Wolbachia pipientis occurs in Aedes aegypti populations in New Mexico and Florida, USA

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Running title: Natural infection of Wolbachia in Aedes aegypti
ABSTRACT

The mosquitoes *Aedes aegypti* (L.) and *Ae. albopictus* Skuse are the major vectors of dengue, Zika, yellow fever and chikungunya viruses worldwide. *Wolbachia*, an endosymbiotic bacterium present in many insects, is being utilized in novel vector control strategies to manipulate mosquito life history and vector competence to curb virus transmission. Earlier studies have found that *Wolbachia* is commonly detected in *Ae. albopictus* but sporadically detected in *Ae. aegypti*. In this study, we used a two-step PCR assay to detect *Wolbachia* in wild-collected samples of *Ae. aegypti*. The PCR products were sequenced to validate amplicons and identify *Wolbachia* strains. A Loop-mediated isothermal amplification (LAMP) assay was developed and used for detecting *Wolbachia* in selected mosquito specimens as well. We found *Wolbachia* in 85/148 (57.4%) wild specimens of *Ae. aegypti* from various cities in New Mexico and in 2/46 (4.3%) wild specimens of *Ae. aegypti* from St. Augustine, Florida. We did not detect *Wolbachia* in 94 samples of *Ae. aegypti* from Deer Park, Harris County, Texas. *Wolbachia* detected in *Ae. aegypti* from both New Mexico and Florida was the wAlbB strain of *Wolbachia pipientis*. A *Wolbachia* positive colony of *Ae. aegypti* was established from pupae collected in Las Cruces, New Mexico in 2018.

KEYWORDS. *Wolbachia*, wAlbB, *Aedes aegypti*, New Mexico, Florida, Texas
INTRODUCTION

Wolbachia are obligate intracellular bacteria found in a wide range of terrestrial arthropods and nematodes (Werren, Baldo, & Clark, 2008). The bacterium was discovered in the reproductive tissues (testes and ovaries) of the mosquito Culex pipiens L. by Hertig and Wolbach in 1924 (Hertig & Wolbach, 1924) and was formally described as Wolbachia pipientis by Hertig in 1936 (Hertig, 1936). About 60-70% of all insect species harbor Wolbachia, including some mosquito species (Hilgenboecker, Hammerstein, Schlattmann, Telschow, & Werren, 2008). The bacterium can be a powerful reproductive manipulator, inducing cytoplasmic incompatibility (CI), parthenogenesis, feminization of males, and male killing (Werren et al., 2008) in various host species. These properties have been exploited for development of Wolbachia as a novel strategy for vector mosquito control. Wolbachia-induced CI favors the reproductive success and spread of colonized females in populations, which can be used to drive desirable traits, including resistance to infection with vector-borne pathogens, into a population. On the other hand, infected mosquito males can cause CI in a population with the presence of different Wolbachia strains or no infection, which can be used for sterile insect technique (SIT) to decrease vector populations (Flores & O'Neill, 2018).

Aedes (Stegomyia) aegypti (L.) and Ae. (Stegomyia) albopictus Skuse are the major vectors for the transmission of several arthropod-borne viruses (arboviruses) among humans, particularly dengue, Zika, yellow fever, and chikungunya viruses. Wolbachia is commonly found in Ae. albopictus (de Albuquerque, Magalhaes, & Ayres, 2011; Joanne et al., 2015; Kittayapong, Baimai, & O'Neill, 2002), but Ae. aegypti was thought not to carry this bacterium (Gloria-Soria, Chiodo, & Powell, 2018; Kittayapong et al., 2002; Kittayapong, Baisley, Baimai, & O'Neill, 2000). However, Wolbachia was found in wild Ae. aegypti in two recent investigations.
Wolbachia 16S ribosomal RNA gene sequencing reads (operational taxonomic units, OTUs) were detected in *Ae. aegypti* larvae collected from one of five sites in Jacksonville, Florida (Coon, Brown, & Strand, 2016) and in a few individuals of *Ae. aegypti* collected from Houston, Texas, although in this latter study, the presence of *Wolbachia* was not be confirmed using PCR (Hegde et al., 2018).

