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 *w*AlbB 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*, *w*AlbB, *Aedes aegypti*, New Mexico, Florida, Texas

1 | 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; Kitrayapong, Baimai, & O'Neill, 2002), but *Ae. aegypti* was thought not to carry this bacterium (Gloria-Soria, Chiodo, & Powell, 2018; Kitrayapong 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 *w*MelPop 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 *w*Mel 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 *w*Mel transinfected line produced complete bidirectional incompatibility with a wildtype line carrying *w*AlbA and *w*AlbB, 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 H_2O. 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 H_2O 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

(http://primerexplorer.jp/lampv5e/index.html). 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 $MgSO_4$, 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 *w*AlbB 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 *w*AlbA and *w*AlbB strains and one carried *w*AlbB 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 *w*AlbB positive, a prevalence of 4.3% (Table 3). The *w*AlbA 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 *w*AlbA and *w*AlbB, and one carried only *w*AlbB. 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 *w*AlbA and *w*AlbB, two carried *w*AlbA only, and nine carried *w*AlbB 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 *w*AlbA and *w*AlbB 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 *w*AblB strain (Mavingui et al., 2012), so the *Wolbachia* detected in the *Ae. aegypti* samples belongs to the *w*AlbB 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 *w*AlbA density varied remarkably in infected males. In that study, 30.8-50.0% of infected male individuals had a low titer of *w*AlbA, which was not detectable by a standard PCR, but detectable by a quantitative PCR assay (Calvitti et al., 2015). The *Wolbachia*

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 *w*MelPop 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 ACCESSEBILITY

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* Rockfeller; 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)

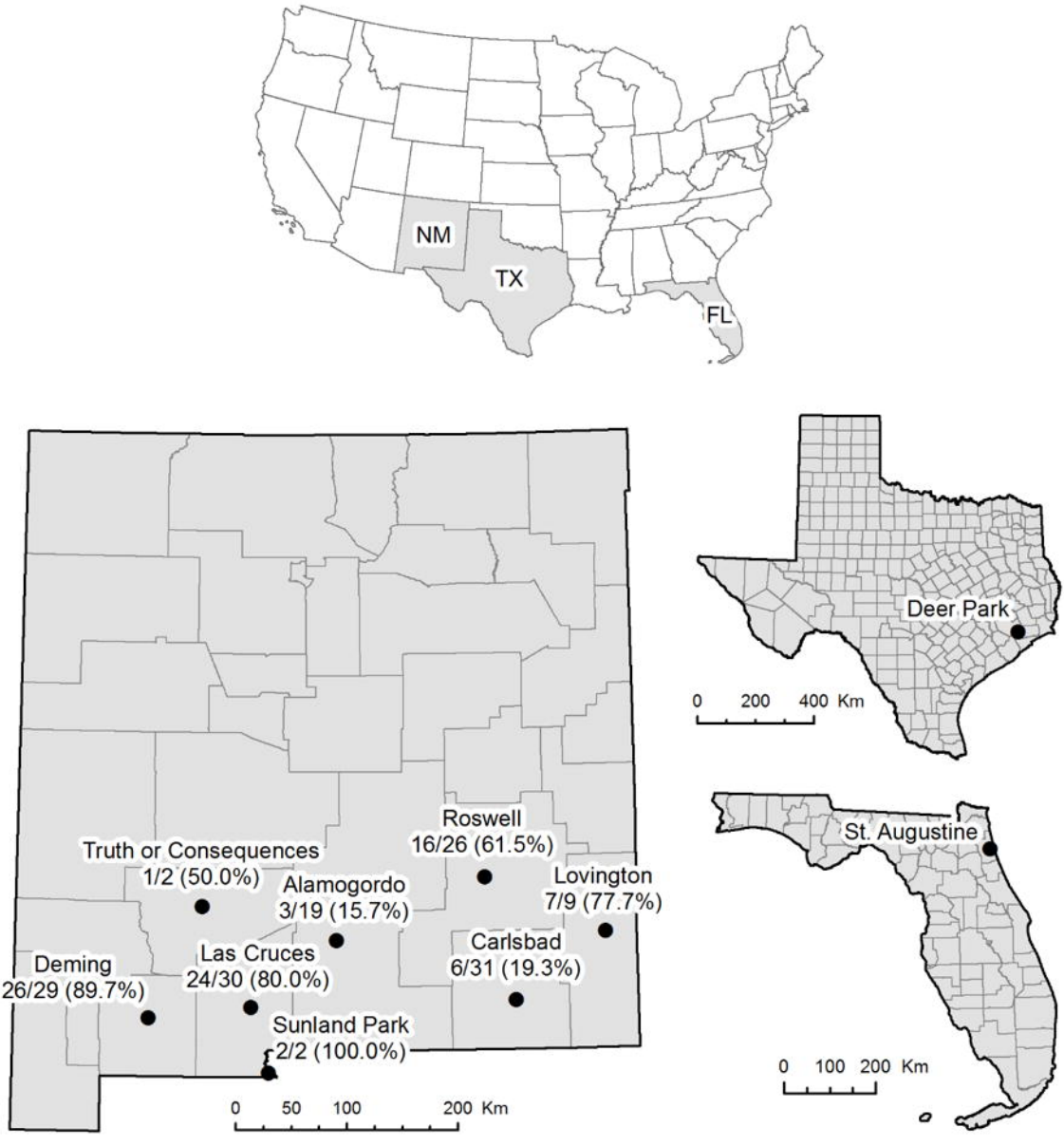
Female (13), TX	9 (69.2)	1 (7.7)	0	10 (76.9)
Male (20), FL	20 (100)	0	0	20 (100)
Female (18), FL	14 (77.8)	0	1 (5.6)	15 (83.3)
Male (2), NM	0	0	1(50.0)	1(50.0)
Female (11), NM	11(100)	0	0	11(100)

