

# UC Merced

## UC Merced Previously Published Works

### Title

Wolbachia in mosquitoes from the Central Valley of California, USA

### Permalink

<https://escholarship.org/uc/item/6c4636z0>

### Journal

Parasites & Vectors, 13(1)

### ISSN

1756-3305

### Authors

Torres, Ryan  
Hernandez, Eunis  
Flores, Valeria  
et al.

### Publication Date

2020-12-01

### DOI

10.1186/s13071-020-04429-z

Peer reviewed

RESEARCH

Open Access



# *Wolbachia* in mosquitoes from the Central Valley of California, USA

Ryan Torres<sup>1</sup>, Eunis Hernandez<sup>1</sup>, Valeria Flores<sup>1</sup>, Jose Luis Ramirez<sup>2</sup> and Andrea L. Joyce<sup>1\*</sup>

## Abstract

**Background:** *Wolbachia* bacteria are widely distributed throughout terrestrial arthropod species. These bacteria can manipulate reproduction and influence the vector competence of their hosts. Recently, *Wolbachia* have been integrated into vector control programmes for mosquito management. A number of supergroups and strains exist for *Wolbachia*, and they have yet to be characterized for many mosquito species. In this study, we examined *Wolbachia* prevalence and their phylogenetic relationship to other *Wolbachia*, using mosquitoes collected in Merced County in the Central Valley of California.

**Methods:** Adult mosquitoes were collected from 85 sites in Merced County, California in 2017 and 2018. Traditional and quantitative PCR were used to investigate the presence or absence and the density of *Wolbachia*, using *Wolbachia*-specific 16S rRNA and *Wolbachia*-surface protein (*wsp*) genes. The supergroup of *Wolbachia* was determined, and Multilocus Sequence Typing (MLST) by sequencing five housekeeping genes (*coxA*, *gatB*, *ftsZ*, *hcpA* and *fbpA*) was also used to determine *Wolbachia* supergroup as well as strain.

**Results:** Over 7100 mosquitoes of 12 species were collected: *Aedes melanimon*, *Ae. nigromaculis*, *Ae. vexans*, *Ae. aegypti*, *Culex pipiens*, *Cx. stigmatosoma*, *Cx. tarsalis*, *Anopheles franciscanus*, *An. freeborni*, *An. punctipennis*, *Culiseta incidens* and *Cs. inornata*. Eight showed evidence of *Wolbachia*. To our knowledge, this study is the first to report detection of *Wolbachia* in five of these species (*Ae. melanimon*, *Cx. stigmatosoma*, *Cx. tarsalis*, *Cs. incidens* and *Cs. inornata*). *Culex pipiens* and *Cx. stigmatosoma* had a high frequency and density of *Wolbachia* infection, which grouped into supergroup B; *Cs. inornata* clustered with supergroup A. MLST comparisons identified *Cx. pipiens* and *Cx. stigmatosoma* as wPip strain type 9 supergroup B. Six species had moderate to low (< 14%) frequencies of *Wolbachia*. Four species were negative, *Ae. nigromaculis*, *An. franciscanus*, *An. freeborni* and *Ae. aegypti*.

**Conclusions:** New records of *Wolbachia* detection were found in mosquitoes from Merced County, California. *Culex stigmatosoma* and *Cs. inornata* were new records for *Wolbachia* supergroup B and A, respectively. Other species with *Wolbachia* occurred with low frequency and low density. Detection of *Wolbachia* in mosquitoes can be used to inform potential vector control applications. Future study of *Wolbachia* within *Cx. stigmatosoma* and *Cs. inornata* in California and through the range of these species could further explore *Wolbachia* infection in these two species.

**Keywords:** *Wolbachia*, Strain characterization, Supergroup, 16S rRNA, Multilocus sequence typing (MLST), *Culex pipiens*, *Culex stigmatosoma*, *Culiseta inornata*, *Aedes melanimon*, *Aedes aegypti*, Vector control

## Background

*Wolbachia pipientis* are a monophyletic group of obligate intracellular bacteria that belong to the order *Rickettsiales*. These endosymbionts were first discovered in the *Culex pipiens* mosquito [1, 2], and are now estimated to infect between 40–52% of arthropod species

\*Correspondence: [ajoyce2@ucmerced.edu](mailto:ajoyce2@ucmerced.edu)

<sup>1</sup> Public Health, University of California, 5200 North Lake Road, Merced, CA 95343, USA

Full list of author information is available at the end of the article



© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

[3, 4]. *Wolbachia* routinely infect their host's reproductive tissues, and they are capable of surviving in a variety of invertebrates [5–7]. *Wolbachia* are known to be transmitted vertically through maternal inheritance and have also been shown to transmit horizontally between species, genera, and orders [8–11]. *Wolbachia* infections can have a diverse range of effects depending on the host species, from commensal, mutualistic, to parasitic interactions [5].

In recent years, *Wolbachia* have been implemented for population control of vector species [12, 13]. This is largely a result of the reproductive alterations that *Wolbachia* induce within their hosts in a strain-specific manner [5, 14]. Such reproductive manipulations include termination of male offspring, feminization of genetic males, parthenogenesis, and cytoplasmic incompatibility [5, 15]. Cytoplasmic incompatibility is the only known phenotype to be expressed within mosquito species [16]; when infected males mate with uninfected females, viable offspring are not produced. Furthermore, *Wolbachia* have been shown to modulate host fitness and vector potential. For instance, studies have shown a protective effect of *Wolbachia* against infection with pathogenic RNA viruses [12, 14, 17–21]. In addition, *Wolbachia*-infections have shown other complex host-specific manipulations: they can have increased or decreased rates of reproductive phenotypes; reduced host life-span and egg viability [6, 22]; impact larval survival [23]; decreased female mosquito biting ability [24]; decreased relative abundance of resident bacteria [25]; and in some cases, increased viral susceptibility and host mortality [26, 27]. Artificial infection of this endosymbiont into arthropod vectors has been shown to impact transmission of vector-borne diseases including lymphatic filariasis, West Nile virus, chikungunya, dengue, Zika, and avian malaria [14, 19, 20, 26].

*Wolbachia* offers a potential effective alternative to traditional chemical pesticide applications for the control of disease vectors, for example through cytoplasmic incompatibility. Cytoplasmic incompatibility was first proposed as a method of biological control for *Culex pipiens fatigans* in 1967, although initially it was not attributed to *Wolbachia* [28]. Since then, the use of *Wolbachia*-mediated incompatible technique strategies have been studied for pest control of a number of insects including *Aedes aegypti*, *Ae. albopictus*, *Ae. polynesiensis*, *Ceratitits capitata*, *Rhagoletis cerasi*, *Glossina morsitans*, *Culex pipiens* and *Cx. quinquefasciatus* [28–34]. This method of control aims to reduce vector populations through the introduction of *Wolbachia*-infected 'sterile' males, which compete with uninfected males for mates at the release site. *Aedes aegypti* do not naturally harbor *Wolbachia*; when *Wolbachia* have been detected in *Ae. aegypti* [35–39], the

range of detected strain types suggest they may be due to environmental contamination [40].

Naturally uninfected arthropod species like *Ae. aegypti* can be amenable to *Wolbachia*-infection through microinjection of the endosymbiont from another insect species into developing embryos [41, 42]. Currently, eight novel strains (*wAlbA*, *wAlbB*, *wAu*, *wMel*, *wMelCS*, *wMelPop-CLA*, *wPip* and *wRi*) have been transfected into *Ae. aegypti* to be evaluated for vector control applications [42–46]. *Aedes aegypti* is widespread in tropical and subtropical regions globally [47, 48], and since its detection in California in 2011 it has become widespread in southern California and the Central Valley [49, 50]. One example of where *Wolbachia*-infected *Ae. aegypti* males have been used to reduce mosquito populations through cytoplasmic incompatibility is through the DeBug Fresno California programme, which released male *Ae. aegypti* with the *wAlbB* strain of *Wolbachia* to reduce local *Ae. aegypti* populations [34]. A second method of using *Wolbachia*-infections for mosquito control relies on the introduction of *Wolbachia*-infected male and female mosquitoes to replace uninfected mosquito populations [12, 51, 52]. *Aedes aegypti* populations (each with a unique *Wolbachia* strain, *wMel* or *wAlbB*), have been introduced into regions of Australia and Malaysia, respectively [12, 52, 53]. Both strains were shown to reduce the incidence of dengue virus infections [53, 54].