Establishing the prevalence of *Wolbachia* in *Ae. aegypti* is critical to public health, because over the past decade, *Ae. aegypti* transinfected with *Wolbachia* have been generated with the goal of blocking transmission of dengue virus (Bian, Xu, Lu, Xie, & Xi, 2010; Bull & Turelli, 2013; Hoffmann et al., 2011; McMeniman et al., 2009; O'Neill, 2018; Walker et al., 2011). This approach was initially aimed at shortening mosquito lifespan below the extrinsic incubation period of the virus (McMeniman et al., 2009), but in the course of these experiments it was discovered that transinfection of *Ae. aegypti* with *Wolbachia* strain wMelPop also blocks dengue and chikungunya virus infections of the mosquito (Moreira et al., 2009). A successful large field trial in Australia showed a stable establishment and slow but steady spread of released *Ae. aegypti* transinfected with wMel in the study area (Schmidt et al., 2017). However, if a population of *Ae. aegypti* were to harbor an autochthonous strain of *Wolbachia*, then this native strain would have a high potential to prevent invasion of a virus-blocking strain that exhibits incompatibility with the native strain (Hoffmann, Ross, & Rasic, 2015). This effect was demonstrated in a study of *Ae. albopictus*, wherein the wMel transinfected line produced complete bidirectional incompatibility with a wildtype line carrying wAlbA and wAlbB, with 0% hatch rate from crossing between females of either strain with males of the other strain (Blagrove, Arias-Goeta, Failloux, & Sinkins, 2012). On the other hand, complete CI could favor SIT for population reduction.
As part of a project to map the distribution of *Ae. aegypti* and *Ae. albopictus* in New Mexico in 2017 and characterize their mosquito microbiota, we initially detected *Wolbachia* in wild-caught *Ae. aegypti* in Las Cruces, New Mexico, via 16S sequencing. A more comprehensive survey was then conducted using a two-step PCR assay, which revealed a 57.4% prevalence of *Wolbachia* carriage in 148 specimens of *Ae. aegypti* collected from eight cities across New Mexico (Figure 1). *Wolbachia* was also detected in two of 48 specimens of *Ae. aegypti* from St. Augustine, Florida (4.2 % prevalence), but was not detected in 94 specimens of *Ae. aegypti* from Deer Park, Harris County, Texas (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Mosquito collections and species identification

*Ae. aegypti* and *Ae. albopictus* mosquitoes were collected using gravid and sentinel traps in New Mexico in 2017 by the SouthWest *Aedes* mosquito Research and Mapping project (SWARM) team, in Florida in 2016 by the Anastasia Mosquito Control District, and in Texas in 2018 by the Harris County Public Health Mosquito and Vector Control Division. The location details of the samples are presented in Table 1 and Figure 1. Mosquitoes were sorted and identified as *Ae. aegypti* or *Ae. albopictus* based on morphology. The species identity was confirmed by a species-diagnostic PCR assay that amplifies species specific fragments of internal transcribed spacer 1 (ITS1) of ribosomal DNA, as described by Higa, Toma, Tsuda, & Miyagi (2010). The primers used in this study are listed in Table S1. The PCR was conducted using 2 × PCR master mix (MCLab, South San Francisco, CA) with ~20 ng DNA, 0.2 μM primers, and cycling parameters as: 35 cycles of denaturing at 94°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 30 seconds with extra 5 min in the last cycle for
final extension. In August 2017, *Ae. aegypti* larvae were collected from a larval habitat in a residential area in Las Cruces and brought back to the laboratory to rear. The eclosed adults were confirmed to carry *Wolbachia* by PCR as described later. Unfortunately, the colony was lost in January 2018. In September 2018, a new colony was initiated from the larvae collected from the same location in August 2017. Again, *Wolbachia* was detected in the adults as described below.

