-t, Shen R-x, Xing D, Zhao C-p, Gao H-t, Wu J-h, et al. Metagenome sequencing reveals the midgut microbiota makeup of Culex pipiens quinquefasciatus and its possible relationship with insecticide resistance. Frontiers in Microbiology. 2021;12:625539.
52. De Freece C, Damiani C, Valzano M, D'amelio S, Cappelli A, Ricci I, Favia G. Detection and isolation of the α-proteobacterium Asaia in Culex mosquitoes. Medical and veterinary entomology. 2014;28(4):438–42.
53. Adly E, Hegazy AA, Kamal M, and Abu-Hussien SH. Midguts of Culex pipiens L. (Diptera: Culicidae) as a potential source of raw milk contamination with pathogens. Scientific Reports, 2022, 12(1):13183.
54. Fouda M, Hassan M, Al-Daly A, and Hammad K. Effect of midgut bacteria of Culex pipiens L. on digestion and reproduction. Journal of the Egyptian Society of Parasitology. 2001;31(3):767-80.
55. Novakova E, Woodhams DC, Rodríguez-Ruano SM, Brucker RM, Leff JW, Maharaj A, et al. Mosquito microbiome dynamics: a background for the prevalence and seasonality of the West Nile virus. Frontiers in Microbiology. 2017;8:526.
56. Bergman A, Hesson JC. Wolbachia prevalence in the vector species Culex pipiens and Culex torrentium in a Sindbis virus-endemic region of Sweden. Parasites & Vectors. 2021;14(1):428.
57. Gonçalves GGA, Feitosa APS, Portela-Júnior NC, de Oliveira CMF, de Lima

Filho JL, Brayner FA, and Alves LC. Use of MALDI-TOF MS to identify the culturable midgut microbiota of laboratory and wild mosquitoes. Acta tropica. 2019;200:105174.

58. Tandina F., Almeras L., Koné AK, Doumbo OK, Raoult D., and Parola P. Use of MALDI-TOF MS and culturomics to identify mosquitoes and their midgut microbiota. Parasites and vectors. 2016;9(1):495