Each *Wolbachia* strain has particular biological characteristics when moved into another vector, and identification of strains is key. Supergroups are used to differentiate major phylogenetic subdivisions within *Wolbachia pipiens* [55]. The 16S rRNA gene and the *Wolbachia*-surface protein (*wsp*) have been used to characterize supergroups [55, 56]. Within supergroups, *Wolbachia* strains are identified and can be characterized by multilocus sequence typing (MLST) which relies on five conserved bacterial housekeeping genes (*gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA*). Strains are commonly characterized based on the host species in which they are first identified [55, 57, 58]. For instance, *wPip* is the strain of *Wolbachia* which was identified from the *Culex pipiens* mosquito species. Mosquitoes can be singly or superinfected with more than one *Wolbachia* strain, or infected with multiple variants of the same strain [31].

Merced County is located in the Central Valley of California and includes a diverse range of habitats and mosquito species. While previous studies have identified the presence or absence of *Wolbachia* within some mosquito species throughout California using traditional PCR [58], the current infection status for species in the Central Valley of California and Merced County is unknown. *Wolbachia*-infected mosquitoes as a method of mosquito

control has great potential globally, and this vector-control method continues to be developed and refined.

The objectives of this study were to determine the presence or absence of *Wolbachia* in twelve mosquito species collected throughout Merced County, and to characterize the *Wolbachia* supergroup and strain for species with detections. Our study expands current knowledge of *Wolbachia* presence in mosquitoes in Merced and in the Central Valley of California, and would aid in the design of future *Wolbachia*-based mosquito control applications.

## Methods

### Mosquito collections

Adult mosquitoes were collected weekly from June to September, in both 2017 and 2018, using Encephalitis Vector Survey (EVS) traps (Bioquip, Rancho Dominguez, CA, USA) in Merced County. Traps sites were selected for habitats known to harbor the different species of mosquitoes. The EVS traps contained (1–2 kg) of dry ice (carbon dioxide) per container as an attractant for host-seeking female mosquitoes. Traps were hung on trees or fences in close proximity to a water source. The GPS coordinates of the site were recorded using a Garmin etrex High Sensitivity GPS unit (Garmin Ltd., Olathe, KS, USA). Traps were placed during the early afternoon and retrieved the following morning. Samples were transported on ice to a – 20 °C laboratory freezer. Adults were identified on a cold plate using a taxonomic key specific to Californian mosquitoes [59] and stored in 1.5 ml Eppendorf tubes until DNA extraction.

*Aedes aegypti* larvae were collected in addition to adults from several sites in Merced, California. Larvae were collected from water sources, transported to the lab, and reared at laboratory temperature in a BugDorm (MegaView Science, Taichung, Taiwan). Emerged adults were stored at – 20 °C and identification was confirmed using a taxonomic key. A map of trapping locations was constructed for 2017 and 2018 using qGIS v3.8.3-Zanzibar [60]. The Census TIGER/Line file for Merced County, California was retrieved ([www.census.gov/cgi-bin/geo/shapefiles2010/main](http://www.census.gov/cgi-bin/geo/shapefiles2010/main)), and site location GPS coordinates were overlaid on the county map.

### DNA extraction

The whole body of the mosquito was used for individual extractions of genomic DNA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's protocol for tissue extraction with a 2 h incubation at 65 °C [61]. Extracted samples were stored at – 20 °C. The DNA quantity was measured using the Qubit® dsDNA HS Assay kit (Life

Technologies, Carlsbad, CA, USA). The quantity of DNA in the samples averaged 10–15 ng/μl.

### Screening samples for *Wolbachia* and relative *Wolbachia* density determination

Presence or absence of *Wolbachia* was determined by amplicon detection of the *Wolbachia*-specific 16S rRNA gene and the general *Wolbachia* surface protein (*wsp*) via qPCR in individual field-collected mosquitoes. For each mosquito species collected, a subset of individuals was screened for *Wolbachia*, and individuals tested came from multiple sites or collection dates (Additional file 1: Table S1). The primer combinations for the *Wolbachia*-16S rRNA gene and *Wolbachia*-surface-protein (*wsp*) used in our assays are detailed on Table 1. The qPCR cycling conditions were those recommended for the master mix and consisted of holding at 95 °C for 10 min and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A melt curve stage at the end of the reaction was included. Each sample was analyzed in duplicates (technical replicates), and a non-template control was included. The qPCR assays were run on Applied Biosystems 5700 Fast Real-time PCR (Applied Biosystems, Foster City, CA, USA).

The relative *Wolbachia* density was determined via qPCR for two species, *Culex pipiens* and *Culex stigmatosoma*. Relative density was determined by measuring the signal amplifications of the *Wolbachia* 16S rRNA or *wsp* gene and the respective reference gene for each mosquito species. The *RpS3* gene was used as a reference gene and primers specific for this location (Table 1) were employed to compare *Wolbachia* densities in the collected samples. The *RpS3* gene is known to be a single copy gene in mosquitoes [62] and is described to be highly conserved [63]. *Culex pipiens* is known to be naturally infected with *Wolbachia* was used as a control to compare the relative *Wolbachia* density to *Cx. stigmatosoma*. Samples were compared and the data was analyzed post-run using the  $\Delta\Delta$  Ct method [64]. Data were evaluated using the GraphPad Prism 8.4.2 statistical software, comparing the two species using Student's t-test.

### Determination of *Wolbachia* supergroups

A subset of samples that screened positive for *Wolbachia* by qPCR were used to characterize the *Wolbachia* supergroup. Samples were run using the *Wolbachia* *wsp* supergroup A and *wsp* supergroup B primers [65] (Table 1). Polymerase chain reaction (PCR) was performed using a mixture of 2 μl of DNA, 1 μl of each forward and reverse primer at 10 μM concentration, 1 μl of Taq polymerase, 5 μl of buffer, 1 μl of dNTPs (2.5 μM) (Takara-Clontech Bio, Mountain View, CA, USA) and 40 μl of sterile water to make the reaction volume of 51 μl. The temperature profile for *wsp* amplification was the following: initial

denaturation for 3 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, a final elongation of 10 min at 72 °C and a final hold at 4 °C, modified from the protocol in Zhou et al. [65]. Amplification was confirmed by visualizing products on an agarose gel. Products were purified using USB Exo-sap-it® (Affymetrix Inc., Santa Clara, CA, USA) PCR cleanup kit. Each forward and reverse sequence reaction was prepared using 1 µM primers, 2 µl purified water, and 10 µl purified PCR product per reaction and sequenced on an Applied Biosystems 3730xl DNA Analyzer at the UC Berkeley DNA Sequencing Facility. Multilocus sequence typing (MLST) was also used to characterize supergroups (described below).

#### Strain characterization by multilocus sequence typing (MLST)

Species with samples which were successfully sequenced for supergroup A or B were also sequenced by multilocus sequence typing (MLST) using the standard primers for five ubiquitous bacterial housekeeping genes: *coxA*, *gatB*, *ftsZ*, *fbpA* and *hcpA* [57] (Table 1). The PCR mix for each gene used a mixture of 2 µl of DNA, 1 µl of each forward and reverse primer at 10 µM concentration, 1 µl of Taq polymerase, 5 µl of buffer, 1 µl of dNTPs (2.5 µM) (Takara-Clontech Bio Inc., Mountain View, CA, USA) and 40 µl of sterile water to make the reaction volume of

51 µl. The PCR temperature profile for four of the genes (*coxA*, *gatB*, *ftsZ* and *hcpA*) was the following: initial denaturation for 2 min at 94 °C, followed by 37 cycles of 30 s at 94 °C, 45 s at 54 °C, and 1.5 min at 72 °C, a final elongation for 10 min at 72 °C and a final hold at 4 °C [57]; the PCR program for the *fbpA* gene was identical except the annealing was for 45 s at 59 °C. PCR amplification was visually confirmed on agarose gels, products purified by USB Exo-sap-it®, and sequencing reactions were similar to those previously described.

#### Sequence analysis

*Wolbachia* surface protein (*wsp*) and the MLST genes (*coxA*, *gatB*, *ftsZ*, *hcpA* and *fbpA*) sequence files were viewed, edited, and aligned in Geneious Prime 2020.05. Consensus sequences were generated and exported as FASTA files. Consensus sequences were queried using the BLASTn program to find sequences with the highest similarity.