### 2.2 DNA isolation, Bacterial 16S rDNA PCR, cloning and sequencing

Mosquito specimens from traps were desiccated in most cases. For each mosquito specimen, the abdomen was separated from the thorax by pulling gently with tweezers that were cleaned with 75% ethanol between samples. Abdomens were used for detecting associated microbiota. Metagenomic DNA was isolated individually from each abdomen using DNAzol following the manufacturer’s protocol (Thermo Fisher Scientific, Waltham, MA). Briefly, one abdomen was homogenized in 100 µl DNAzol, and centrifuged at 12,000 ×g for 10 min. Supernatant was transferred to a new tube and 50 µl ethanol was added, and the tube was centrifuged at 12,000 ×g for 10 min for DNA precipitation. The DNA pellet was dissolved in 30 µl H₂O. A bacterial 16S rDNA fragment covering V1 to V3 was amplified from individual DNA using primers 27F and 519R (Table S1), as we previously reported (Wang, Gilbreath, Kukutla, Yan, & Xu, 2011). PCR was run using 2× PCR master mix with 0.2 µM primers and cycling parameters: 35 cycles of denaturing at 94°C for 15 seconds, annealing at 52°C for 15 seconds, and extension at 72°C for 30 seconds with extra 5 min in the last cycle for final extension. PCR products were purified and cloned into the plasmid pMiniT 2.0 using a NEB PCR cloning kit (New England Biolabs, Ipswich, MA) following manufacturer’s instructions. Colony PCR was conducted to amplify the inserts using SP6 forward and T7 reverse primers. PCR products with a size of ~ 500 bp were sent for Sanger sequencing at a commercial provider (MCLab).
2.3 | Wolbachia PCR assays and sequencing

PCR assays using primer sets for the *Wolbachia* *gatB* and *ftsZ* gene, derived from (Baldo et al., 2006) (Table S1), were used for detecting *Wolbachia* in mosquitoes. PCR was run using 2× PCR master mix (MCLab) with a primer concentration of 0.2 µM and the following cycling parameters: 35 cycles of denaturing at 94°C for 15 seconds, annealing at a temperature optimal for the amplicon (Table S1) for 15 seconds, and extension at 72°C for 30 seconds with an extra 5 min in the last cycle for final extension. For specimens that showed a faint band or no visible band after a *Wolbachia* target PCR, a second round PCR was conducted. The first round PCR product was diluted 100 times with H₂O and 1 µl was used as template for the second PCR. In 85 *Wolbachia* positive specimens from NM, 8 showed a clear band in the first PCR and the remaining specimens showed a faint or no band in the first PCR but a clear band in second PCR.

To validate the *Wolbachia* detection in *Ae. aegypti*, we designed primers to amplify fragments from two *Wolbachia* genes encoding phosphoesterase (PE) and diaminopimelate epimerase (DE) based on the draft genomes of *w*AlbB (Mavingui et al., 2012) and *w*AlbA (GenBank accession NWVK00000000.1). The sequences of the two genes have distinctive inter-strain differences, enabling the design of strain specific primers (Table S1). A subset of specimens that were positive from the first or second PCR were subjected to the validation PCR and sequencing. The products were sequenced at MCLab, and the sequences were deposited in GenBank; the accession numbers are presented in Table S2.

2.4 | Loop-mediated isothermal amplification (LAMP) assay

Loop-mediated isothermal amplification (LAMP) was developed as an additional assay for the detection of *Wolbachia* in mosquitoes. Oligonucleotides for LAMP were designed using Primer Explorer V5 software available on the website
The sequences of oligos for the 16S rRNA gene are listed in Table S1. The LAMP reactions were conducted using a NEB LAMP kit with Bst 3.0 (M0374, NEB). The reaction mixture, consisting of 1X isothermal amplification buffer II, 6 mM MgSO₄, 1.4 mM of each of the deoxynucleotide triphosphates (dNTPs), 1.6 μM Forward Inner Primer/Backward Inner Primer, 0.4 μM F3/B3 primers, 0.8 μM Loop Forward/Backward, 8U of Bst 3.0 DNA polymerase, and 1 μl genomic DNA in a total volume of 25 μl, was incubated at 65°C for 60 min in a T100 Thermal Cycler (Bio-Rad). The amplified products (10 μl) were run on 2% agarose gel and visualized under UV light. For all of the tests, a positive control (DNA from a female *Wolbachia*-infected *Ae. albopictus*), a negative control (DNA from a female *Ae. aegypti* Rockefeller strain) and a system negative control (nuclease-free water) were used.