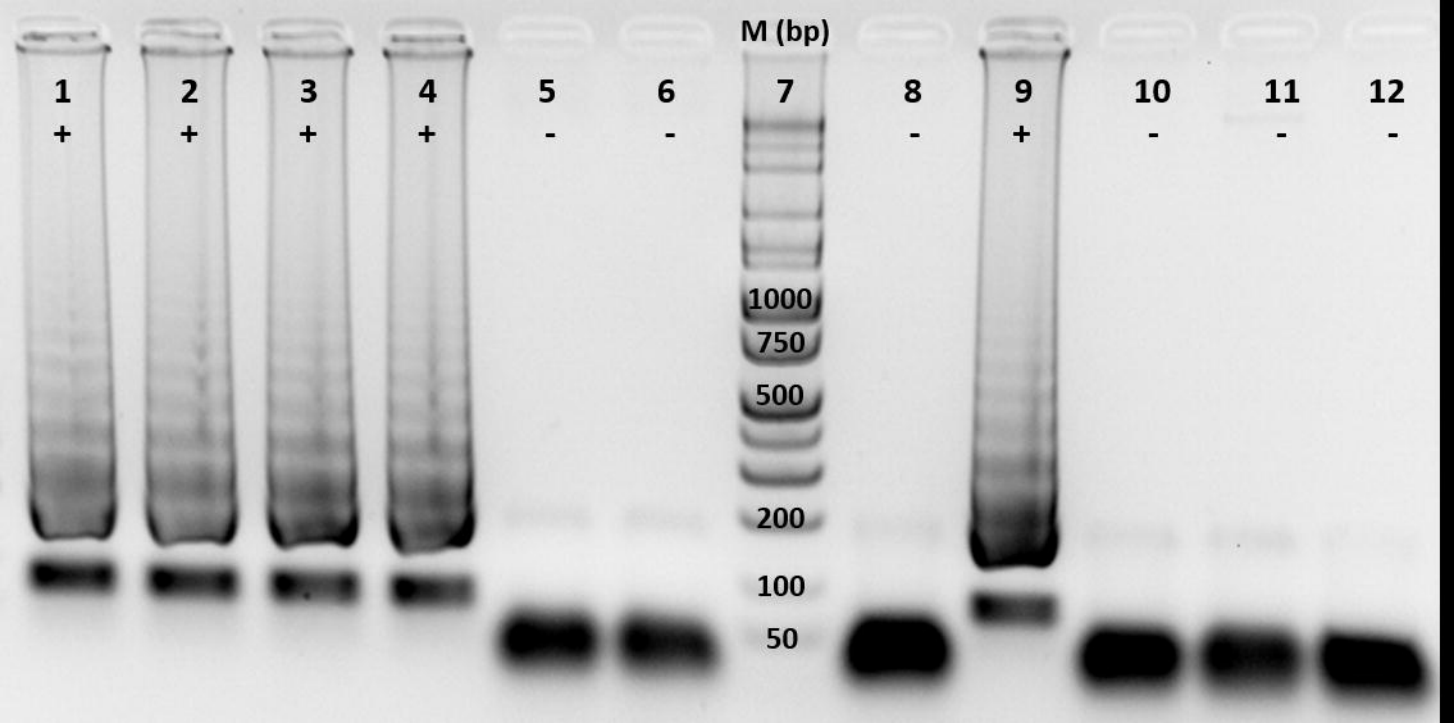
Table S1. Primer sets used in the study

Species identification PCR		Annealing Tm	Reference
18SFHIN	GTAAGCTTCCTTTGTACACACCGCCCGT	55	Higa eta l. (2010)
aeg.r1	TAACGGACACCGTTCTAGGCCCT		
alb.r1	GTACTAGGCTCACTGCCACTGA		
Bacterial 16S rRNA gene			
27F	GAGTTTGATCNTGGCTCAG	50	Wang et al. (2011)
519R	GTNTTACNGCGGCKGCTG		
gatB			Baldo et al. (2006)
wAlbB_gatB_F	TAAGAATCGCAAGAATTCAC	50	
wAlbB_gatB_R	TGGYAAYTCRGGYAAAGATGA		
wAlbA_gatB_F	TTTAGAGCAAGATGCAGGRAAGAGCG	50	
wAlbA_gatB_R	TGGYAAYTCRGGYAAAGATGA		
fitsZ			Baldo et al. (2006)
wAlbB_ftsZ_F	AAAGATAGCCATATGCTCTTT	50	
wAlbB_ftsZ_R	CATTGCTTTACCCATCTCA		
wAlbA_ftsZ_F	AAAGATAGTCATATGCTTTTC	50	
wAlbA_ftsZ_R	CATCGCTTTGCCCATCTCG		
Phosphoprotein phosphatase			This study
wAlbB_PP_F	CGCAGCTCAATTAACAATACC	55	
wAlbB_PP_R	CAATCAGCGTACCAAGCTTCT		
wAlbA_PP_F	ACTCAATTAACAATGGTGATCAT	55	
wAlbA_PP_R	AGCTCTCTTACAACTCTCATGG		
Diaminopimelate epimerase (EC 5.1.1.7)			This study
wAlbB_DE_F	TGTGAATGCGATCCCCTTTATC	55	
wAlbB_DE_R	TATCGCCAGTCATAAATATATTTCC		