*Wolbachia* supergroup sequences from this study were combined with high similarity sequences from GenBank and others from a study by Carvajal et al. [38] to produce a Neighbor-Joining tree. Included in the tree were consensus sequences of 18 samples from this study [*Cx. pipiens* (*n* = 8) *Culex stigmatosoma* (*n* = 9) and *Culiseta inornata* (*n* = 1)] and an additional 15 *wsp* sequence

**Table 1** Primer sequences used for diagnostic testing of *Wolbachia*

Test	Gene target	PCR product (bp)	Primer name	Sequence (5'-3')	References
<i>Wolbachia</i> presence	16S rRNA	438	W16S-F W16S-R	CATACCTATTCGAAGGGATAG TTGCGGGACTTAACCCAACA	[56, 95, 96]
	<i>wsp</i> (General)	185	<i>wsp</i> -F <i>wsp</i> -R	GCATTTGTTAYAAAATGGACGA GGAGTGATAGGCATATCTTCAAT	[97]
<i>Wolbachia</i> density	<i>RpS3</i>	70	RpS3-F RpS3-R	AGCGTGCCAAGTCGATGAG ACGTACTCGTTGCACGGATCTC	[98]
Supergroup A/B identification	<i>wsp</i> (Supergroup A)	556	136F 691R	TGAAATTTTACCTCTTTT AAAAATTAACGCTACTCCA	[65]
	<i>wsp</i> (Supergroup B)	442	81F 522R	TGGTCCAATAAGTGATGAAGAAAC ACCAGCTTTTGCTTGATA	
Multilocus sequence typing	<i>gatB</i>	396	gatB_F1 gatB_R1	GAKTTAAAYCGYGCAGGBGTT TGGYAAAYTCRGGYAAAGATGA	[57]
	<i>coxA</i>	402	coxA_F1 coxA_R1	TTGGRGCRATYAACCTTATAG CTAAAGACTTTKACRCCAGT	
	<i>hcpA</i>	444	hcpA_F1 hcpA_R1	GAAATARCAGTTGCTGCAAA GAAAGTYRAGCAAGYCTCG	
	<i>ftsZ</i>	435	ftsZ_F1 ftsZ_R1	ATYATGGARCATAAARGATAG TCRAGYAATGGATTRGATAT	
	<i>fbpA</i>	429	fbpA_F1 fbpA_R1	GCTGCTCCRCTTGGYWTGAT CCRCCAGARAAAAYACTATTC	

files from GenBank which represented 11 genera previously confirmed with detections of *Wolbachia*. The species selected for comparison were *Aedes albopictus* (AF020058, AF020059), *Brugia malayi* (AJ252061), *Culex pipiens* (AF020061), *Culex quinquefasciatus* (AF020060), *Dirofilaria immitis* (AJ252062), *Drosophila melanogaster* (AF020072), *Drosophila simulans* (AF020070), *Glossina austeni* (AF020077), *Glossina morsitans* (AF020079), *Muscidifurax uniraptor* (AF020071) and *Phlebotomus papatasi* (AF020082) [38], and three additional sequences (*Loxoblemmus* sp. MG97910, *Myrmecophilus* sp. MK995471 and *Cerapachys augustae* KC137155) of high similarity. These 33 sequences were subjected to multiple sequence alignment using the ClustalW algorithm in MEGA 7.0. The Gamma distributed, Tamura 3-parameter substitution model was chosen based on the lowest Bayesian information criterion. A Neighbor-Joining tree was constructed using 1000 bootstraps in MEGA 7.0 [66].

*Wolbachia* strains were characterized by concatenating the *coxA*, *gatB*, *ftsZ*, *hcpA* and *fbpA* gene sequences from each sample in Geneious. Following concatenation, each sequence was exported in FASTA format and queried against the *Wolbachia* MLST database (<https://pubmlst.org/Wolbachia/>) to determine allelic profiles [57, 67]. An exact match with the queried database was necessary to distinguish profile composition. All sequences were submitted to Genbank.

## Results

### Mosquito collections, identification and abundance

In total, 12 mosquito species from 4 genera were collected from 85 sites within Merced county in 2017 and 2018 (Table 2, Additional file 1: Table S1). There was a total of 7150 mosquitoes identified to species. The species collected were the following: *Aedes melanimon*, *Aedes vexans*, *Aedes nigromaculis*, *Aedes aegypti*, *Culex stigmatosoma*, *Culex pipiens*, *Culex tarsalis*, *Anopheles franciscanus*, *Anopheles freeborni*, *Anopheles punctipennis*, *Culiseta incidens*, *Culiseta inornata* (Table 2). These species represent the diversity of nearly every mosquito from the region where trapping occurred [59]. The 85 trap sites were in the vicinity of 8 cities within Merced county: Atwater, Ballico, Hilmar, Le Grand, Los Banos, Merced, Snelling and Winton (Fig. 1, Table 2). Each mosquito species was trapped from two to five different regions of the county (Table 2, Additional file 1: Table S1), to provide geographic diversity in samples which were tested. Some mosquito species were more abundant than others. For example, *Cx. pipiens* and *Cx. tarsalis* were trapped in cities as well as in rural sites (Additional file 1: Table S1). *Ae. melanimon* and *Ae.*

*vexans* adults were most abundant within rural wetland habitats. *Aedes aegypti* was found in several Merced neighborhoods and near the Merced Zoo. *Anopheles franciscanus*, *An. freeborni* and *An. punctipennis* were found at rural riparian sites. *Culex stigmatosoma* were numerous at a semi-natural rural site near dairy runoff. *Aedes nigromaculis*, *Cs. incidens* and *Cs. inornata* were collected from rural and residential properties.

### *Wolbachia* screening with qPCR

For each species, 30–50 mosquitoes were typically screened for the presence or absence of *Wolbachia*, except for a few species which had smaller numbers of individuals collected (Table 2). A total of 406 mosquitoes were screened for *Wolbachia* prevalence using qPCR, and all mosquitoes screened were females. *Wolbachia* was detected within 73 of the 406 samples tested, and sites with mosquitoes positive for *Wolbachia* were found throughout the county (Table 2, Additional file 1: Table S1). Eight species within four genera tested positive for *Wolbachia* (Table 2). The frequency and percent of samples positive for each species from highest to lowest was the following: *Cx. stigmatosoma* (30/34; 88.2%), *Cx. pipiens* (31/37; 83.8%), *Cs. inornata* (1/7; 14.3%), *Ae. melanimon* (6/55; 10.9%), *An. punctipennis* (1/19; 5.3%), *Cx. tarsalis* (1/26; 3.9%), *Ae. vexans* (2/52; 3.9%) and *Cs. incidens* (1/42; 2.4%) (Table 2). Species where no *Wolbachia* was detected were *An. freeborni*, *An. franciscanus*, *Ae. nigromaculis* and *Ae. aegypti* (Table 2).

Each species was screened by qPCR for *Wolbachia* with two primers. For *Cx. pipiens* and *Cx. stigmatosoma*, all individuals were positive for *Wolbachia* when tested with both genes (*16S* rRNA and *wsp*) (Table 2). In a few cases, one primer would detect *Wolbachia*, while another would not (Table 2). For *Ae. melanimon*, *Cx. tarsalis*, *Cs. incidens*, *Cs. inornata*, *An. punctipennis* and *Ae. vexans*, *Wolbachia* was detected in very few individuals (Table 2). For *Cs. incidens* and *Cs. inornata*, both primers detected only one positive individual (Table 2). Six individuals were positive detections with the *16S* rRNA primer set but were negative with *wsp* (one *An. punctipennis*, one *Cx. tarsalis*, two *Ae. vexans* and two *Ae. melanimon*). Only one sample was negative with *16S* rRNA but positive for *wsp* (*Ae. melanimon*) (Table 2, Additional file 1: Table S1).

To evaluate the relative *Wolbachia* density of the two *Culex* spp., we conducted a relative comparison using qPCR for 30 individuals each of *Culex stigmatosoma* and *Cx. pipiens*, the later which was used as a control. The relative *Wolbachia* density comparison indicated no significant difference between the two species (*16S*, t-test,  $t = 0.80$ ,  $df = 48$ ,  $P = 0.43$ ; *wsp*, t-test,  $t = -1.34$ ,  $df = 48$ ,  $P = 0.18$ ).

### **Wolbachia supergroup identification**

*Wolbachia* supergroup identification was carried out by PCR of samples using general *wsp* supergroup A and supergroup B primers. A total of 18 *Wolbachia* surface protein sequences were generated from three species, *Cx. pipiens* ( $n = 8$ ), *Cx. stigmatosoma* ( $n = 9$ ) and *Cs. inornata* ( $n = 1$ ). *Wsp* sequences were not successfully obtained from the other species with low frequency *Wolbachia* detections (Table 2). The sequences produced in this study were combined with an additional 15 *wsp* sequences from GenBank for supergroup comparison (described above). The *Cx. pipiens* and *Cx. stigmatosoma* individuals grouped with the reference supergroup B samples, and *Cs. inornata* grouped with supergroup A reference samples (Fig. 2).