3 | RESULTS

3.1 | Prevalence of *Wolbachia* in sympatric *Ae. aegypti* and *Ae. albopictus* populations in New Mexico

*Aedes aegypti* and *Ae. albopictus* occur in the state of New Mexico (Hahn et al., 2017; Hahn et al., 2016). In 2017, we conducted a survey to map the distribution and characterize the microbiomes of both species in New Mexico. In an effort to profile bacteriomes associated with wild *Ae. aegypti*, a bacterial 16S ribosomal RNA gene fragment was amplified by PCR using primers 27F and 519R. The PCR products were cloned and sequenced to identify bacterial taxa, yielding a total of 80 sequences from 8 individuals collected from Las Cruces. Surprisingly, 10 sequences of *Wolbachia* 16S amplicons were identified in 6 of 8 individuals. We then conducted a large survey by screening 148 specimens collected in 2017 from eight cities across New
Mexico, which largely span the distribution of Ae. aegypti in the state (Figure 1), by amplifying Wolbachia gatB or ftsZ (Table 2). Selected positive PCR products were validated by sequencing. Out of 148 specimens tested, 85 were wAlbB strain positive, for a total Wolbachia prevalence of 57.4% (Tables 2 & 3). We assayed for Wolbachia in Ae. albopictus collected from the region as well. Ae. albopictus is less common in New Mexico and is only present on the eastern part of the state. In each of the cities (Clovis, Portales, and Roswell (Figure 1)) in which Ae. albopictus has been collected by us to date, Ae. aegypti also was collected. Among the 13 specimens that were available for screening, 11 carried both the wAlbA and wAlbB strains and one carried wAlbB only. The overall Wolbachia prevalence in Ae. albopictus in New Mexico was 92.3% (Table 4). We also amplified DNA fragments of two additional Wolbachia genes, encoding PE and DE. The amplicons of gatB, ftsZ, PE and DE were confirmed by sequencing. Representative sequences were deposited in GenBank (Table S2).

In addition, Wolbachia was detected in 2 of 4 females and 2 of 4 males of Ae. aegypti adults that eclosed from pupae collected in Las Cruces, NM, in August 2017. The infection persisted in F1 as well, 14/33 females (42.4%) and 14 of 14 males (100%) of F1 adults examined were positive for Wolbachia. Unfortunately, the colony was lost in January 2018 because of a failure of egg hatching. In September 2018, a new colony of Ae. aegypti was initiated from the pupae collected from the same location in August 2017. Three adults (two females, one male) from this site were examined and found to be positive for Wolbachia by two rounds of ftsZ PCR. More adults were tested for Wolbachia by LAMP assay; 7/9 (77.8%) females and 3/3 (100%) males were positive.

3.2 | Prevalence of Wolbachia in Ae. aegypti and Ae. albopictus in Florida
Coon and colleagues previously reported the presence of *Wolbachia* in the larvae of *Ae. aegypti* collected from Jacksonville, FL (Coon et al., 2016). We therefore examined specimens of *Ae. aegypti* collected in July 2016 from St. Augustine, Florida, which is approximately 50 miles south from Jacksonville. Among 46 specimens screened, one male and one female were wAlbB positive, a prevalence of 4.3% (Table 3). The wAlbA strain was not detected in these specimens. As expected, *Wolbachia* infection occurred at high prevalence in *Ae. albopictus*. Among the 38 specimens tested, 35 were co-infected with wAlbA and wAlbB, and one carried only wAlbB. The overall *Wolbachia* prevalence was 92.1% (Table 4). *Ae. aegypti* and *Ae. albopictus* were sampled at different sites in St. Augustine (Table 1).

### 3.3 | Prevalence of *Wolbachia* in *Ae. aegypti* and *Ae. albopictus* in southeastern Texas

In 2018 we screened 98 *Ae. aegypti* and 32 *Ae. albopictus* collected from a neighborhood in Deer Park, Harris County, Texas (Table 1). No *Wolbachia* was detected in *Ae. aegypti*. The overall prevalence of *Wolbachia* infection was 81.3% in *Ae. albopictus*. Among the 32 specimens positive for Wolbachia, 15 carried both wAlbA and wAlbB, two carried wAlbA only, and nine carried wAlbB only (Table 4).

