5-2017

Genetic Variation in Two Economically Important Arthropod Vectors of Citrus Diseases

Alejandra Fuentes
The University of Texas Rio Grande Valley

GENETIC VARIATION IN TWO ECONOMICALLY IMPORTANT ARTHROPOD VECTORS OF CITRUS DISEASES

A Thesis

by

ALEJANDRA FUENTES

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2017

Major Subject: Biology

GENETIC VARIATION IN TWO ECONOMICALLY IMPORTANT ARTHROPOD VECTORS OF CITRUS DISEASES

A Thesis
by
ALEJANDRA FUENTES

COMMITTEE MEMBERS

Dr. Alexis Racelis
Chair of Committee

Dr. Erin Schuenzel
Committee Member

Dr. Matthew Terry
Committee Member

Dr. W. Evan Braswell
Committee Member

Dr. Raul Ruiz-Arce
Committee Member

May 2017

ABSTRACT

Fuentes, Alejandra, Genetic Variation in Two Economically Important Arthropod Vectors of Citrus Diseases. Master of Science (MS), May, 2017, 56 pp., 13 tables, 6 figures, references, 93 titles.

Arthropod vectors are agriculturally and ecologically important because they transmit numerous plant pathogens. Commonly, control strategies for vector-borne plant pathogens focus on suppression of vector populations. Genetic variation studies in pest populations provide useful information for biological control, understanding pesticide resistance, and inferring global movement patterns. In chapter I, genetic variation of worldwide populations of *Diaphorina citri*, the vector of *Citrus* greening disease, was examined to assess potential sites of origin of invasive populations. The results showed population structure at regional levels, suggesting limited gene flow and revealing patterns of invasion. In chapter II, I explored the community of potential *Citrus* leprosis vectors, *Brevipalpus* species, on *Citrus* plants. These communities showed low genetic diversity, lack of association with *Citrus* species, and a high proportion of disease vectors. Population genetic analysis with Mexican and Brazilian mites revealed no population structure suggesting that these vectors can migrate unhindered between these areas.

DEDICATION

The completion of this thesis would not have been possible without the support of my loving family. To my parents, Paula and Ignacio, who are my inspiration and motivation to follow through all my endeavors. To my sister, Paulina and my brother Nacho, whom I have always looked up to. To my aunt Irene whose hard work and determination has been an example that inspired me to accomplish all my dreams. Thank you for all the love, support, and patience.

ACKNOWLEDGEMENTS

I would like to give special thanks to Dr. Evan Braswell for all the support while doing my research and writing my thesis. His commitment to this project has made the completion of this thesis possible – thank you for being an exemplary mentor. I would also like to thank Dr. Raul Ruiz-Arce for his valuable suggestions to the analyses included, and his consistent feedback during the writing process. I would like to acknowledge the chair of my thesis committee, Dr. Alexis Racelis for giving me the opportunity to be part of his program, and for providing me with guidance through my academic career. I would like to acknowledge the assistance given to me by my coworkers at the USDA-APHIS Mission Laboratory and the many collaborators that supported this research. Many thanks for the contributions in the completion of sample collections, data processing, and your continued support in the writing of this thesis. I would also like to thank my friend Ramiro Patino for continuously being there for me all these years.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

GENETIC VARIATION AND POPULATION STRUCTURE OF DIAPHORINA CITRI USING CYTOCRHOME OXIDASE I SEQUENCING

Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama [Hemiptera: Psyllidae], is a major pest of citrus. In addition to physical damages to citrus, this phloem-feeding insect is the most effective vector of citrus greening disease *Candidatus* Liberibacter asiaticus, and *Candidatus* Liberibacter americanus, the bacterium responsible for Huanglongbing (HLB) disease (Hall & McCollum, 2011). Nymphs and adults of *D. citri* acquire the bacterial pathogen when feeding on infected citrus plants. Once the bacteria are inside the vector insect, it can colonize and propagate in the digestive tract, invading the salivary glands thus enabling *D. citri* to infect other citrus plants while feeding (Chu et al. 2016).

Citrus greening disease is considered the most serious disease of citrus in the world (da Graca & Korsten, 2004). The effects from this disease have a substantial economic impact because the majority of commercial citrus species are adversely affected by this bacterium (McClean & Schwarz, 1970). The affected trees develop diverse symptoms including mottling of the leaves and chlorosis, and invisible symptoms including root death (Tsai & Liu, 2000; Johnson et al. 2013). The quality and size of the fruit produced by infected trees is negatively affected, resulting in small, discolored fruit with a bitter taste (Halbert & Manjunath, 2004). At

present, the primary method to control HLB is destructive removal of infected trees to prevent further infections (Ayres et al. 2005; Lopez-Collado et al. 2013). Therefore, as a key vector for HLB, ACP represents a dangerous threat to regions that are still free of this disease (Bove, 2006).

The ACP was first described in Taiwan in 1907, and it is thought to be native to southwestern Asia (Halbert & Manjunath, 2004; Mead, 1977). Specifically, it has been suggested that *D. citri* evolved in India, in association with a species of orange jessamine *Murraya* (Beattie et al. 2008; Hollis et al. 1987). At the present, it has invaded many countries causing significant damages to the agricultural communities in the rest of Asia, including China, Myanmar, Taiwan, Philippine Islands, Malaysia, Indonesia, Sri Lanka, Pakistan, Thailand, Nepal, Hong Kong, Ryukyu Islands, Afghanistan, Saudi Arabia, Yemen, the Philippine Islands, Papua New Guinea, Reunion, and Mauritius (Hall et al 2013; Boykin et al. 2012; Hall et al. 2008). ACP was first reported in South America in 1942, and now is common to citrus growing regions in Brazil, Argentina, Venezuela, Paraguay, and Uruguay (Costa Lima, 1942). It is also pervasive in Central America, reported in Mexico, Costa Rica, Belize and Honduras (Hall et al. 2008; Halbert & Nunez 2004), and most of the Caribbean islands including Bahamas, Cayman Islands, Cuba, Jamaica, Dominican Republic, Guadeloupe, Abaco Island, Grand Bahama Island, and Puerto Rico (Pluke et al. 2008; Hall et al. 2008).

In the US, ACP has also invaded the Pacific Islands of Hawaii and Guam and has been reported in the states of Alabama, Arizona, California, Georgia, Louisiana, Mississippi and South Carolina (French et al. 2001; Hall et al. 2013; Hall et al. 2011; De Leon et al. 2010). Of pressing concern, ACP is being actively managed in California, Florida, and Texas, three of the most important citrus regions in the US. In sum, citrus losses associated with ACP and citrus

greening has been estimated to exceed US$3.6 billion in a 5-year period, only in Florida (Hodges & Spreen, 2012), and its spread continues to threaten the entire citrus industry worldwide. Minimizing further infestations by *D. citri* is paramount to help reduce the spread of citrus greening disease.

Invasive species like the ACP are often subject to intensive evolutionary pressures, including bottlenecks. This process can result in the development of populations and subpopulations with different ecological traits, such as host preference, mating preference, development time, pathogenicity, vector capacity and susceptibility to natural enemies (Guidolin et al. 2014; Jourdie et al. 2010; Remais et al. 2011). As such, knowledge of the genetic variation and population structure of an invasive pest species can help identify the geographical origin of the invader, and this information can also be useful for biological control programs (Guillemaud et al. 2011).

Mitochondrial DNA analysis has been widely used for phylogenetic inferences and population genetic inferences (Guidolin et al. 2014; Boykin et al. 2012; Avise et al. 2000). Previously, the global genetic diversity of *D. citri* was studied in 15 different countries using mitochondrial cytochrome oxidase I (mtCOI) sequencing, by Boykin et al. (2012). Their study detected 8 haplotypes divided in two major haplotype groups South West Asia (SWA), and South East Asia (SEA). They proposed two independent introductions for the New World (Boykin et al. 2012), as suggested by a previous study on the genetic diversity of *D. citri* (De Leon et al. 2011). However, due to the limited sampling in the Old World they could not resolve the geographic origin of *D. citri*. The authors suggested that further sampling, especially from the Middle East and Asian countries was needed to better define the genetic diversity of this insect.