### **Wolbachia strain characterization**

There were five individual *Cx. pipiens* which had 5 MLST genes (*coxA*, *gatB*, *ftsZ*, *hcpA* and *fbpA*) successfully sequenced (*Cx. pipiens* nos. 29, 31, 32, 34 and 35) and they were matches with strain type 9, *wPip* supergroup B *Wolbachia* in the MLST database (Table 3). Four additional *Cx. pipiens* were similar at 3 or 4 of the five gene sequences to strain type 9 *wPip*; however, these had a low quality *hcpA* sequences and exact match of that allele could not be confirmed.

For *Cx. stigmatosoma*, two samples had complete gene sequences for the five MLST genes (*coxA*, *gatB*, *ftsZ*, *hcpA* and *fbpA*); the allelic profile for *Cx. stigmatosoma* samples 10 and 15 from Ballico were a match for the five sequences retrieved from several *Cx. pipiens* samples (nos. 29, 31, 32, and 35), and these were characterized as *Wolbachia wPip* supergroup B strain-type 9 (Table 3).

The two *Cx. stigmatosoma* individuals had four of the five MLST genes sequenced and also matched strain type 9, but only partial sequences were obtained for the *hcpA* gene. The *hcpA* locus has been observed with variable sequence lengths, ranging from 435 to 477 bp (pubmlst.org/*Wolbachia*). Five additional samples (*Cx. stigmatosoma* nos. 16,17, 20, 25, 38) were sequenced at 3 or 4 of the 5 genes, which also had matching profiles to *Wolbachia* housekeeping genes (*coxA*, *ftsZ* and *fbpA*) from this study.

One *Cs. inornata* sample had a detection of *Wolbachia* with 16S rRNA gene, and this individual was used to generate sequence data for the five MLST genes. The one *Cs. inornata* had four sequences (*fbpA*, *gatB*, *coxA*, *ftsZ*) which had mlst allele matches; these sequences matched *fbpA* allele 277, *gatB* 312, *coxA* 236, and *ftsZ* 154, while *hcpA* had no match [67]. For *Cs. inornata*, the *wsp* sequence grouped with others in supergroup A.

### **Discussion**

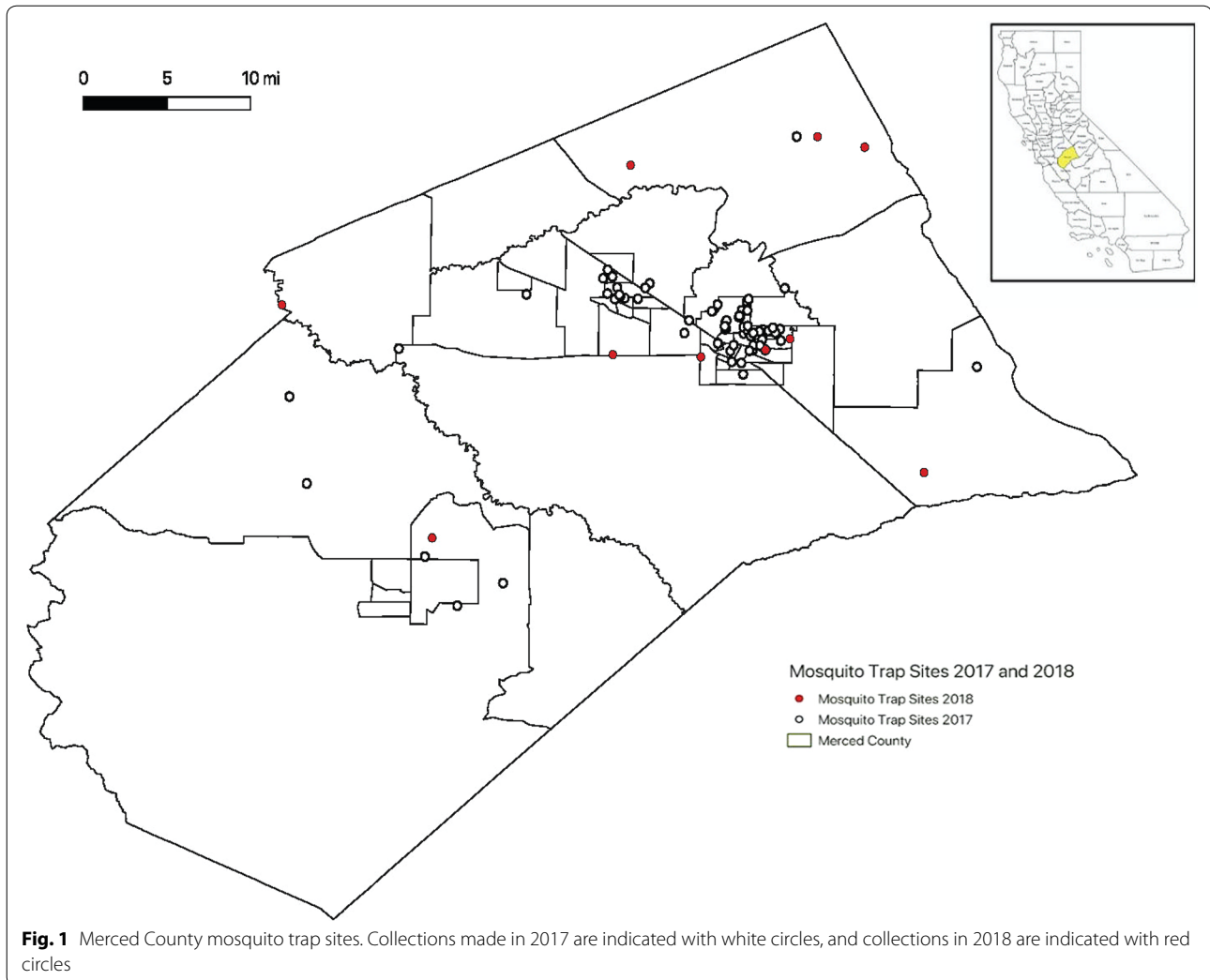
This study screened 12 field-collected mosquito species in the Central Valley of California for the presence or absence of *Wolbachia*, and for species with *Wolbachia* detections, attempted to characterize the supergroup and strain type. The 12 mosquito species identified and screened were the following: *Ae. melanimon*, *Ae. nigromaculis*, *Ae. vexans*, *Ae. aegypti*, *Cx. pipiens*, *Cx. stigmatosoma*, *Cx. tarsalis*, *An. franciscanus*, *An. freeborni*, *An. punctipennis*, *Cs. incidens* and *Cs. inornata*. *Wolbachia* was detected in eight of the mosquito species. To our knowledge, this study is the first to report *Wolbachia* detection in five of these species (*Ae. melanimon*, *Cx. stigmatosoma*, *Cx. tarsalis*, *Cs. incidens* and *Cs. inornata*), while three species which were positive in

**Table 2** Mosquito species collected and screened for *Wolbachia* by qPCR of 16S rRNA gene and *WSP*

Mosquito species	Total trapped	Atwater	Ballico	Hilmar	Le Grand	Los Banos	Merced	Snelling	<i>wsp</i> <sup>a</sup>	16S <sup>a</sup>	Total <sup>b</sup>
<i>Ae. melanimon</i>	1827	–	–	5/26	–	1/20	–	0/9	4/55	5/55	6/55 (10.9%)
<i>Ae. nigromaculis</i>	12	–	0/1	–	–	– 0/8	0/3	–	–	–	0/12 (0%)
<i>Ae. vexans</i>	488	–	–	2/36	–	0/16	–	–	0/52	2/52	2/52 (3.9%)
<i>Ae. aegypti</i>	60	–	–	–	–	–	0/60	–	–	–	0/60 (0%)
<i>Cx. pipiens</i>	994	5/5	15/15	–	–	–	10/10	1/7	31/37	31/37	31/37 (83.8%)
<i>Cx. stigmatosoma</i>	36	2/2	28/28	–	–	–	0/1	0/3	30/34	30/34	30/34 (88.2%)
<i>Cx. tarsalis</i>	3878	–	1/15	–	0/5	–	0/4	0/2	0/26	1/26	1/26 (3.9%)
<i>An. franciscanus</i>	2	–	–	–	–	–	–	0/2	–	–	0/2 (0%)
<i>An. freeborni</i>	221	–	0/29	0/1	0/22	–	0/1	0/7	–	–	0/60 (0%)
<i>An. punctipennis</i>	19	–	–	–	–	0/1	–	1/18	0/19	1/19	1/19 (5.3%)
<i>Culiseta incidens</i>	94	–	–	–	0/1	–	1/35	0/6	1/42	1/42	1/42 (2.4%)
Total	7150	7/7	44/88	7/63	0/28	1/45	11/114	3/60	67/406	72/406	73/406