### 3.4 | LAMP assays

The LAMP assay is a sensitive method for detecting low-abundance target DNA in a sample (Notomi et al., 2000). Goncalves Dda et al. (2014) developed a LAMP assay for *Wolbachia* detection in insects (Goncalves Dda, Cassimiro, de Oliveira, Rodrigues, & Moreira, 2014). Recently, Bhadra *et al.* (2018) reported a specific and sensitive assay that combines LAMP and oligonucleotide strand displacement (OSD) for detecting both species identity and
Wolbachia (Bhadra et al., 2018). The Wolbachia density appears to be low in most infected specimens of Ae. aegypti. To corroborate the results from the Wolbachia PCR assay, we developed a LAMP assay to detect Wolbachia. The LAMP assay was sensitive and able to detect target Wolbachia DNA in infected Ae. aegypti directly. As shown in Figure 2, Wolbachia positive samples yield a ladder of bands between 200 bp-1kb, and a ~100 bp band. Wolbachia negative samples show an accumulation of oligos around 50 bp. The infected Ae. albopictus specimen could be detected at 100 times dilution of template, but not at 500 times dilution, while the infected Ae. aegypti specimen could not be detected at 20 times dilution. Figure 2 shows a representative result of LAMP assay on the Wolbachia positive and negative specimens from New Mexico, Florida, and Texas.

4 | DISCUSSION

Wolbachia is commonly associated with wild Ae. albopictus around the world. However, Wolbachia has not been detected in wild Ae. aegypti in previous surveys, except for two recent reports (Coon et al., 2016; Hegde et al., 2018). In the study by Coon et al. (2016), two Wolbachia 16S rDNA OTUs were detected in a pool of 30 larvae of Ae. aegypti that were collected from one of five larval sites in Jacksonville, Florida in June 2014. The Wolbachia OTUs were detected in another collection from the same location in July 2014, and both wAlbA and wAlbB were detected based on the sequence comparison of several Wolbachia genes. The prevalence of Wolbachia in the Ae. aegypti population was not investigated in this study. In a study by Hegde et al. (2018), a small number of Wolbachia 16S rDNA reads were detected in a few Ae. aegypti individuals collected from Houston, Texas, but the result could not be independently validated by PCR using several Wolbachia genes. Our survey of Florida mosquitoes was consistent with
previous results, detecting a low prevalence (4.3%) of Wolbachia in Ae. aegypti from St. Augustine, Florida (Table 3). No Wolbachia was detected in 94 Ae. aegypti specimens from Deer Park, Texas. In contrast, our analysis of wild populations in eight cities of New Mexico Ae. aegypti revealed a high prevalence (15.8-100%, average of 57.4%) of Wolbachia, a level unprecedented for this species. The infection was validated by sequencing the PCR amplicons from the ftsZ, gatB, DE and PE genes (Table S2). These sequences were identical to the sequences of respective genes in the genome of wAlbB strain (Mavingui et al., 2012), so the Wolbachia detected in the Ae. aegypti samples belongs to the wAlbB strain.

Recently, Gloria-Soria et al. (2018) reported a survey of Wolbachia in 2,663 specimens of Ae. aegypti from 27 counties, including 60 from Las Cruces, New Mexico, and no Wolbachia was detected in these samples. The screening in their survey was conducted on DNA pools of up to 20 individuals. In our survey, we screened individual mosquitoes, the Wolbachia density in Ae. aegypti was low, and most positive specimens were identified by two rounds of PCR. We tested the sensitivity of our PCR assay using a DNA pool mixed with 19 Wolbachia negative individuals and one positive individual. Wolbachia could be detected in the pool containing DNA from one positive Ae. albopictus specimen, but not in the pool containing DNA from one positive Ae. aegypti specimen (data not shown). It has been documented that Wolbachia titer is variable in infected individuals of Ae. albopictus (Ahantarig, Trinachartvanit, & Kittayapong, 2008; Calvitti, Marini, Desiderio, Puggioli, & Moretti, 2015). In wild-caught Ae. albopictus in North and Central Italy, the wAlbA density varied remarkably in infected males. In that study, 30.8-50.0% of infected male individuals had a low titer of wAlbA, which was not detectable by a standard PCR, but detectable by a quantitative PCR assay (Calvitti et al., 2015).
load was low in the *Ae. aegypti* samples in our study. Therefore, we hypothesize that assay sensitivity explains the different results between our study and Gloria-Soria et al. (2018)’s survey.