Because of the limited knowledge on the genetic diversity of *D. citri*, identifying the source or pathway of the Asian citrus psyllid is difficult. Additional data from geographic regions where this pest occurs is needed. This information would also aid in making decisions concerning pest management programs. The objective of this study is to: (I) examine the genetic variation of *D. citri* using COI sequences in samples from different geographic locations and (II) to examine these collections for population structure in order to further populate the dataset used for the pathway analysis of this pest.

Materials and Methods

Insect Collection

A total of 883 *D. citri* samples were collected from different citrus host-plants, and made available to us by collectors from 18 different countries between 2008 and 2012 (Table 1.1). Additionally, 225 *D. citri* COI sequences deposited in GenBank by Boykin et al. (2012), Lashkari et al. (2014), and Chaitanya et al. (2013 – not published) were included in the global phylogenetic analysis (Table 1. 2).

Genomic DNA isolation, Polymerase Chain Reaction (PCR) amplification, and sequencing

Total genomic DNA was isolated from each individual using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). The primers (forward: DCITRI COI-L 5'- AGG AGG TGG AGA CCC AAT CT-3') and (reverse: DCITRI COI-R 5'- TCA ATT GGG GGA GAG TTT TG-3') designed by Boykin et al. (2012) were used to amplify a partial fragment of COI from *D. citri*.

Table 1.1. Geographical origin of *D. citri* samples analyzed in this study.

Site no.	Country	Population Site	n	Latitude	Longitude	Year	Host
1	American	American Samoa, Amanave Village	1	-14.32664 S	170.82886 W	2011	*Citrus limon*
2	Samoa (22)	American Samoa, Amouli Village	1	-14.27298 S	170.58499 W	2012	*Citrus aurantium*
3		American Samoa, Aoa Village	1	-14.26147 S	170.58553 W	2011	*Citrus aurantifolia*
4		American Samoa, Asili Village	1	-14.33071 S	170.79608 W	2011	*Citrus aurantium*
5		American Samoa, Auasi Village	1	-14.27056 S	170.57408 W	2012	*Citrus aurantifolia*
6		American Samoa, Fagaalu Village	1	-14.28962 S	170.68767 W	2011	*Citrus aurantifolia*
7		American Samoa, Fagaitua Village	1	-14.26783 S	170.6153 W	2011	*Citrus aurantifolia*
8		American Samoa, Fagatogo Village	1	-14.27962 S	170.69231 W	2011	*Citrus aurantium*
9		American Samoa, Faleniu Village	1	-14.32985 S	170.74478 W	2011	*Citrus limon*
10		American Samoa, Fatumafuti Village	1	-14.29742 S	170.67842 W	2012	*Citrus aurantifolia*
11		American Samoa, Futiga Village	1	-14.34895 S	170.75926 W	2011	*Citrus aurantifolia*
12		American Samoa, Leone Village	1	-14.33727 S	170.78508 W	2012	*Citrus limon*
13		American Samoa, Malaeimi Village	1	-14.32152 S	170.74036 W	2012	*Citrus aurantifolia*
14		American Samoa, Matu'u Village	1	-14.29853 S	170.68532 W	2012	*Citrus paradisi*
15		American Samoa, Nua Village	1	-14.32516 S	170.80875 W	2011	*Citrus aurantifolia*
16		American Samoa, Nuuuli Village	1	-14.31373 S	170.71385 W	2011	*Citrus reticulata*
17		American Samoa, Pago Pago Village	1	-14.27842 S	170.70582 W	2011	*Citrus aurantifolia*
18		American Samoa, Pavaia'i Village	1	-14.33314 S	170.75023 W	2011	*Citrus aurantifolia*
19		American Samoa, Tafuna Village	1	-14.33472 S	170.72968 W	2011	*Citrus limon*
20		American Samoa, Tula Village	1	-14.25253 S	170.56743 W	2011	*Citrus aurantifolia*
21		American Samoa, Utumea Village	1	-14.32851 S	170.81506 W	2012	*Citrus aurantifolia*
22		American Samoa, Utusi'a Village	1	-14.2706 S	170.61887 W	2012	*Citrus aurantifolia*
23	Argentina (15)	Argentina:Bella Vista, Corrientes	4	-28.507731 S	-59.044857 W		
24		Argentina:Federacion, Entre Rios	3	-30.985036 S	-57.919842 W		
25		Argentina:Fraile Pintado, Jujuy	4	-23.944207 S	-64.803149 W		
26		Argentina:Yuchan, Salta	4	-23.433330 S	-64.16667 W		
27	Barbados (72)	Barbados, Golden Grove	33	13.156757 N	-59450503 W	2010	*Citrus sp.*
28		Barbados, St. Michael, Clermont	39	13.145170 N	-59.617539 W	2010	*Citrus sp.*
29	Belize (9)	Belize, Stann Creek	9	16.811663 N	-88.401604 W	2009	
30	China (55)	China Yunnan, Xishuangbanna	4	21.11440 N	101.41234 E	2010	*Murraya paniculata*
31		China: Hong Kong, Pak Kong	3	22.379476 N	114.258182 E	2010	*Murraya paniculata*
32		China: Jiangxi Province, Chongyl	1	25.40433 N	114.18285 E	2011	*Citrus sp.*
33		China: Gangzhou	40	23.129110 N	113.264385 E		
34		China: Zhejaing	7	30.267443 N	120.152792 E		
35	Colombia (12)	Colombia: Armero Guayabal	4	5.030556 N	-74.885556 W		*Citrus aurantifolia*
36		Colombia: Municipio de Coello	4	4.354029 N	-74.863576 W		*Citrus aurantifolia*
37		Colombia: Municipio de Guamo	4	4.195320 N	-75.00753 W		*Citrus aurantifolia*
38	Costa Rica (10)	Costa Rica	10	9.748917 N	-83.753428 W		
39	Mexico (30)	Mexico: Tijuana, Baja California	1	32.454655 N	-116.916855 W	2010	*Citrus sinensis*
40		Mexico: Tijuana, Baja California	1	32.521307 N	-116.935036 W	2010	*Citrus sinensis*
41		Mexico: Hermosillo, Sonora	6	29.072967 N	-110.955919 W	2008	
42		Mexico: Jalpan, Queretaro	2	21.207300 N	-99.472267 W	2012	
43		Mexico: Nuevo Mugica, Michoacan	2	19.429241 N	-102.084917 W	2012	*Citrus aurantifolia*
44		Mexico: Puerto Vallarta, Jalisco	2	20.653407 N	-105.225332 W	2012	*Citrus latifolia*
45		Mexico: Rio Grande, Oaxaca	1	16.010481 N	-97.433525 W	2012	*Citrus latifolia*
46		Mexico: Los Mochis, Sinaloa	2	25.790466 N	-108.985882 W	2012	*Citrus aurantifolia*
47		Mexico: Ciudad Obregon, Sonora	2	27.482773 N	-109.930367 W	2012	*Citrus sinensis*
48		Mexico: Huimanguillo, Tabasco	2	17.830278 N	-93.391389 W	2012	*Citrus sinensis*
49		Mexico:Tamuin, San Luis Potosi	2	22.00000 N	-98.783333 W	2012	*Citrus sinensis*
50		Mexico: Tecoman, Colima	1	18.908889 N	-103.874722 W	2012	*Citrus aurantifolia*
51		Mexico: Tepic, Nayarit	2	21.504165 N	-104.894589 W	2012	*Citrus latifolia*
52		Mexico: Ucum, Quintana Roo	2	18.502535 N	-88.518226 W	2012	*Citrus latifolia*
53		Mexico: Cazones, Veracruz	1	20.723230 N	-97.31353 W	2010	*Citrus paradisi*
54		Mexcio: Mtz de la Torre, Veracruz	1	20.061513 N	-97.054526 W	2012	*Citrus latifolia*
55	Pakistan (374)	Pakistan: Bhalwal	39	32.275141 N	72.904714 E	2010	*Citrus sp.*
56		Pakistan: Faisalabad	13	31.418714 N	73.079107 E	2009	*Citrus sp.*
57		Pakistan: Lalian	40	31.825260 N	72.80274 E	2009	*Citrus sp.*
58		Pakistan: Mandi Bahauddin	41	32.588169 N	73.497343 E	2010	*Citrus sp.*
59		Pakistan: Sahiwal	42	30.661181 N	73.108576 E	2009	
60		Pakistan: SGD	41	32.003973 N	72.723141 E	2010	
61		Pakistan: Shahpur	18	32.286612 N	72.430253 E	2010	*Citrus*
62		Pakistan: Shamasabad Jhang	39	31.592714 N	73.050454 E	2010	*Citrus sp.*
63		Pakistan: Toba Tek Singh	40	30.966667 N	72.483333 E	2010	*Citrus sp.*
64		Pakistan: Toba Tek Singh	40	30.966667 N	72.483333 E	2010	*Citrus sp.*
65		Pakistan: Multan	10	30.198381 N	71.468703 E		
66		Pakistan: Singh & Sargoda	11	32.113041 N	73.935097 E		
67	Paraguay (7)	Paraguay: Itapua	7	-26.792362 S	-55.668964 W		