<sup>a</sup> Number positive/Number tested

<sup>b</sup> Percent of samples screened positive for *Wolbachia* by either *wsp* or 16S rRNA. Collections details for all mosquitoes are detailed in Additional file 1: Table S1



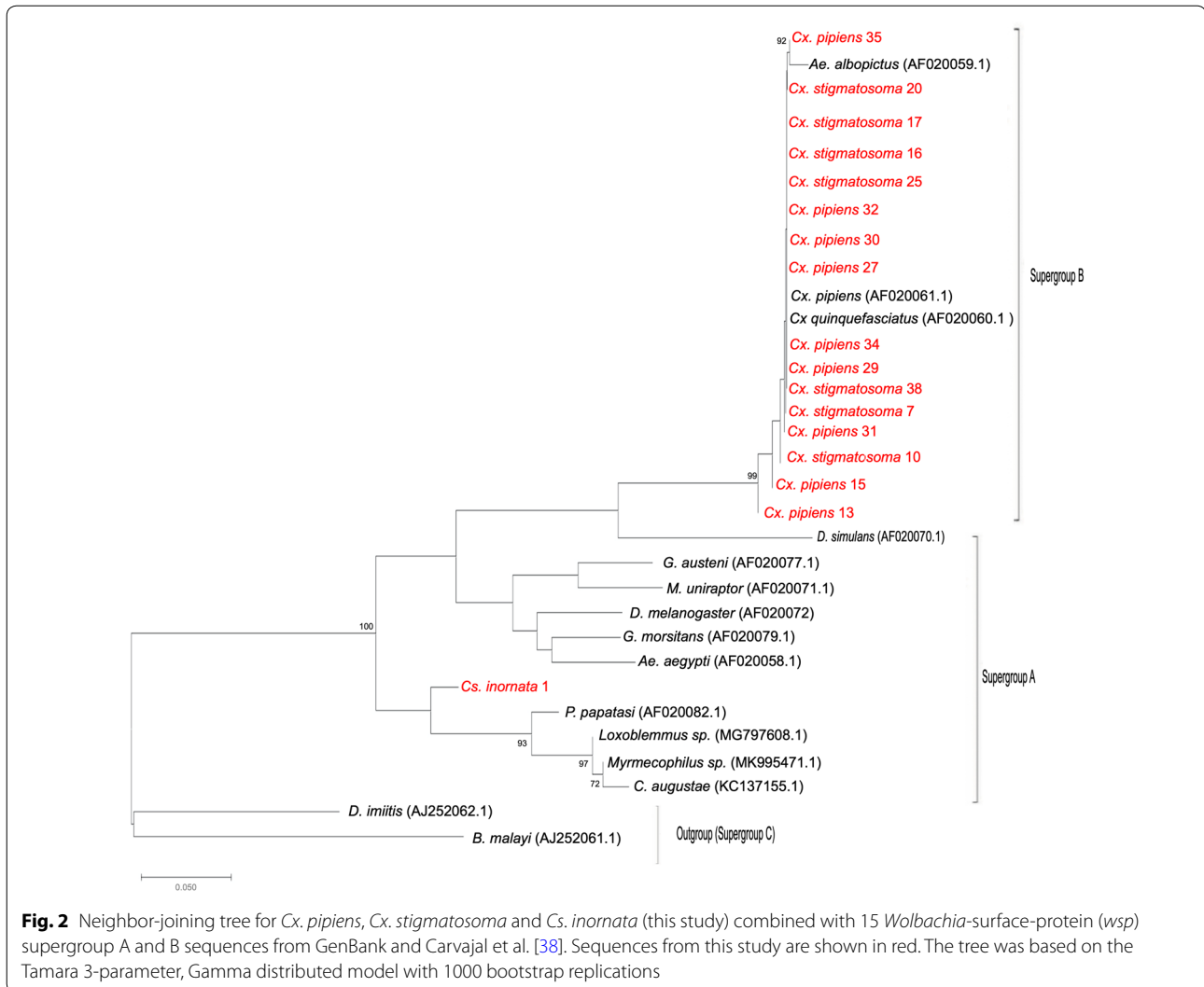
this study have been previously reported in the literature (*Ae. vexans*, *Cx. pipiens* and *An. punctipennis*). The *Wolbachia* supergroup was determined for two of these new records (*Cx. stigmatosoma* and *Cs. inornata*), and the strain was characterized for *Cx. stigmatosoma* using MLST. The other species with detections of *Wolbachia* had a very low prevalence (frequency) and could not be sequenced.

The two mosquito species which were positive for *Wolbachia* at high frequencies (prevalence) were *Cx. pipiens* and *Cx. stigmatosoma*. The other six species showed detections of *Wolbachia* at low prevalence (< 13%). Furthermore, when the relative *Wolbachia* density was compared between *Cx. pipiens* and *Cx. stigmatosoma*, there was no statistical difference indicating that these two species potentially hold similar *Wolbachia* densities. Further assessment *via* absolute quantification of *Wolbachia* would further confirm this finding. In addition, future

work with *Cx. stigmatosoma* could investigate maternal transmission to provide supporting evidence for *Wolbachia* infection. The inability to sequence *Wolbachia* in the species with low *Wolbachia* prevalence could be due to a low *Wolbachia* density. One species, *Cs. inornata*, had a low *Wolbachia* prevalence (13%), yet the *wspA* sequence was obtained which allowed it to be tentatively classified into supergroup A. Four of five MLST genes were sequenced for *Cs. inornata* in this study. This *Wolbachia* isolate may potentially represent a new *Wolbachia* strain, but further research would be needed with additional samples collected to replicate detection of *Wolbachia*.

*Wolbachia* infections were previously reported in *Cx. pipiens* [58, 68], *An. punctipennis* [69], and *Ae. vexans* [70]. Although *Wolbachia* has been previously detected in *An. punctipennis* and *Ae. vexans*, currently there is no description of a strain type for these mosquitoes.





Our study did not detect *Wolbachia* in several mosquito species including *An. freeborni*, *An. franciscanus*, *Ae. nigromaculis* and *Ae. aegypti*. Although a few studies have indicated *Wolbachia* detection in *Ae. aegypti* [36–39], others found absence of infection in this species [40, 71] and suggest that the variability of strains found in previous studies on *Ae. aegypti* may indicate environmental contamination rather than a true *Wolbachia* infection. Ross et al. [40] recommend that to confirm *Wolbachia* infection, experiments should be run to demonstrate maternal transmission or to visualize *Wolbachia* in the mosquito using a method such as fluorescent *in situ* hybridization (FISH), in addition to determining sequences. *Culex pipiens* is well known for its infection with *Wolbachia*, as *Wolbachia pipientis* was first described from this mosquito species [1, 2]. Previous research identified *wPip* supergroup B infections in the *Cx. pipiens* species complex in five California populations

[58]. Since then more than 60 *wPip* haplotypes have been identified [31, 72]. Our study screened *Cx. pipiens* from four sites and found individuals from all sites carrying *Wolbachia*. In the present study, the MLST results for *Cx. pipiens* found strain type 9 supergroup B among samples with complete allelic profile data. These were all acquired from the Ballico collection site. Isolates of strain type 9 have been documented in *Cx. pipiens* and *Cx. quinquefasciatus* [67]. Other studies have found *Cx. pipiens* with strain type 9 in Placer County, California and Tompkins County, New York; while *Wolbachia*-infected *Cx. quinquefasciatus* were found in Hawaii, Midway and Kenya [57, 73].

Interestingly, our study also found a new *Wolbachia* detection record for *Cx. stigmatosoma*. This species is highly ornithophilic [74] and often found in urban residential areas and near farms. It prefers foul water sources like street drains and dairy lagoons for oviposition [75].