The striking variation in the prevalence of *Wolbachia* carriage in different populations of *Ae. aegypti* among the three states and among cities within New Mexico raises several compelling questions. Chief among these are ‘what factors contribute to the low density of *Wolbachia* in *Ae. aegypti* relative to *Ae. albopictus*?’, and ‘what makes the New Mexico populations more susceptible/hospitable to *Wolbachia* than other populations?’

*Wolbachia* density variation is common in natural populations of different insect hosts (Ahantarig et al., 2008; Unckless, Boelio, Herren, & Jaenike, 2009), however, it is not clear whether the variation is driven by genetic or environmental variation or both. A recent study revealed an amplification of a genome region that harbors a cluster of eight genes, called Octomom, which is responsible for the over-replication and virulence of *wMelPop* in *Drosophila melanogaster*. The copy number of Octomom correlates with *Wolbachia* titers (Chrostek & Teixeira, 2015). Environmental factors, for instance, temperature may play a role as well. A recent study in *D. melanogaster* demonstrated that *Wolbachia* abundance was higher when host flies developed at lower temperature (13°C and 23°C compared to 31°C) (Moghadam et al., 2018). Additionally, mosquito genetic background may affect *Wolbachia* prevalence. It has been shown that *Ae. aegypti* populations from Las Cruces, New Mexico, Houston, Texas, and four locations of Florida are genetically distinct (Pless et al., 2017). Further studies are needed to tease out the roles of these potential drivers of *Wolbachia* presence and abundance in *Ae. aegypti*.

Another critical question raised by our study is how *Ae. aegypti* acquired a strain of *Wolbachia* associated with *Ae. albopictus* and how this strain impacts *Ae. aegypti* life history. *Ae. aegypti* can be artificially transinfected with different *Wolbachia* strains, and the artificial
infection can be introduced into natural populations and spread in nature (Frentiu et al., 2014; Hoffmann et al., 2011; Schmidt et al., 2017). The \( w \)AlbB has been successfully introduced into \( Ae. \ aegypti \) to form a line with inherited infection (Xi, Khoo, & Dobson, 2005). Interestingly, the Toll and IMD pathways favor establishment and maintenance of \( w \)AlbB infection in the line; the knockdown of Toll and IMD by RNA interference reduces the \( w \)AlbB load, while the transgenic activation of Toll and IMD increases the load (Pan et al., 2018). It appears that transinfected \( Wolbachia \) is able to exploit host immunity for a symbiotic association. Our survey revealed the prevalence of \( w \)AlbB in \( Ae. \ aegypti \) natural populations in New Mexico, and infected colonies could be established from wild-collected mosquitoes. This provides an opportunity to study the natural \( Ae. \ aegypti-Wolbachia \) association and its impact on various mosquito life traits, such as reproductive manipulation and interference with viral transmission.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

AUTHORS’ CONTRIBUTIONS

JX conceived the study design. KJLM, DD, KH and MD collected mosquitoes. AK, WY, JJ, CS AKK conducted assays and data analysis. JX, MB and AKK drafted the manuscript, KAH, DD, MD, MB, IAH and RX critically reviewed the manuscript. All authors read and approved the manuscript.

DATA ACCESSIBILITY

DNA sequences were deposited in GenBank under accession number MH732668-MH732670, MH734116-MH734121.

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Figure Legends

Figure 1. Maps of the sites where Ae. aegypti mosquitoes were sampled in this study. No. of Wolbachia positive/No. of tested (%) was displayed in NM sampling sites.

Figure 2. LAMP detection of Wolbachia 16S rDNA 1,2 = Ae. aegypti (NM); 3,4 = Ae. aegypti (FL); 5,6 = Ae. aegypti (TX); 7 = Marker; 8 = Ae. aegypti (NM-1, 1:20 dilution); 9 = Ae. albopictus (NM, 1:100 dilution); 10 = Ae. albopictus (NM, 1:500 dilution); 11 = Ae. aegypti Rockefeller; 12 = No template control. +: Wolbachia positive; -: Wolbachia negative.