Site no.	Country	Population Site	n	Latitude	Longitude	Year	Host
68	Puerto Rico (59)	Puerto Rico: Isabela, Guerrero	19	18.473889 N	-67.048056 W	2010	*Citrus sinensis*
69		Puerto Rico: Arecibo	38	18.454893 N	-66.758134 W		*Citrus sp.*
70		Puerto Rico: Carolina, Vista Mar	2	18.436944 N	-65.981944 W		
71		Puerto Rico	2				
72	Reunion (4)	Reunion Island: Saint Pierre	4	-21.332838 N	55.471843 E	2011	*Murraya*
73	Saudi Arabia (4)	Saudi Arabia	4	23.885942 N	45.079162 E		
74	Singapore (3)	Singapore: Chinese Gardens	1	1.20358 N	103.43867 E	2010	*Murraya paniculata*
75		Singapore: Kim Seng Road Park	1	1.17604 N	103.4995 E	2010	*Murraya paniculata*
76		Singapore: Winsland Place	1	1.17952 N	103.50393 E	2010	*Murraya paniculata*
77	Thailand (29)	Thailand: NST, Twin Lotus Hotel	13	8.391284 N	99.978176 E	2010	*Murraya paniculata*
78		Thailand: Bangkok	16	13.786902 N	100.512641 E	2010	*Murraya paniculata*
79	Trinidad (19)	Trinidad: Grand Bazaar	19	37.169463 N	-104.500541 W	2010	*Citrus limettoides*
80	Uruguay (6)	Uruguay: Itapebi, Salto	6	-31.287202 S	-57.7.5548 W		
81	USA (153)	USA: AZ Yuma	2	32.692651 N	-114.627692 W	2010	*Citrus lemon*
82		USA: AZ Yuma	1	32.490834 N	-114.76772 W	2010	*Citrus lemon*
83		USA: LA, Kenner	1	29.996357 N	-90238538 W	2011	*Citrus sp.*
84		USA: LA, Grammercy	1	30.061528 N	-90.696012 W	2011	*Citrus unshui*
85		USA: SC, Port Royal	1	32.376735 N	-80.69475 W	2012	*Citrus sinensis*
86		USA: CA, Los Angeles County	3	34.052227 N	-118.243660 W	2009	
87		USA: CA, Imperial County	9	33.011369 N	-115.473355 W	2010	
88		USA: HI, Hilo, Homelani	16	19.718755 N	-155.089629 W	2010	*Murraya paniculata*
89		USA, TX, Edinburg,	81	26.301737 N	-98.163343 W	2010	*Murraya paniculata*
91		USA: TX, North East of Hidalgo	19	26.313640 N	-97.8634.16 W	2010	*Citrus sp.*
92		USA: TX, Corpus Christi	2	27.800583 N	-97.396381 W	2008	*Citrus sp.*
93		USA: TX, Presidio, Presidio County	2	29.560738 N	-104372146 W	2008	*Citrus so.*
94		USA: TX, Houston, Harris County	2	29.760427 N	-95.369803 W	2008	*Citrus sp.*
95		USA: TX, Uvalde	2	29.209684 N	-99.786168 W		
96		USA: TX, Tilden	2	28.461508 N	-98.549378 W		
97		USA: TX, San Antonio	2	29.457978 N	-98.460466 W		
98		USA: TX, Refugio	2	28.292619 N	-97271013 W		

The Polymerase Chain Reaction (PCR) contained 16.77µl of DNAase free water, 2.50µl of 10X Taq Buffer (TaKaRa Bio Inc, Mountain View, CA), 2.60µl of dNTP mixture 2.5µM each (TaKaRa), 0.13µl TaKaRa Ex Taq polymerase, 1µl of each primer at 10µM and 1µl of DNA template for a total volume of 25µl. The amplification was conducted in Applied Biosystems (Foster City, CA) Gene Amp® PCR System 9700 thermal cycler and the following cycling parameters were used: 94°C for 2 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 53°C, of 1 min at 72°C extension and a final extension of 72°C for 10 minute, as described in Boykin et al. (2012).

Table 1.2. Accession numbers of *D. citri* sequences recovered from GenBank included in the analyses.

Country	Accession No.	Author	Country	Accession No.	Author
USA: Florida	FJ190167-FJ190176	Boykin et al. 2012	Mexico	FJ190300-FJ190309	Boykin et al. 2012
	FJ190182-FJ190191		Mauritius	FJ190312-J190316	
	FJ190193-FJ190227		Reunion	FJ190317-FJ190318	
	FJ190232-FJ190259		Brazil	FJ190321-FJ190327	
	FJ190277-FJ190278		Brazil	FJ190329-FJ190333	
	FJ190310-FJ190311		Indonesia	FJ190336	
	FJ190372-FJ190377		Saudi Arabia	FJ190337-FJ190340	
USA: Texas	FJ190177-FJ190181		India	FJ190342-FJ190345	
Brazil	FJ190228-FJ190231		Guadeloupe	FJ190346-FJ190356	
Puerto Rico	FJ190260-FJ190263		China	FJ190357-FJ190364	
Indonesia	FJ190263-FJ190271		China	FJ190366-FJ190369	
Indonesia	FJ190280-FJ190282		Vietnam	FJ190272-FJ190276	
Taiwan	FJ190284-FJ190287		Vietnam	FJ190278-FJ190282	
Pakistan	FJ190288-FJ190292		Pakistan	KC509561-KC509572	Lashkari et al. 2013
Thailand	FJ190293-FJ190294		India	KF702297-KF702306	Chaitanya et al. 2013 (unpublished)
China	FJ190297-FJ190-299				

The PCR products were stained with Blue/Orange 6X loading Dye (Promega, Madison, WI) and visualized with ethidium bromide on 1.5% electrophoresis agarose gels at 90V for 90 minutes in 1X TAE buffer. Documentation of these gels was performed using UVP Gel-Doc-it^{TS2} imager (Upland, CA). Before sequencing, the PCR products were purified with Exo-SAP-IT^{TM} (Affimetrix, Santa Clara, CA), using the company protocol. Sequencing of PCR products was performed using bidirectional sequencing and 3' BigDye-labeled dideoxynucleotide triphosphates (v. 3.1 dye terminators, Applied Biosystems, Foster City, CA, USA), and run on an ABI 3730XL DNA Analyzer with the ABI Data Collection Program v. 2.0 at the Huck Institute's Nucleic Acid facility at Pennsylvania State University. Sequences were edited using

Sequencher® v. 5.0 (Gene Coders Corp. Ann Arbor, MI). The alignment was constructed in MEGA v. 6.0 (Tamura et al. 2013) using Clustal W (Thompson et al. 1994). Sequences were trimmed of primers to 678bp.

Genetic Diversity and Population Structure

Haplotypes (h) were identified using DnaSP v. 5.10.01 (Rozas et al. 2003). DNA Pairwise genetic distances were estimated in MEGA v.6. The number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (μ) the average number of pairwise differences (k) (Tajima, 1983), and the Tajima's D (Tajima, 1989) and Fu and Li's D and F (Fu & Li, 1993) tests were determined with DnaSP v. 5.10.01 (Rozas et al. 2003). Tajima's and Fu and Li's tests were performed to test for neutral evolution and evaluate the potential for recent population expansion or contraction. General population genetics statistics were calculated to determine probable center of origins based on diversity. A haplotype network, which include haplotype frequencies, was constructed in PopArt (Leigh & Bryant, 2015) using the TCS statistical algorithm (Clement et al. 2000). An analysis of molecular variance (AMOVA) was performed to determine the percentage of variation among geographic groups, among populations, and within groups, were performed in ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010).