wAlbA_DE_F	ACTGAGTGTGACACTCTTCACT	55	
wAlbA_DE_R	CTGTTGGGCAGTCAGATATCC		
LAMP, 16S ribosomal RNA gene		N/A	This study
F3	CTGGAACTGAGATACGGTC		
B3	TTACGCCCAATAATTCCGA		
FIP	TCTTCACTCATGCGGCATGGCAGTGGGGAATATTGGACAA		
BIP	AGGAAGATAATGACGGTACTCACAGATAACGCTAGCCCTCTCC		
LF	CTGGATCAGGCTTTCGCCC		
LB	AGTCCTGGCTAACTCCGTG		

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	*w*AlbB	MH734116
D4a	*Ae. aegypti*	gatB	*w*AlbB	MH734120
LC11	*Ae. albopictus*	diaminopimelate epimerase (DE)	*w*AlbA	MH734119
FL139	*Ae. aegypti*	diaminopimelate epimerase (DE)	*w*AlbB	MH734118
TX120	*Ae. albopictus*	phosphoesterase (PE)	*w*AlbA	MH734121
FL8	*Ae. aegypti*	phosphoesterase (PE)	*w*AlbB	MH734117


NM
TX
FL
Roswell
16/26 (61.5%)
Truth or Consequences
1/2 (50.0%)
Alamogordo
3/19 (15.7%)
Lovington
7/9 (77.7%)
Las Cruces
24/30 (80.0%)
Carlsbad
6/31 (19.3%)
Deming
26/29 (89.7%)
Sunland Park
2/2 (100.0%)
0 50 100 200 Km
Deer Park
0 200 400 Km
St. Augustine
0 100 200 Km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