**Table 3** Multilocus sequence typing (MLST) to identify *Wolbachia* strains

Species	Sample #	MLST gene		MLST gene		ftsZ	fbpA	Strain	SG <sup>a</sup>	Strain #
		gatB	coxA	hcpA	fbpA					
<i>Cx. pipiens</i>	Cxpip 29	4	3	354	444	22	444	wPip	B	9
	Cxpip 31	4	3	354	444	22	444	wPip	B	9
	Cxpip 32	4	3	354	444	22	444	wPip	B	9
	Cxpip 34	4	227	354	444	22	444	wPip	B	9
	Cxpip 35	4	3	354	444	22	444	wPip	B	9
<i>Cx. stigmatosoma</i>	Cxstig 15	4	3	354	444	22	444	wPip	B	9
	Cxstig 10	148	3	354	444	22	444	wPip	B	9

<sup>a</sup> Supergroup

These types of habitat are similar to those where *Cx. pipiens* can also be found. This species is known to occur throughout the western USA to Mexico, Central America and northern South America [75, 76]. *Culex stigmatosoma* is a competent vector of West Nile virus, and is capable of transmitting St Louis encephalitis and avian malaria [76, 77].

*Culex stigmatosoma* had MLST sequences produced from two different collection sites. One site was a rural semi-natural habitat near a dairy (Ballico), and another was a rural farm in Atwater. At the first site, *Cx. stigmatosoma* had sequences from the five MLST genes that were an identical match for those from *Wolbachia* strain type 9 (ST-9) *wPip* infection in the MLST database, sequences which were identical to those characterized from *Cx. pipiens* tested in this study (Table 3). Although the five MLST housekeeping genes sequenced from *Cx. stigmatosoma* matched those of *Cx. pipiens* for strain type 9 *wPip*, it would be worthwhile to examine differences in *Wolbachia* from these two species using a more comprehensive method such as comparative genomics before concluding the two species harbor the same strain [78]. Bleidorn & Gerth (2018) discussed the limits of the MLST for *Wolbachia* strain characterization; one of these is that several of the MLST genes used to characterize *Wolbachia* strains evolve slowly, and may not sufficiently differentiate among strains where significant biological differences may exist. In this study, *Cx. stigmatosoma* was not likely to be misidentified as adult *Cx. pipiens*. Adult *Cx. stigmatosoma* more closely resemble *Cx. tarsalis*, but the two species are distinguished by distinct markings on ventral abdominal segments [59]. *Culex stigmatosoma* had a high prevalence (frequency) of individuals with detections of *Wolbachia*. The second collection site (Atwater) where *Cx. stigmatosoma* was positive for *Wolbachia* in this study also had an individual with MLST alleles match those of *Cx. pipiens* *Wolbachia* strain type as well (strain 9). This species could represent a new *Wolbachia* infection, not just a detection of *Wolbachia*. However, further studies would be needed to provide evidence of infection which are complementary to sequencing, such as FISH or loop mediated isothermal amplification (LAMP) [40].

The *Wolbachia* similarity observed between the two species above (*Cx. pipiens* and *Cx. stigmatosoma*) is not unusual. In fact, several studies have documented high similarity among some *Wolbachia*-infections in hosts within the same genus [57, 79, 80]. In Italy, evidence of natural *wPip* *Wolbachia* infections have been identified within *Culex modestus* and *Culex torrentium* mosquito species, and there was no observable divergence in *wsp* sequences when compared to field collected *Cx. pipiens* [79]. Another example was documented in Portugal,

where low prevalence *Wolbachia*-infections were found in *Culex theileri* and indistinguishable from *Cx. pipiens* by 16S rRNA, *ank2* and *pk1* genes [80]. Furthermore, the results of restricted fragment length polymorphisms (RFLP) suggested a shared *wPip* haplotype I infection among both *Cx. theileri* and *Cx. pipiens*. Thus, it is not surprising that two closely related *Culex* species in the present study could harbor very similar or closely related *Wolbachia* strains.

*Culiseta inornata* had several MLST genes match those in the MLST database. When grouped in the supergroup phylogeny with other vector species, *Cs. inornata* was closely related to supergroup A infections previously reported in a dipteran, *Phlebotomus papatasi* (sand fly) and an orthopteran (*Loxoblemmus* spp.) (Fig. 2). *Culiseta inornata* in this study was collected in a semi-natural riparian habitat along the Merced River. This species is predominant in rural areas, and is capable of vectoring West Nile virus, western equine encephalitis, St Louis encephalitis, Japanese encephalitis, California encephalitis and avian malaria [76, 81, 82]. This species occurs throughout the United States, with a known presence in 46 states from California to New York and the range also expands north into Canada [75, 83]. *Culiseta inornata* persists through the winter months, which could have implications for the seasonality of arbovirus transmission. Given that *Cs. inornata* transmits a number of vector-borne diseases, further study to investigate *Wolbachia* within this species could be worthwhile, since *Wolbachia* can influence vector competence. Moreover, future research could investigate whether *Wolbachia* in this species persist within other populations in California or other regions.

Several other mosquito species had *Wolbachia* at a low frequency or density. Some of these species have been previously tested through traditional PCR, but perhaps escaped detection due to the lower sensitivity of traditional PCR compared to qPCR [58]. Our study detected *Ae. melanimon* with *Wolbachia* at a low frequency. This study is the first record of *Ae. melanimon* with detection of *Wolbachia*, but additional tests as previously described would be needed to confirm infection [40]. *Aedes melanimon* are widely distributed throughout western and southwestern USA and Canada [75, 84, 85]. This species prefers to oviposit in or around irrigated pastures, ponds and fields. *Aedes melanimon* is the primary vector of California encephalitis and is capable of transmitting western equine encephalitis and West Nile virus [76, 86, 87]. Past literature has identified *Ae. melanimon* to have a secondary role in maintenance of western equine encephalitis virus within the Central Valley of California, and has identified this mosquito as preferentially feeding on humans and other mammals [88, 89]. Along with *Ae.*

*melanimon*, several other species (*An. punctipennis*, *Cx. tarsalis* and *Cs. incidens*) had very low prevalence (all less than 10%), perhaps due to horizontal transmission [90]. Recently, Shaikovich et al. [90] suggested that *Wolbachia* diversity is likely attributed to horizontal transfer and strain recombination. By utilizing one-allele-criterion (OAC) phylogenetic networks, the authors suggest a link between the *Ae. albopictus* (wAlbB) *Wolbachia* strain and *Wolbachia* from ants; furthermore, that supergroup B strains from mosquitoes are linked with *Wolbachia* from Lepidoptera [90]. Routes of horizontal transmission have been shown to occur through parasitism, shared habitats, and predation [11, 91–93].

## Conclusions

Our survey of *Wolbachia* infections in Merced county mosquitoes identified new *Wolbachia* detections, providing information to support current and future *Wolbachia*-mediated vector control applications. As noted, it will be important to confirm *Wolbachia* detections are true infections by providing evidence in addition to *Wolbachia* sequences. *Wolbachia*-based approaches have been implemented within vector control strategies by propagation of a desired strain within an uninfected population, or by inducing cytoplasmic incompatibility through mating incompatibility. Successful integration depends on the strain chosen for its effects on the novel host [94]. Characterizing new *Wolbachia* strains and determining their mosquito host species are critical to efforts to further develop *Wolbachia*-mediated vector control applications.

## Supplementary information

**Supplementary information** accompanies this paper at <https://doi.org/10.1186/s13071-020-04429-z>.

**Additional file 1: Table S1.** Mosquito collections in Merced county.

## Acknowledgements

We thank the Merced County Mosquito Abatement District (MCMAD) for supporting this research, and the following people from MCMAD for assistance with this project: Rhiannon Jones, Jason Bakken, and Allan Inman. The following individuals from the University of California Merced assisted with the project: Jocelyn Acosta, Hannah Parolini, Ricardo Hernandez, Mackenzie Hoyt and Michaela Hoyt. The Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases assisted with funding, and was funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). The USDA is an equal opportunity employer.

## Authors' contributions

Study design: ALJ and JLR. Data collection: RT, EH, VF, JLR and ALJ. Data analysis: RT, VF, EH, JLR and ALJ. Manuscript preparation: RT, ALJ and JLR. All authors read and approved the final manuscript.

## Funding

This study was supported by funding from Merced County Mosquito Abatement District. In addition, RT was supported by a trainee grant from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases, which is

funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

## Availability of data and materials

The datasets generated during this study consist of sequences submitted to GenBank under the accession numbers MW125593–MW125610 (*wsp*), MW133153–MW133170 (*ftsZ*), MW133171–MW133187 (*coxA*), MW133188–MW133204 (*fbpA*), MW133205–MW133220 (*gatB*), and MW133221–MW133228 (*hcpA*).