Phylogenetic and haplotypes analyses

JModelTest 2 (Darriba et al. 2012) was used to identify the best substitution model for the DNA sequence data. A Bayesian phylogenetic reconstruction was then conducted using Mr.Bayes v.3.2.3 (Ronquist & Huelsenbeck, 2003; Miller et al. 2010) in Cipres allowing transitions and transversions to have different rate. The analysis was performed using a Monte

Carlo Markov Chain (MCMC) method. Four chains were run independently for 10,000,000 generations with sampling conducted every 1,000 generations. The COI sequence of the hackberry petiole gall psyllid (*Pachypsylla venusta*) was selected as an out-group for the analyses (accession number: NC_006157). Additionally, a phylogenetic analysis using maximum likelihood methods was performed in PhyML v. 3.0 (Guindon et al. 2010), using the nearest-neighbor interchange tree rearrangement and 1,000 bootstrap replications.

Results

Genetic Variation

The final DNA sequence alignment of mitochondrial COI from 1,108 individuals was 678bp and contained a total of 29 polymorphic sites, 12 parsimoniously informative sites, and no indels. There were 18 synonymous changes and 11 non-synonymous. This resulted in 28 haplotypes shown by samples gathered from among the 26 locations analyzed for the study (Table 1.3). Of these haplotypes, 16/28 (57.14%) were identified from single individuals (singletons). The genetic diversity for the entire sequence dataset was low (<1%), and the maximum number of substitutions was 5bp for the most diverse haplotype (Dcit-20-Texas). Nucleotide and haplotype diversity were 0.00293 and 0.734 respectively. Four haplotypes were observed among 95.84% of these individuals. These included Dcit-1 368/1108 (33.21%), Dcit-2 158/1108(14.27%), Dcit-3 160/1108 (14.45%) and Dcit-06 376/1108 (33.96%). The average number of haplotypes per collection was 2.19 and ranged from 1 to 10 haplotypes per site for the entire dataset. The majority (92.3%) of these haplotypes were private to a single geographic region: fourteen haplotypes were unique to South West Asia (SWA), five for North and Central America (NCA), four for South East Asia (SEA), and two for the Caribbean (CAR).

Table 1.3. Geographic distribution and frequency of the 28 haplotypes of *D. citri* observed in this study.

Haplotype	Clade/Subclade	Origin of population (n = # of individuals)
Dcit-01	B1	USA (229), Mexico (33), Belize (9), Costa Rica (10), American Samoa (22), Brazil (4), Puerto Rico (14), Saudi Arabia (8), Iran (10), Pakistan (9), India (4), China (11), Indonesia (4), Reunion (1).
Dcit-02	B2	Brazil (12), Paraguay (7), Argentina (15), Uruguay (6), China (59), Taiwan (4), Indonesia (9), Thailand (22), Singapore (3), Mauritius (5), Reunion (6) Vietnam (10)
Dcit-03	B3	USA (13), Puerto Rico (48), Guadeloupe (8), Barbados (72), Trinidad (19)
Dcit-04	B1	Mexico (6)
Dcit-05	B1	China (3)
Dcit-06	A	Colombia (12), Pakistan (361), India (3)
Dcit-07	B1	USA (1)
Dcit-08	B2	China (1)
Dcit-09	A	Pakistan (1)
Dcit-10	A	Pakistan (1)
Dcit-11	A	Pakistan (3)
Dcit-12	A	Pakistan (1)
Dcit-13	B3	Guadeloupe (3)
Dcit-14	A	Pakistan (1)
Dcit-15	A	Pakistan (2)
Dcit-16	A	Pakistan (1)
Dcit-17	A	Pakistan (1)
Dcit-18	B2	Thailand (9)
Dcit-19	B2	USA (2)
Dcit-20	B3	USA (1)
Dcit-21	B1	Mexico (1)
Dcit-22	B2	China (1)
Dcit-23	B3	India (2)
Dcit-24	A	India (1)
Dcit-25	A	India (1)
Dcit-26	A	India (1)
Dcit-27	A	India (1)
Dcit-28	A	India (1)

Phylogenetic Analyses

The HKY85 + I + G substitution model was selected as the best model based on Akaike Information Criterion ranking. Modeltest revealed a proportion of invariable sites of 0.1860, a transition/transversion ratio of 1.6314, and gamma shape parameter of 0.6100. The Bayesian (Fig. 1.1) and ML (not shown) phylogenetic analyses showed concordant topologies. The Bayesian analysis and the constructed phylogenetic network (Fig. 1.2) support the presence of geographic structure for *D. citri*. This analysis revealed strong support for major and minor clades. In the Bayesian analysis, two major clades were observed (clade A and clade B). Clade A contains 14 haplotypes and shows strong support (PP=1.00), and clade B moderate support (.67). Three subclades (Subclade B1, B2, and B3) were observed in clade B. Each of these three subclades showed strong branch support (Fig. 1.1).

Population Structure

Geographic association was observed for both major clades and subclade structure (Fig. 1). For example, in Pakistan 372/381 (97.64%) fall within clade A, as well as 10/14 (71.43%), of the individuals from India, and 13/13 (100%) of the individuals from Colombia. This clade included fourteen haplotypes: Dcit-06, Dcit-09 through Dcit12, Dcit-14 through Dcit-17, and Dcit-24 through Dcit-28. The haplotype network shows a star-like shape arrangement seen among haplotypes from Clade A. Dcit-06 is the most common central haplotype for this clade with the remaining closely related haplotypes expressed by no more than three individuals. The haplotypes are not more than three mutational steps from the central haplotype (Dcit-06).

Clade B consisted of subclades B1, B2 and B3. Subclade B1, includes five haplotypes: Dcit-01, Dcit-04, Dcit-05, Dcit-07 and Dcit-21. Dcit-01 is the most geographically distributed

haplotype seen in the study. The remaining four haplotypes are not more than four mutational steps from the most frequent haplotype (Dcit-01), they are locally restricted to a single collection site each. This clade included individuals from 14 of the 26 countries; US 230/246 (93.49%), Mexico 40/40 (100.0%), Belize 9/9 (100%), Costa Rica 10/10 (100%), American Samoa 22/22 (100.0%), Saudi Arabia 8/8 (100.0%), Iran 10/10 (100.0%), Brazil 4/16 (25.00%), Puerto Rico 14/62 (22.58%), Pakistan 9/381 (2.36%), India 4/14 (28.57%), China 11/75 (14.67%), Indonesia 4/13 (30.77%), Reunion 1/7 (14.29%).

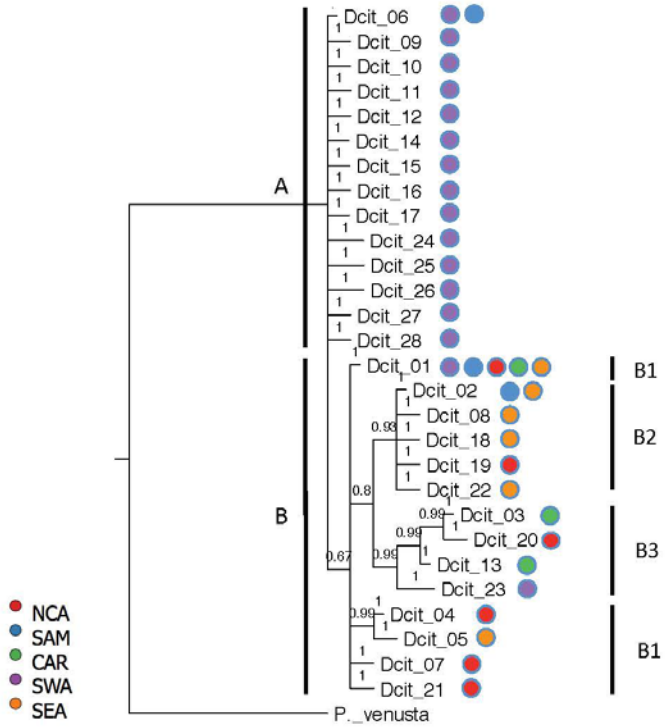

Figure 1.1. Bayesian tree showing the phylogeny of the 28 haplotypes of *D. citri*. Two major clades are observed: A and B, where B is further divided in subclades: B1, B2 and B3.