Additional files

Table S1. Primer sets used in the study

Table S2. GenBank accession numbers of Wolbachia sequences
### Table 1. Mosquito collections in Florida, New Mexico, Florida

| Species  | Location         | Coordinates (Latitude, Longitude) | Collection Date |
|----------|------------------|-----------------------------------|-----------------|
| Ae. aegypti | St. Augustine, FL | 29.895, -81.313                    | June, 2016      |
| Ae. albopictus | St. Augustine, FL | 29.890, -81.332                    | June, 2016      |
| Ae. aegypti | Deer Park, TX     | 29.693, -95.115                    | May, 2018       |
| Ae. albopictus | Deer Park, TX     | 29.693, -95.115                    | May, 2018       |
| Ae. aegypti | 8 cities, NM      | see Table 3                        | May-Nov, 2017   |
| Ae. albopictus | 8 cities, NM      | see Table 3                        | May-Nov, 2017   |

### Table 2. *Wolbachia* infection in *Aedes aegypti* populations in New Mexico from May-November 2016

| City (n)            | Infection rate (%) | Coordinates of collection sites (Latitude, Longitude) |
|---------------------|--------------------|------------------------------------------------------|
| Alamogordo (19)     | 3 (15.8)           | 32.861, -105.979; 32.918, -105.936                    |
| Carlsbad (31)       | 6 (19.4)           | 32.356, -104.248; 32.440, -104.240; 32.427, -104.223 |
| Deming (29)         | 26 (89.7)          | 32.251, -107.763; 32.245, -107.761; 32.262, -107.745 |
| Las Cruces (80)     | 24 (80.0)          | 32.296, -106.732; 32.357, -106.769; 32.396, -106.816 |
| Lovington (9)       | 7 (77.8)           | 21.491, -103.364                                    |
| Sunland Park (2)    | 16 (61.5)          | 31.816, -106.603                                    |
| Roswell (26)        | 2 (100)            | 33.378, -104.513; 33.416, -104.529                   |
| Truth or Consequences (2) | 1 (50.0) | 33.120, -107.272; 33.203, -107.228                   |
| Total               | 85 (57.4)          |                                                      |

### Table 3. *Wolbachia* infection in *Aedes aegypti* in New Mexico and Florida

| Specimens (n)       | *Wolbachia* strain A & B (%) | A (%)         | B (%)         | Total No. (%) |
|---------------------|-------------------------------|---------------|---------------|---------------|
| Male(51), NM        | 0                             | 0             | 28(54.9)      | 28(54.9)      |
| Female(97), NM      | 0                             | 0             | 57(58.8)      | 57(58.8)      |
| Male (18), FL       | 0                             | 0             | 1(5.5)        | 1(5.5)        |
| Female (28), FL     | 0                             | 0             | 1(3.6)        | 1(3.6)        |