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Author details

<sup>1</sup> Public Health, University of California, 5200 North Lake Road, Merced, CA 95343, USA. <sup>2</sup> USDA-ARS, NCAUR, Crop Protection Research, 1815 N. University, Peoria, IL 61604, USA.

Received: 29 June 2020 Accepted: 24 October 2020

Published online: 10 November 2020

## References

- Hertig M, Wolbach SB. Studies on *Rickettsia*-like micro-organisms in insects. *J Med Res*. 1924;44:329–74.
- Hertig M. The rickettsia, *Wolbachia pipiens* (gen. et sp. N.) and associated inclusions of the mosquito, *Culex pipiens*. *Parasitology*. 1936;28:453–86.
- Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiol Lett*. 2008;281:215–20.
- Zug R, Hammerstein P. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE*. 2012;7:38544.
- Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol*. 2008;6:741–51.
- Axford JK, Ross PA, Yeap HL, Callahan AG, Hoffmann AA. Fitness of wAlbB *Wolbachia* infection in *Aedes aegypti*: parameter estimates in an outcrossed background and potential for population invasion. *Am J Trop Med Hyg*. 2016;94:507–16.
- Ma Y, Chen W, Li Z, Zhang F, Gao Y, Luan Y. Revisiting the phylogeny of *Wolbachia* in Collembola. *Ecol Evol*. 2017;7:2009–17.
- Werren JH, Zhang W, Guo LR. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc R Soc B-Biol Sci*. 1995;261:55–63.
- Werren JH. Biology of *Wolbachia*. *Ann Rev Entomol*. 1997;42:587–609.
- Zug R, Hammerstein P. Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biol Rev*. 2014;90:89–111.
- Ahmed MZ, Breinholt JW, Kawahara AY. Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. *BMC Evol Biol*. 2016;16:118.
- Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*. 2011;476:451–7.
- O'Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, et al. Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. *Gates Open Res*. 2019;2:36.
- Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, van den Hurk A, et al. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS Negl Trop Dis*. 2014;8:2688.
- Saridaki A, Bourtzis K. *Wolbachia*-induced reproductive parasitism and applications. *Entomol Hell*. 2017;18:3.

16. Sinkins SP. *Wolbachia* and cytoplasmic incompatibility in mosquitoes. *Insect Biochem Mol Biol.* 2004;34:723–9.
17. Teixeira L, Ferreira A, Ashburner M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.* 2008;6:e2.
18. Brownlie JC, Johnson KN. Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 2009;17:348–54.
19. Bian G, Xu Y, Lu P, Xie Y, Xi Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathog.* 2010;6:e1000833.
20. Schultz MJ, Isern S, Michael SF, Corley RB, Connor JH, Frydman HM. Variable inhibition of Zika virus replication by different *Wolbachia* strains in mosquito cell cultures. *J Virol.* 2017;91:339.
21. Dutra HLC, Rocha MN, Dias FBS, Mansur SB, Caragata EP, Moreira LA. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host Microbe.* 2016;19:771–4.
22. Yeap HL, Mee P, Walker T, Weeks AR, O'Neill SL, Johnson P, et al. Dynamics of the “popcorn” *Wolbachia* infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. *Genetics.* 2011;187:583–95.
23. Ross PA, Endersby NM, Hoffmann AA. Costs of three *Wolbachia* infections on the survival of *Aedes aegypti* larvae under starving conditions. *PLoS Negl Trop Dis.* 2016;10:e0004320.
24. Turley AP, Moreira LA, O'Neill SL, McGraw EA. *Wolbachia* infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. *PLoS Negl Trop Dis.* 2009;9:e516.
25. Duan X, Sun J, Wang L, Shu X, Guo Y, Keiichiro M, et al. Recent infection by *Wolbachia* alters microbial communities in wild *Laodelphax striatellus* populations. *Microbiome.* 2020;8:104.
26. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell.* 2009;139:1268–78.
27. Dodson BL, Hughes GL, Paul O, Matachiero AC, Kramer LD, Rasgon JL. *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Negl Trop Dis.* 2014;8:e2965.
28. Laven H. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature.* 1967;216:383–4.
29. Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proc Natl Acad Sci USA.* 2004;101:15042–5.
30. Alam U, Medlock J, Brelsfoard C, Pais R, Lohs C, Balmant S, et al. *Wolbachia* symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly *Glossina morsitans*. *PLoS Pathog.* 2011;7:e1002415.
31. Atyame CM, Delsuc F, Pasteur N, Weill M, Duron O. Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. *Mol Biol Evol.* 2011;28:2761–72.
32. Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calviti M, Moreira LA, et al. Harnessing mosquito–*Wolbachia* symbiosis for vector and disease control. *Acta Trop.* 2014;132:5150–63.
33. Zhang D, Lees RS, Xi Z, Gilles RL, Bourtzis K. Combining the sterile insect technique with *Wolbachia*-based approaches: II—a safer approach to *Aedes albopictus* population suppression programmes, designed to minimize the consequences of inadvertent female releases. *PLoS ONE.* 2015;10:e0135194.
34. Crawford JE, Clarke DW, Criswell V, Desnoyer M, Cornel D, Deegan B, et al. Efficient production of male *Wolbachia*-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. *Nat Biotechnol.* 2020;38:484–92.
35. Coon KL, Brown MR, Strand MR. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Mol Ecol.* 2016;25:5806–26.
36. Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN. Mosquito vector-associated microbiota: metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. *Ecol Evol.* 2018;8:1352–68.
37. Balaji S, Jayachandran S, Prabakaran SR. Evidence for the natural occurrence of *Wolbachia* in *Aedes aegypti* mosquitoes. *FEMS Microbiol Lett.* 2019;366:fnz055.
38. Carvajal TM, Hashimoto K, Harnandika RK, Amalin DM, Watanabe K. Detection of *Wolbachia* in field-collected *Aedes aegypti* mosquitoes in metropolitan Manila, Philippines. *Parasit Vectors.* 2019;12:361.
39. Kulkarni A, Yu W, Jiang J, Sanchez C, Karna AK, Martinez KJ, et al. *Wolbachia pipiensis* occurs in *Aedes aegypti* populations in New Mexico and Florida, USA. *Ecol Evol.* 2019;9:6148–56.
40. Ross PA, Callahan AG, Yang Q, Jasper M, Arif MAK, Afizah AN, et al. An elusive endosymbiont: does *Wolbachia* occur naturally in *Aedes aegypti*? *Ecol Evol.* 2020;10:1581–91.
41. Ruang-Areerate T, Kittayapong P. *Wolbachia* transinfection in *Aedes aegypti*: a potential gene driver of dengue vectors. *Proc Natl Acad Sci USA.* 2006;103:12534–9.
42. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, et al. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature.* 2011;476:450–3.
43. Xi Z, Khoo CC, Dobson SL. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science.* 2005;310:326–8.
44. Fraser JE, De Bruyne JT, Iturbe-Ormaetxe I, Stephnell J, Burns RL, Flores HA, O'Neill SL. Novel *Wolbachia*-transinfected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotypes. *PLoS Pathog.* 2017;13:e1006751.
45. Ant TH, Herd CS, Geoghegan V, Hoffmann AA, Sinkins SP. The *Wolbachia* strain wAu provides highly efficient virus transmission blocking in *Aedes aegypti*. *PLoS Pathog.* 2018;14:e1006815.
46. Flores HA, O'Neill SL. Controlling vector-borne diseases by releasing modified mosquitoes. *Nat Rev Microbiol.* 2018;16:508–18.
47. Gloria-Soria A, Ayala D, Bheecarry A, Calderon-Argudas O, Chadee DD, Chiappero M, et al. Global genetic diversity of *Aedes aegypti*. *Mol Ecol.* 2016;25:5377–95.
48. Joyce AL, Torres MM, Torres R, Moreno M. Genetic variability of the *Aedes aegypti* (Diptera: Culicidae) mosquito in El Salvador, vector of dengue, yellow fever, chikungunya, and Zika. *Parasit Vectors.* 2018;11:637.
49. Porse CC, Kramer V, Yoshimizu MH, Metzger M, Hu R, Padgett K, Vugia DJ. Public health response to *Aedes aegypti* and *Ae. albopictus* mosquitoes invading California, USA. *Emerg Infect Dis.* 2015;21:1827–9.
50. CDPH (California Department of Public Health). Map and city list of *Aedes aegypti* and *Aedes albopictus* mosquitoes in CA, 2011–2020. 2020. <https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/AedesDistributionMap.pdf>.
51. Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL, Billington K, Axford JK, et al. Stability of the wMel *Wolbachia* infection following invasion into *Aedes aegypti* populations. *PLoS Negl Trop Dis.* 2014;8:e3115.
52. Schmidt TL, Barton NH, Rašić G, Turley AP, Montgomery BL, Iturbe-Ormaetxe I, et al. Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS Biol.* 2017;15:e2001894.
53. Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, et al. Establishment of *Wolbachia* strain wAlbB in Malaysian populations of *Aedes aegypti* for dengue control. *Curr Biol.* 2019;29:4241–4248.e5.
54. O'Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, et al. Scaled deployment of *Wolbachia* to protect the community from *Aedes* transmitted arboviruses. *Gates Open Res.* 2018;2:36.
55. Baldo L, Werren JH. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. *Curr Microbiol.* 2007;55:81–7.
56. O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc Natl Acad Sci USA.* 1992;89:2699–702.
57. Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, et al. Multilocus sequence typing system for the endosymbiont *Wolbachia pipiensis*. *Appl Environ Microbiol.* 2006;72:7098–110.
58. Rasgon JL, Scott TW. An initial survey for *Wolbachia* (Rickettsiales: Rickettsiaceae) infections in selected California mosquitoes (Diptera: Culicidae). *J Med Entomol.* 2004;41:255–7.
59. Meyer RP, Durso SL. Identification of the mosquitoes of California. Sacramento: Mosquito and Vector Control Association; 1993.
60. QGIS. QGIS Geographic Information System. Open Source Geospatial Foundation Project. 2019; <https://qgis.org/en/site/>
61. Qiagen. DNeasy tissue handbook. Valencia: Qiagen; 2006.