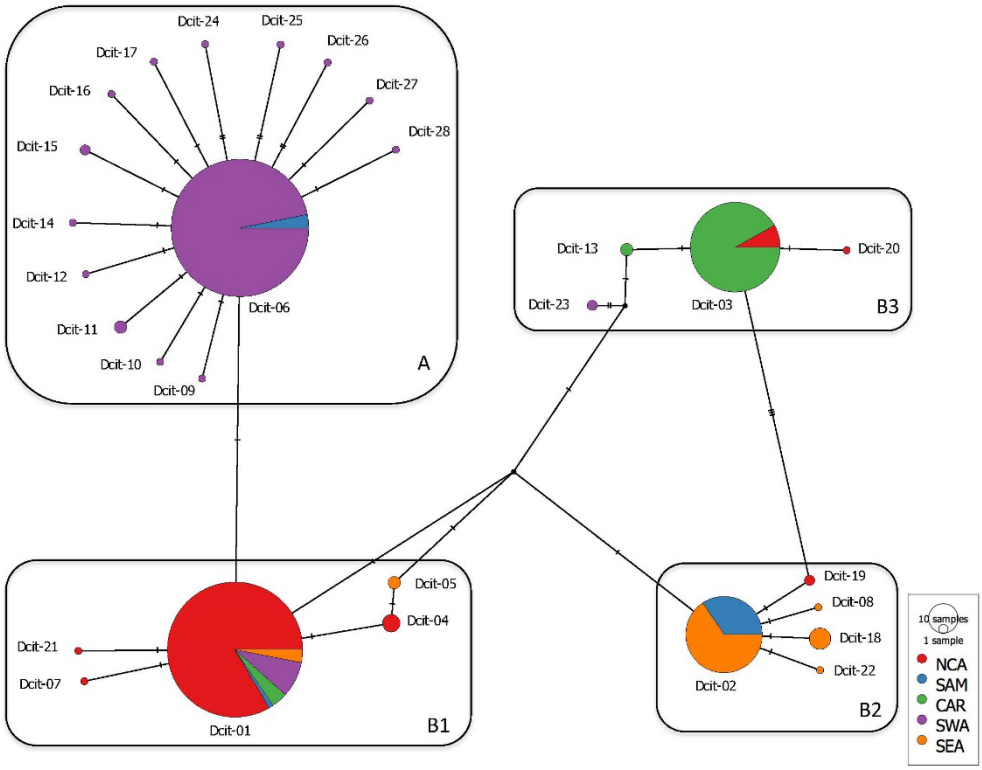

Figure 1.2. Haplotype TCS network showing the genetic relationship of 28 haplotypes recovered from this study. Circles represent haplotypes and connecting lines represent patterns of relationship. Hash marks represent additional mutational steps. Colored circles represent haplotypes observed in the regions associated with their color (NCA – North and Central America, SAM – South America, CAR – The Caribbean, SWA – South West Asia, and SEA – South East Asia). Size of each colored circle is proportional to the observed frequency of the haplotype, and proportion of samples from each region is indicated by the proportion of color associated with that region. Black circles represent inferred haplotypes.

Subclade B2 includes five haplotypes (Dcit-02, Dcit-08, Dcit-18, Dcit-19, and Dcit-22). The most common and widespread haplotype is Dcit-02. The other four haplotypes are no more than two mutational steps away from Dcit-02, and are limited to a single collection site each. This clade contain 7/7 (100.0%) of the individuals from Paraguay, 15/15 (100.0%) of the

samples from Argentina, 6/6 (100.0%) of the samples from Uruguay, 61/75 (81.33% of the individuals from China, 4/4 (100.0%) of the specimens from Taiwan, 2/246 (0.81%) of the samples collected in the US, 10/10 (100.0%) of the samples from Vietnam, 9/13 (69.23%) of the specimens from Indonesia, 31/31 (100.0%) of the samples from Thailand, 3/3 (100.0%) of the individuals from Singapore, 5/5 (100.0%) of the individuals collected in Mauritius, and 6/7 (85.71%) of the specimens from Reunion.

Subclade B3 included haplotypes Dcit-03, Dcit-13, Dcit-20 and Dcit-23. The most common haplotype is Dcit-03. The three other haplotypes were not more than six mutational steps away and were limited to a single collection site each. This clade contains 13/246 (5.28%) of the individuals from the US, 48/62 (77.42%), of the individuals from Puerto Rico, 8/11 (78.73%) of the samples collected in Guadeloupe, 72/72 (100.0%), of the individuals gathered in Barbados, and 19/19 (100.0%), of the individuals from Trinidad.

Using natural geographic barriers, previously posed hypothesis, and observed patterns of distribution, the data were delineated into five populations: North and Central America (NCA), South America (SAM), The Caribbean (CAR), South West Asia (SWA) and South East Asia (SEA). The AMOVA confirmed the significance of population structure for the five geographic populations (Table 1.4). The AMOVA rejected the null hypothesis that the five populations are homogeneous. The highest source of variation (75.92%) was observed within regions, according to the five populations we mention above, for those combinations we tested. Variation was also observed among populations (10.05%), and within populations (14.03%).

Table 1.4. AMOVA results to test for population structure for *D. citri* mtCOI. Values in **bold** are significant with p=0.00000.

			Among populations			
	Within populations		Among regions		Within regions	
Group Division	% var	F_{ST}	% var	F_{SC}	% var	F_{CT}
Region (n=5)	14.03	**0.8596**	10.05	**0.1729**	75.92	**0.7592**
N. America/C. America vs. The Caribbean S. America vs. South West Asia vs. South East Asia						

Regional Population Summary

South West Asia population. In all, 413 individuals were successfully sequenced. A total of 16 haplotypes were observed in this region. We observed a higher nucleotide diversity (0.00447), and a lower haplotype diversity (0.218), as compared with the estimated overall nucleotide and haplotype diversity (Table 1.5). The most common haplotype recovered in this region is haplotype Dcit-6. This haplotype was observed in 364 (88.13%) of SWA individuals. This haplotype was observed in 361/374 (97%) individuals from Pakistan, 3/14 (21.42%) of the individuals from India, and 12/12 (100%) of the psyllids gathered from Colombia. We observed that most of the diversity for the entire dataset belonged within Pakistan and India. Collectively, the haplotype diversity for these two countries was estimated at Hd=0.149.

Eight of the sixteen haplotypes were exclusive to Pakistan psyllids (n=381). Dcit-11 was observed in three individuals from Pakistan and Dcit-16 was observed in two. The remaining five haplotypes (Dcit-9, -10, -14, -16 and -17) seen in samples from Pakistan were recovered from a single individual each. Among the 14 samples from India, six haplotypes were seen in

seven psyllids and were found to be exclusive to that country. Haplotype Dcit-23 was observed in two individuals. The remaining five haplotypes (Dcit-24-28) were recovered from a single individual each. All the individuals from Saudi Arabia (n=8) and Iran (n=10) showed haplotype Dcit-1. Demographic analyses for this population revealed significant values for Tajima's D (-2.27; P<0.02), Fu and Li's D (-5.64: P<0.02), and Fu and Li's F (5.14: P<0.02) (Table 1.5). For SWA populations the Fst value was significant and relatively high (0.86623 P<0.05), suggesting that there is population structure within these populations.

Table 1.5. Genetic diversity of *D. citri* populations analyzed in this study. Values in **bold** are statistically significant with p= < 0.02. The values on the columns represents Tajima's D (D_1), Fu and Li's D (D_2), Fu and Li's F (F), sample size (*n*), average number of nucleotide differences (k), segregating sites (S), nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (*H*d).