### Table 4. *Wolbachia* strain distribution in *Aedes albopictus* in Texas, Florida, and New Mexico

| Specimens (n)       | *Wolbachia* strain A & B (%) | A (%)         | B (%)         | Total No. (%) |
|---------------------|-------------------------------|---------------|---------------|---------------|
| Male (19), TX       | 6 (31.6)                      | 1 (5.3)       | 9(47.4)       | 16 (84.2)     |
| Group           | Count (Percentage) | Value 1 (Percentage) | Value 2 | Value 3 | Value 4 (Percentage) |
|-----------------|--------------------|----------------------|---------|---------|----------------------|
| Female (13), TX | 9 (69.2)           | 1 (7.7)              | 0       | 10 (76.9) |
| Male (20), FL   | 20 (100)           | 0                    | 0       | 20 (100)  |
| Female (18), FL | 14 (77.8)          | 0                    | 1 (5.6) | 15 (83.3) |
| Male (2), NM    | 0                  | 0                    | 1 (50.0)| 1 (50.0)  |
| Female (11), NM | 11 (100)           | 0                    | 0       | 11 (100)  |
| Species identification PCR                      | Annealing Tm | Reference                  |
|---------------------------------------------|--------------|----------------------------|
| 18SFHIN                                     | GTAAGCTTCTTTGTACACACCGCCGCT | 55 | Higa et al. (2010)         |
| aeg.r1                                      | TAACGGACACCGTTCTTAGGCCCT    |           |                            |
| alb.r1                                      | GTACTAGGCTCAGTCGGCCACTG    |           |                            |
| **Bacterial 16S rRNA gene**                 |              |                           |
| 27F                                         | GAGTTTGATCTCNGGTTCAG       | 50 | Wang et al. (2011)         |
| 519R                                        | GTNTACNGCGGCGGCTG          |       |                            |
| gatB                                        |                           |       | Baldo et al. (2006)        |
| wAlbB_gatB_F                               | TAAGAATCGCAAGATTTACAC      | 50 |                           |
| wAlbB_gatB_R                               | TGGYAAATCGGGAAGAGATGA      |       |                            |
| wAlbA_gatB_F                               | TTAGAGCAAGATCCAGGAGAGGAGG | 50 |                           |
| wAlbA_gatB_R                               | TGGYAAATCGGGAAGAGATGA      |       |                            |
| ftsZ                                        |                           |       | Baldo et al. (2006)        |
| wAlbB_ftsZ_F                               | AAAGATAGCCATATGCTTTTT     | 50 |                           |
| wAlbB_ftsZ_R                               | CATGGGTTTTACCCATCTCA       |       |                            |
| wAlbA_ftsZ_F                               | AAAGATAGCTATATGCTTTTC     | 50 |                           |
| wAlbA_ftsZ_R                               | CATGGGTTTTACCCATCTCG       |       |                            |
| **Phosphoprotein phosphatase**              | This study             |     |                            |
| wAlbB_PP_F                                 | CGCAAGCTCAATTAACAATACC     | 55 |                           |
| wAlbB_PP_R                                 | CAATCAGCTTACCGGAAGCTTTCT  |       |                            |
| wAlbA_PP_F                                 | ACTCAATTAATAGCTAGATCAT    | 55 |                           |
| wAlbA_PP_R                                 | AGCTCTCTTTCACTCATGCG       |       |                            |
| **Diaminopimelate epimerase (EC 5.1.1.7)**  | This study             |     |                            |
| wAlbB_DE_F                                 | TGTTGAAATGGATGCACCCCAAT   | 55 |                           |
| wAlbB_DE_R                                 | TATGCCGACACATATAATTTCCT   |       |                            |
| wAlbA_DE_F                                 | ACTGGATGTGACACTCTCCACT    | 55 |                           |
| wAlbA_DE_R                                 | CTGGTGGGCAAGATAGATATGCC   |       |                            |
| **LAMP, 16S ribosomal RNA gene**            | N/A            | This study               |    |
| F3                                          | CTGGAACTGAGATACGGCTC      |       |                            |
| B3                                          | TTACGCCCCAAATAATCCGA      |       |                            |
| FIP                                         | TCTTCACTCATGGCGCATGGCATGGGAATATTGGGACAA | 55 |                            |
| BIP                                         | AGGAAGATAATGACGGGTACTCACAGATAACGCTAGGCCCTCCTCC |       |                            |
| LF                                          | CGGATCAGGCGTTGCCCGCC      |       |                            |
| LB                                          | AGTCCCTGGCTAACTCCGTG      |       |                            |
Table S2. GenBank accession numbers of *Wolbachia* sequences

| Sequence ID | Host mosquito   | *Wolbachia* gene          | *Wolbachia* strain | GenBank accession |
|-------------|-----------------|---------------------------|--------------------|-------------------|
| S77         | *Ae. aegypti*   | 16S rRNA                  | N/A                | MH732668          |
| S56         | *Ae. aegypti*   | 16S rRNA                  | N/A                | MH732669          |
| S66         | *Ae. aegypti*   | 16S rRNA                  | N/A                | MH732670          |
| LC40        | *Ae. aegypti*   | ftsZ                      | wAlbB              | MH734116          |
| D4a         | *Ae. aegypti*   | gatB                      | wAlbB              | MH734120          |
| LC11        | *Ae. albopictus*| diaminopimelate epimerase (DE) | wAlbA              | MH734119          |
| FL139       | *Ae. aegypti*   | diaminopimelate epimerase (DE) | wAlbB              | MH734118          |
| TX120       | *Ae. albopictus*| phosphoesterase (PE)      | wAlbA              | MH734121          |
| FL8         | *Ae. aegypti*   | phosphoesterase (PE)      | wAlbB              | MH734117          |