62. Li L, Fallon AM. Mosquito ribosomal protein S3 lacks a critical glutamine residue associated with DNA repair activity in homologous *Drosophila* proteins. *Arch Insect Biochem Physiol*. 2006;4:188–96.
63. Park K, Kwak I. Gene expression of ribosomal protein mRNA in *Chironomus riparius*: effects of endocrine disruptor chemicals and antibiotics. *Comp Biochem Physiol C Toxicol Pharmacol*. 2012;2:113–20.
64. Livak KJ, Schmittgen TD. Analysis of real time gene expression data using real-time quantitative PCR and the 2<sup>-ΔΔCT</sup> method. *Methods*. 2001;25:402–8.
65. Zhou W, Rousset F, O'Neill S. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc R Soc Lond B-Biol Sci*. 1998;265:509–15.
66. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4:406–25.
67. Jolley KA, Maiden MCJ. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinform*. 2010;11:595.
68. Rasgon JL, Scott TW. *Wolbachia* and cytoplasmic incompatibility in the California *Culex pipiens* mosquito species complex: parameter estimates and infection dynamics in natural populations. *Genetics*. 2003;165:2029–38.
69. Muturi EJ, Ramirez JL, Rooney AP, Kim C. Comparative analysis of gut microbiota of mosquito communities in Central Illinois. *PLoS Negl Trop Dis*. 2017;11:e0005377.
70. Wiwatanaratnabutr I. Geographic distribution of *Wolbachia*-infections in mosquitoes from Thailand. *J Invertebr Pathol*. 2013;114:337–40.
71. Gloria-Soria A, Chiodo TG, Powell JR. Lack of evidence for natural *Wolbachia* infections in *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*. 2018;55:1354–6.
72. Sinkins SP, Walker T, Lynd AR, Steven AR, Makepeace BL, Godfray HC, Parkhill J. *Wolbachia* variability and host effects on crossing types in *Culex* mosquitoes. *Nature*. 2005;436:252–60.
73. Atkinson CT, Watcher-Weatherwax W, Lapointe D. Genetic diversity of *Wolbachia* endosymbionts in *Culex quinquefasciatus* from Hawai'i, Midway Atoll, and Samoa. Technical Report. Hilo, Hi. Hawaii Cooperative Studies Unit (HCSU); 2016. <https://hdl.handle.net/10790/2671>.
74. McPhatter LP, Su T, Williams G, Cheng ML, Dhillon M, Gerry AC. Host-feeding patterns of *Culex stigmatosoma* (Diptera: Culicidae) in southern California. *J Med Entomol*. 2017;54:1750–7.
75. Carpenter SJ, LaCasse WJ. Mosquitoes of North America north of Mexico. Berkeley: University of California Press; 1955.
76. Goddard LB, Roth AE, Reisen WK, Scott TW. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis*. 2002;8:1385–91.
77. Reisen WK, Milby MM, Presser SB, Hardy JL. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles basin of California, 1987–1990. *J Med Entomol*. 1992;29:582–98.
78. Bleidorn C, Gerth M. A critical re-evaluation of multilocus sequence typing (MLST) efforts in *Wolbachia*. *FEMS Microbiol Ecol*. 2018;94:1–11.
79. Ricci I, Cancrini G, Gabrielli S, D'amelio S, Favia G. Searching for *Wolbachia* (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): large polymerase chain reaction survey and new identifications. *J Med Entomol*. 2002;39:562–7.
80. De Pinho MV, Mendes AM, Maurício IL, Calado MM, Novo MT, Belo S, Almeida APG. Molecular detection of *Wolbachia pipientis* in natural populations of mosquito vectors of *Dirofilaria immitis* from continental Portugal: first detection in *Culex theileri*. *Med Vet Entomol*. 2016;30:301–9.
81. Hammon WM, Reeves WC. Laboratory transmission of St. Louis encephalitis virus by three genera of mosquitoes. *J Exp Med*. 1943;78:241–53.
82. Work TM, Washino RK, Van Riper C. Comparative susceptibility of *Culex tarsalis*, *Anopheles franciscanus* and *Culiseta inornata* (Diptera: Culicidae) to *Plasmodium relictum* (Haemosporidia: Plasmodiidae). *J Med Entomol*. 1990;27:68–71.
83. Ward RD. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. 2nd ed. Gainesville: University Press of Florida; 2005.
84. Richards CS. *Aedes melanimon* Dyar and related species. *Can Entomol*. 1956;88:261–9.
85. Burgess L. Note on *Aedes melanimon* Dyar, a mosquito new to Canada (Diptera: Culicidae). *Can Entomol*. 1957;89:532.
86. Hammon WM, Reeves WC, Sather G. California encephalitis virus, a newly described agent. II. Isolations and attempts to identify and characterize the agent. *J Immunol*. 1952;69:493–510.
87. Jensen T, Washino RK. An assessment of the biological capacity of a Sacramento Valley population of *Aedes melanimon* to vector arboviruses. *Am J Trop Med Hyg*. 1991;44:355–63.
88. Hardy JL. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945–1985. *Am J Trop Med Hyg*. 1987;37:185–325.
89. Tempelis CH, Washino RK. Host-feeding patterns of *Culex tarsalis*, with notes on other species. *J Med Entomol*. 1967;4:315–8.
90. Shaikevich E, Bogacheva A, Rakova V, Ganushkina L, Illinsky Y. *Wolbachia* symbionts in mosquitoes: intra- and intersupergroup recombinations, horizontal transmission and evolution. *Mol Phylogenet Evol*. 2019;134:24–34.
91. Huigens ME, de Almeida RP, Boons PA, Luck RF, Stouthamer R. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc R Soc Lond B*. 2004;271:509–15.
92. Heath BD, Butcher RDJ, Whitfield WGF, Hubbard SF. Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. *Curr Biol*. 1999;6:313–6.
93. Li S, Ahmed MZ, Lv N, Shi P, Wang X, Huang J, et al. Plant-mediated horizontal transmission of *Wolbachia* between whiteflies. *ISME J*. 2017;11:1019–28.
94. Hoffmann AA, Ross PA, Rašić G. *Wolbachia* strains for disease control: ecological and evolutionary considerations. *Evol Appl*. 2015;8:751–68.
95. Werren JH, Windsor DM. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc R Soc Lond B*. 2000;267:1277–85.
96. Gomes FM, Hixson BL, Tyner MDW, Ramirez JL, Canepa GE, Alves e Silva TL, et al. Effect of naturally occurring *Wolbachia* in *Anopheles gambiae* s.l. mosquitoes from Mali on *Plasmodium falciparum* malaria transmission. *Proc Natl Acad Sci USA*. 2017;47:12566–71.
97. Osborne SE, Leong YS, O'Neill SL, Johnson KN. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog*. 2009;5:e100656.
98. Lee SF, White VL, Weeks AR, Hoffmann AA, Endersby NM. High-throughput PCR assays to monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. *Appl Environ Microbiol*. 2012;78:4740–3.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.