Population	*n*	D_1	D_2	*F*	k	S	π	h	*H*d
1. South America	56	1.60671	0.88016	1.28383	1.17403	3	0.00173	3	0.447
2. South West Asia	413	**-2.27012**	**-5.64237**	**-5.1706**	**0.27800**	**20**	**0.00447**	**16**	**0.218**
3. North and Central America	327	-1.51335	-1.37741	-1.71555	0.41782	9	0.00062	7	0.14
4. The Caribbean	164	-1.12119	0.89984	0.66641	0.65831	4	0.00097	3	0.19
5. South East Asia	148	-0.94481	0.08597	-0.31304	0.59801	6	0.00088	6	0.354

South East Asia (SEA) population. In all, 148 sequences were analyzed, and a total of six haplotypes were recovered for psyllids from this region. The haplotype (Hd) and nucleotide (μ) diversity for this region was of 0.354 and 0.00088, respectively. Haplotype Dcit-2 was observed in 118/148 (79.72%) of the SEA individuals, and it was the only haplotype observed in individuals from Taiwan (n=4), Vietnam (n=10), Singapore (n=3) and Mauritius (n=5). In China, five haplotypes were identified, three of which were exclusive to this country (Dcit-05, Dcit-08, and Dcit-22) and made up a small percent of the sample (4%, 1%, and 1%, respectively). The

remaining haplotypes, Dcit-1 and Dcit-2, were observed in eleven individuals (14.67%) and fifty-nine (78.66%) individuals, respectively. Haplotype Dcit-18 was only observed in 9/31 (29.03%) individuals from Thailand. Demographic analyses in this region using haplotype (Hd) and nucleotide (μ) diversity revealed non-significant values for Tajima's D (-0.88), Fu and Li's D (-0.79), and F (-0.97). The Fst value for this region was significant ($P<0.05$), and relatively high (0.80376), suggesting the existence of population structure.

North and Central America (NCA) population. A total of 327 specimens were successfully sequenced for collections from this region, and seven haplotypes were observed. Six of the haplotypes were recovered from psyllids gathered in a single collection site each. The haplotype and nucleotide diversity of this population was of 0.734 and 0.00294, respectively. The majority of the samples from this region were observed in subclade B1 311/327 (95.1%). Haplotype Dcit-1 was the most common haplotype in this group; it was seen in 92.66% of the NCA individuals. This haplotype was seen in psyllids from the five geographical regions analyzed. In Edinburg, Texas, USA, 13/119 (10.92%) individuals showed haplotype Dcit-3, consistent with samples gathered in the Caribbean. Haplotype Dcit-4 was only observed in 6 individuals and was unique to a specific location in Yucatan, Mexico. In Los Angeles, California, US, two individuals showed haplotype Dcit-19. Haplotype Dcit-7, Dcit-20 and Dcit-21 were observed in a single individual each, from psyllids gathered in St. Lucie, Florida, Edinburg, Texas and Los Mochis, Sinaloa, Mexico respectively. Demographic analyses using haplotype (Hd) and nucleotide (μ) diversity estimates resulted in non-significant results for Tajima's D (-1.51), Fu and Li's D (-1.37), and F (-1.71). The Fst value for this region was significant ($P<0.03$), but relatively low (0.10132), suggesting no population structure.

South America population (SAM). Sequences from 56 individuals were included in the analyses. Only three haplotypes were recovered from individuals gathered in this geographic region. A total of 71.43% of the individuals expressed haplotype Dcit-2, the most common haplotype found in SEA. This haplotype occurs in 44/56 of the analyzed individuals. These individuals were found in Argentina, Brazil, Paraguay, and Uruguay. Haplotype Dcit-2 is included in subclade B2. The second most common haplotype, Dcit-6, was found in all individuals from Columbia (12/12) is common to SWA collections. Haplotype Dcit-6 in included in clade A. Four individuals, all from a single location in Brazil, expressed haplotype Dcit-1. Haplotype Dcit-1 is found in subclade B1. Demographic analyses in this population using haplotype (Hd) and nucleotide (μ) diversity revealed no significant values for Tajima's D (1.60), Fu and Li's D (0.88), and F (1.28). The Fst value for this region was significant ($P<0.05$) and relatively high (0.81727), suggesting the presence of population structure.

Caribbean population. For the Caribbean region, sequences from a total of 164 individuals were analyzed, and three haplotypes were recovered. Haplotype Dcit-3 was observed in 89.63% of the collected CAR samples. Additionally, this haplotype was recovered from 13 individuals from Texas. In Puerto Rico fourteen individuals showed haplotype Dcit-1, which also occurs in NCA, SAM, SWA and SEA. Three individuals (27%) from Guadeloupe exhibited haplotype Dcit-13, which was unique to this region. Demographic analyses in this population using haplotype (Hd) and nucleotide (μ) diversity revealed no significant values for Tajima's D (-1.12), Fu and Li's D (0.89), and F (0.66). The Fst value for this region was significant ($P<0.002$), but relatively low, suggesting no population structure for this geographic region.

Discussion

Genetic Diversity

A total of 1,108 DNA sequences from the COI mitochondrial region were analyzed. The analysis revealed the existence of 28 haplotypes and a haplotype and nucleotide diversity of 0.723 and 0.00294, respectively. Our results are in accordance with previous reports of the relatively low genetic diversity of *D. citri* in worldwide populations (Boykin et al. 2012). Because of this low genetic diversity, these results further suggest that *D. citri* is a single species and not a species complex. However, the population structure indicates barriers to gene flow. The numerous private haplotypes observed suggest differentiation and limited gene flow between the five different populations.

It is possible that this genetic isolation is caused by geographic barriers or biotic factors, such as infection with the endosymbiont *Wolbachia*. Recent studies indicated that 66% of all insect species may be infected with this bacterium (Hilgenboecker et al. 2008), and *Wolbachia* has been found infecting *D. citri*. Infection with *Wolbachia* often causes reproductive abnormalities issues including cytoplasmic incompability (CI) (Saha et al. 2012). It has been suggested that infection with different *Wolbachia* strains may lead to reproductive isolation between populations (Riegler & Stauffer, 2002). An association of COI sequences for *D. citri* and *Wolbachia* strains has been observed. US, Mexico, Belize and American Samoa *D. citri* samples were infected with *Wolbachia* strain ST-FL, as well as samples from Pakistan and Colombia (Stelinski pers. comm.). These results suggest that NAM and SWA populations might be infected with the same *Wolbachia* strain. Similarly, *D. citri* samples from China, Singapore and Argentina carried *Wolbachia* strain ST-173, a different strain than the one from SWA and

NAM. Another strain of *Wolbachia* was associated with samples from the CAR, Puerto Rico, Trinidad and Barbados (Stelinski pers. comm.). These results further suggest possible isolation between populations infected with different *Wolbachia* strains.

The information provided in this study is also important for the development of effective biological control programs to control *D. citri* populations. The parasitoid *Tamarixia radiata* has been extensively used as a biological control agent for *D. citri* (Etienne et al. 2001; Hoy & Nguyen, 2001). *T. radiata* has been successfully introduced to several countries, including Reunion Island, Taiwan, Mauritius, Guadeloupe, Florida, the Philippines and Indonesia, with varied results in parasitism rates of *D. citri* (Lashkari et al. 2013; Qureshi et al. 2009). In Reunion, biological control with Pakistani *T. radiata* reduced psyllid populations and helped to mitigate the impact of citrus greening (Chien & Chu, 1996). However, when *T. radiata* (originating from Taiwan and South Vietnam) was released in Florida, the reported parasitism was lower than 20% (Michaud, 2004; De Leon & Setamou, 2010). The use of genetic analysis that identify the different evolutionary lineages or strains of *T. radiata* and *D. citri* (Barr et al. 2009) may improve parasitism rates and effectiveness of these biocontrol programs. It is possible that *T. radiata* populations, as a biological control agent, are more effective for *D. citri* populations originated in the same geographic region. However, other ecological factors should be considered to understand variation in rates of parasitism (Boykin et al. 2012).

Phylogenetic Analyses

Based on the phylogenetic tree and haplotype network it was not possible to determine the ancestral haplotype for *D. citri*. Previous analyses in the geographic origin of *D. citri* had proposed Dcit-01 as the ancestral haplotype (Boykin et al. 2012; Lashkari et al. 2013), based on

frequency and geographic distribution, and suggested a southwestern Asia origin. However, this assumption was based on limited sampling in this geographical region. Previously, only two haplotypes were reported for SWA: Dcit-01 and Dcit-06. In this analysis, additional samples from SWA were included, and a higher diversity was observed, as 13 haplotypes were recovered from this region. Even though Dcit-06 was the most frequent haplotype in SWA it has a limited geographic distribution as it was only observed in Pakistan, and in a few individuals from India and Colombia. The rest of the countries included in SWA (Saudi Arabia n=8; Iran n=10) expressed haplotype Dcit-01 only. More sampling from this region, especially from India, where the two haplotypes co-occur, is needed to confirm the geographic origin of *D. citri*.

SWA collections exhibited a relatively high nucleotide diversity but low haplotype diversity. The haplotype network shows a star-shaped network for this region with a common central haplotype (Dcit-06), and a relatively high number of low-frequency haplotypes. These observations suggest that this is an expanding population, possibly caused by a recent introduction (Aris-Brosou & Excoffier, 1996). The diversity estimates generated for each region suggested a possible SEA origin. Relative to SWA (Hd=0.218), SEA (Hd=0.354) has a high haplotype diversity, low nucleotide diversity, fewer segregation sites, and a high average number of nucleotide differences.

In the present analysis, as well from previous analyses, Dcit-01 was the most geographically distributed haplotype, and it was present in all five geographical regions analyzed. However, it was more frequent in NAM populations (92.66%), when compared with the other geographical regions (SWA 7.51%; SAM 7.14%; CAR 8.55%; SEA 10.20%), suggesting a single introduction for NAM. This haplotype was the most frequent haplotype for all the collections from the New World (US, Mexico, Belize, Costa Rica), and it was also present

and frequent for some countries in the Old World. In Saudi Arabia and Iran, Dcit-01 was the only haplotype found. It is possible that Dcit-01 originated in SWA, where is present and relatively frequent. However, more sampling from this region is necessary to confirm this.

Haplotype Dcit-02 was the most common haplotype for SEA and SAM populations, suggesting that this Asian region is the geographic origin for South America psyllid populations. However, haplotype Dcit-01 was also present in Brazil, where it also co-occurred with Dcit-02, suggesting two independent introductions for this country. It is possible that Dcit-01 was introduced from SWA, or from China or Indonesia, where this haplotype co-occurs with Dcit-02. In addition, haplotype Dcit-06 was found in twelve individuals from Colombia, suggesting a possible third introduction for South America.

Haplotype Dcit-03 was found mostly in the CAR region with thirteen individuals showing this haplotype from Texas collections. Dcit-03 clustered within subclade B3 along with other haplotypes recovered from Texas (Dcit-20), Guadeloupe (Dcit-13) and India (Dcit-23). Since a closer relative has been found in India (Dcit-23), SWA could be a possible origin for this haplotype. Because this haplotype was found in some of the Texas samples, it is possible that a second introduction from the CAR to the US has recently occurred.

Population Structure

Even though relatively low genetic diversity was observed, the geographical distribution of the different haplotypes suggested the existence of population structure within the five different geographic regions. The analysis suggests the existence of five different populations: South West Asia (SWA), South East Asia (SEA), North and Central America (NCA), South America (SAM), and the Caribbean (CAR), and four major haplotype groups or clades (Clade A,

subclade B1, subclade B2 and subclade B3). A substantial portion of the haplotypes found in this study were private for each region, suggesting limited gene flow among the five geographic regions.

CHAPTER II

DIVERSITY OF BREVIPALPUS MITES IN SOUTH TEXAS FROM DIFFERENT CITRUS HOST PLANTS

Introduction

Citrus leprosis is among the most serious diseases of citrus in the world because it can ultimately kill citrus trees (Rodrigues, 2000). Citrus leprosis causes severe damages to leaves, branches, and fruits, leading to the weakening of these branches, eventually resulting in the death of the infected citrus trees. Damaged fruits usually fall and cannot be used for fresh commercialization. This disease nearly destroyed the citrus industry of Florida between 1900 and 1925 (Knorr & Price, 1958; Knorr, 1968), with an estimated production loss ranging from 35 to 75%. Leprosis was completely eradicated in Florida because adverse weather conditions and intensive use of sulfur miticide dramatically reduced the vector populations (Kitajima, et al. 2010). Since its eradication in Florida, distribution of citrus leposis has been largely restricted to South America causing significant economic losses in Argentina, Bolivia, Brazil, Colombia, Paraguay, Uruguay, and Venezuela (Locali et al. 2003; Caceres et al. 2013). Leprosis has spread to Central America, where it has been reported in Panama, Honduras, Guatemala, Costa Rica, Nicaragua, El Salvador, Belize, and most recently Mexico (Mejia et al. 2005; Gomez et al. 2005; Leon et al. 2006; Izquierdo-Castillo et al. 2011). Citrus leprosis is associated with two different viral groups referred to as cytoplasmic and nuclear *Citrus leprosis virus*. The cytoplasmic types

(CiLV-C1 and CiLV-C2) produce viral particles in the cytoplasm of infected plant cells, and the nuclear type (CiLV-N) produces viral particles mainly in the nucleus (Roy et al. 2015). CiLV can naturally infect *Citrus* spp., especially oranges (*Citrus sinensis*). Other citrus species, such as mandarin (*C. reticulata, C. reshni* and *C. deliciosa*) have been reported to be less susceptible to infections under natural conditions (Bastaniel et al. 2006). No other natural hosts have been reported for CiLV (Rodrigues et al. 2003).

Citrus leprosis is vectored by mites included in the *Brevipalpus* genus (Acari: Tenuipalpidae). These mites are commonly referred as false spider mites and the genus includes some of the most important plant feeding mite species in the world (Beard et al. 2015; Childers et al. 2003). *Brevipalpus* mites are elongated and dorsoventrally flattened, with a characteristic reddish color and usually slow moving. Frequently, they are difficult to detect and identify because of their small size, varying between 200 and 400μm. *Brevipalpus* mites reproduce by parthenogenesis (thelytokous), with females producing females and males being rare to find (Helle et al. 1980). Female and male individuals have 2 chromosomes and are haploid (Pijnacker et al. 1980). Their life cycle consists of four active stages: larvae, protonymph, deutonymph and adult (Childers et al. 2003).

Brevipalpus mites have been reported to be present in tropical and subtropical regions around the world (Kitajima et al. 2010). The genus contains more than 300 species, including *B. californicus, B. obovatus*, and *B. phoenicis*. These three species have been found infesting almost 1000 different plant species in over 100 plant families worldwide (Childers et al. 2003). Presently, it is not completely known which species within the genus *Brevipalpus* are capable of vectoring any of the citrus leprosis viruses. Transmission of leprosis has been associated with *B. californicus, B. obovatus*, and *B. phoenicis*, but the identity of the mite vectors species for

citrus leprosis has not been confirmed (Roy et al. 2015). However, *B. yothersi* (former synonym of *B. phoenicis* s. I), is considered the main vector in Brazil (Musumeci & Rossetti, 1963; Rodrigues et al. 2003), and *B. californicus* has been associated with the transmission of this disease in Mexico (Roy et al. 2015).

Citrus leprosis is currently not present in the United States. However, the three potential vectors of this disease (*B. phoenicis, B. californicus, B. obovatus*) have been reported to be established in Texas citrus (Mata et al. 2010; Chen et al. 2007; Ghai & Shenhmar, 1984; Dean, 1959; French & Rakha, 1994). This is of chief concern for the citrus industry, but the proper identification of these three distinct species is made difficult by morphological similarities, and its taxonomy remains uncertain. Moreover, it has been suggested that a species complex may exist within one or more of these three species because of their limited morphological variation, extensive host range and geographical distributions, and the number of plant-specific viruses they transmit (Childers et al. 2003).

The diversity of *Brevipalpus* mites in South Texas citrus plants was studied using morphology and molecular data by Mata et al. (2010). The analysis included molecular identification using molecular fingerprinting (RAPD and AFLP). In their sampling, they reported *B. californicus* and *B. phoenicis* to be present in Texas citrus. The most abundant species was *B. phoenicis* (Mata et al. 2010). However, the *B. phoenicis* species group was recently re-described and identification to species-level for this genus was changed from relying mainly on morphological identification to include sequencing of a Cytochrome Oxidase I (COI) fragment (Beard et al. 2015; Sanchez-Velazquez et al. 2015; Navia et al. 2013). Therefore, reassessment of the diversity for *Brevipalpus* mites gathered in the border region of South Texas using molecular information is needed.

The purpose of this study was to determine species composition and distribution of these mites in South Texas citrus plants. *Brevipalpus* mites were collected from different locations in South Texas and identified using COI sequence information. Information regarding the diversity for members from the genus *Brevipalpus* in South Texas would likely be helpful in the development of effective control strategies for mite species that transmit citrus leprosis.

Materials and Methods

Collection of *Brevipalpus* mites in South Texas

Brevipalpus mites were collected from four different citrus species among residential areas and citrus orchards from different geographic sites in South Texas. Samples consisted of leaves and fruits that were inspected under the stereomicroscope at 50x to determine the presence of *Brevipalpus* mites. Plant samples were separately washed in a 1L Erlenmeyer flask containing 700ml of hot tap water, one drop of MAZU DF 230SC (Emerald Materials, Vancouver, WA, US) solution and one drop of Tween 80 (Sigma-Aldrich, St. Louis, MO, US). The samples were vigorously washed for one minute to release the mites from the plant tissue. The solution was poured into two overlapping sieves, 300μm and 53μm (Thermo Fisher Scientific, Waltham, MA, US). The 300μm sieve retained the debris and the 53μm sieve retained the mites. Recovered material in the 53μm sieve was washed with 70% ethanol and the filtrate recovered in a Syracuse plate. The ethanol solution was inspected under the stereomicroscope (50x). Mites were collected using a fine brush and stored on vials with 100% ethanol. Mites from all stages were collected but only adults were used for molecular identification.

Molecular Identification

Total genomic DNA was extracted from individual mites using the InviMag Forensic kit (STRATEC, Birkenfeld, Germany), on a KingFisher 96 machine from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, US). Mites were placed on a 96 deep, well plate containing 100µl of Lysis Buffer M, 10µl of Proteinase K, and 20µl of Carrier-RNA, from the mentioned kit. A sonic water bath was used during lysis treatment for 30 min at 52°C. The rest of the extraction process was performed using the manufacture's protocol. Extracted DNA was eluted in 100µl of Elution Buffer D. A fragment of the cytochrome oxidase I (COI) gene was amplified by Polymerase Chain Reaction (PCR) using the primers DNF 5' TAC AGC TCC TAT AGA TAA AAC 3' and DNR 5' TGA TTT TTT GGT CAC CCA GAA G 3'. PCR was performed using OmniMix HS Lyophilized universal PCR reagent beads from Cepheid (Sunnyvale, CA, US). PCR reactions were performed using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster, CA, US). Amplification reactions consisted of 25µl volumes containing 20.5µl of H_2O, 1µl of each primer (10mM), and 2µl of DNA template. PCR reactions were denatured at 94°C for 5 min, followed by 40 cycles of 30 sec denaturation at 94°C, 30 sec of annealing at 54°C and 1 min extension at 68°C, with a final elongation at 68°C for 5 min after the completion of all cycles. PCR products were visualized on 1% agarose gels using ethidium bromide to confirm the presence of the amplified fragment and then purified using Exo-SAP-IT (Affimetrix, Santa Clara, CA) as per manufacturer instructions prior to sequencing. The amplified fragments were of approximately 450 bp. Samples were sent to GENEWIZ (South Plainfield, NJ, US), for bi-directional sequencing.

Species Diversity and Population Structure on *Citrus* Host Plants

Sequences were edited on Geneious v. 10.0.2 (http://www.geneious.com, Kearse et al. 2012). An alignment was constructed on MEGA v.7.0 (Tamura et al. 2013) using Clustal W (Thompson et al. 1994). For consistency, all sequences were truncated to 435bp. Maximum likelihood (ML) and maximum parsimony (MP) analyses were done using MEGA v. 7.0. Phylogenetic reconstruction using ML was performed using the General Time Reversible model of evolution with 500 pseudo replicates to obtain branch support values for the ML tree construction. Previously reported sequences from *Brevipalpus* species were retrieved from GenBank and included in our analyses. A *Cenopalpus pulcher* and a *Tetranychus urticae* sequences were used as outgroups. Reference sequences and outgroup accession numbers are listed in (Table 2.1). A haplotype network was constructed using TCS v1.21 (Clement et al. 2000) methods with 95% confidence in PopArt (Leigh & Bryant, 2015) in order to compare the frequencies and distribution of haplotypes recovered from the different host plant studied.

To evaluate the partitioning of the genetic variation among populations according to the four-citrus host plants, an Analysis of Molecular Variance (AMOVA) was performed in ARLEQUIN v. 3.5 (Excoffier et al. 2010), with 10,000 permutations. Samples collected in other citrus plants (5) were excluded from this analysis since they included a single individual each.

The correlation between genetic and geographic distances for *B. yothersi* and *B. californicus* was analyzed using the Isolation By Distance (IBD) web service (http://ibdws.sdsu.edu/~ibdws, Jensen et al. 2005). Only the samples with complete geographic information were included in this analysis. The number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (μ), the average number of pairwise differences (k) (Tajima, 1983), the

Tajima's D (Tajima, 1989), and Fu and Li's D and F tests (Fu & Li, 1993) were determined with DnaSP v. 5.10.01 (Rozas et al. 2003).

Table 2.1. GenBank accession numbers of previously published *Brevipalpus* sequences used as references.

Species	Accession No.	Author
B. yothersi	KF955008	Sanchez-Velazquez et al. 2015
B. yothersi	KF954959	Sanchez-Velazquez et al. 2015
B. phoenicis	KC291389	Navia et al. 2013
B. papayensis	KF954950	Sanchez-Velazquez et al. 2015
B. obovatus	KC344714	Navia et al. 2013
B. chilensis	KC244721	Navia et al. 2013
B. chilensis	KC344729	Navia et al. 2013
B. californicus	DQ789591	Groot et al. 2006
B. californicus	DQ789594	Groot et al. 2006

Genetic Variation in Populations from South Texas, Mexico, and Brazil

Sequences of *B. yothersi* and *B. californicus* were retrieved from GenBank to observe the genetic variation for these species among the three countries (Table 2.2). To ensure comparability between datasets, sequences were truncated to 352bp to match available data for *B. yothersi* from Mexico and Brazil, and 425bp for *B. californicus* from Mexico. An alignment was constructed in MEGA v. 7.0. A Maximum likelihood analysis was performed using the parameters, evolutionary model, outgroup and reference sequences previously mentioned. An AMOVA was performed in ARLEQUIN v. 3.5 (Excoffier et al. 2010), to examine for patterns in the population structure for B. *yothersi* collections using the parameters described above. The AMOVA was performed to test for the null hypothesis that the three populations (South Texas, Mexico, and Brazil) are equal.

Table 2.2. Accession numbers of *Brevipalpus* sequences recovered from GenBank included in the analyses.

Country	Accession No.	Author
Brazil	KF954958- KF954958	Sanchez-Velazquez et al. 2015
Mexico	KF9549-KF95008	Sanchez-Velazquez et al. 2015
Mexico	KX100162-KX100321	Salinas-Vargas et al. 2016

Results

Species Diversity and Population Structure on *Citrus* Host Plants

The final alignment included 216 sequences for the 435bp COI fragment. Sequences contained 71 variable sites and 53 parsimoniously informative sites. The phylogenetic analysis confirmed the existence of four species of *Brevipalpus* in South Texas citrus, *B. californicus* (155), *B. yothersi* (57), Species "A" (1), and Species "B" (3) (Not shown). Sequences for Species A and Species B did not match any of the reference sequences included in this analysis (Table 2.1).

The haplotype network showed the arrangement for 23 haplotypes, nine haplotypes belonging to *B. californicus*, eleven haplotypes to *B. yothersi*, one haplotype for Species "A", and two haplotypes for Species "B" (Figure 2.1). Out of these, 15 were recovered from a single individual each (HAP01, HAP04-HAP07, HAP11-HAP16, and HAP19-HAP23). Nucleotide and haplotype diversity was of 0.03332 and 0.549, respectively. There were five discrete networks recovered from the TCS analysis: one for *B. yothersi*, two for *B. californicus*, one for Species A, and one for Species B (Fig. 2.1). The most abundant haplotype was HAP03 (*B. californicus*) with 143 samples showing this haplotype. HAP01 was seen from all four different citrus host plants sampled. The second most abundant haplotype was HAP02 (*B. yothersi*), observed in 29

samples, and similarly, this haplotype was recovered from the four citrus plant species. The distribution of haplotypes among the different citrus host plants is shown in Table 2.3.

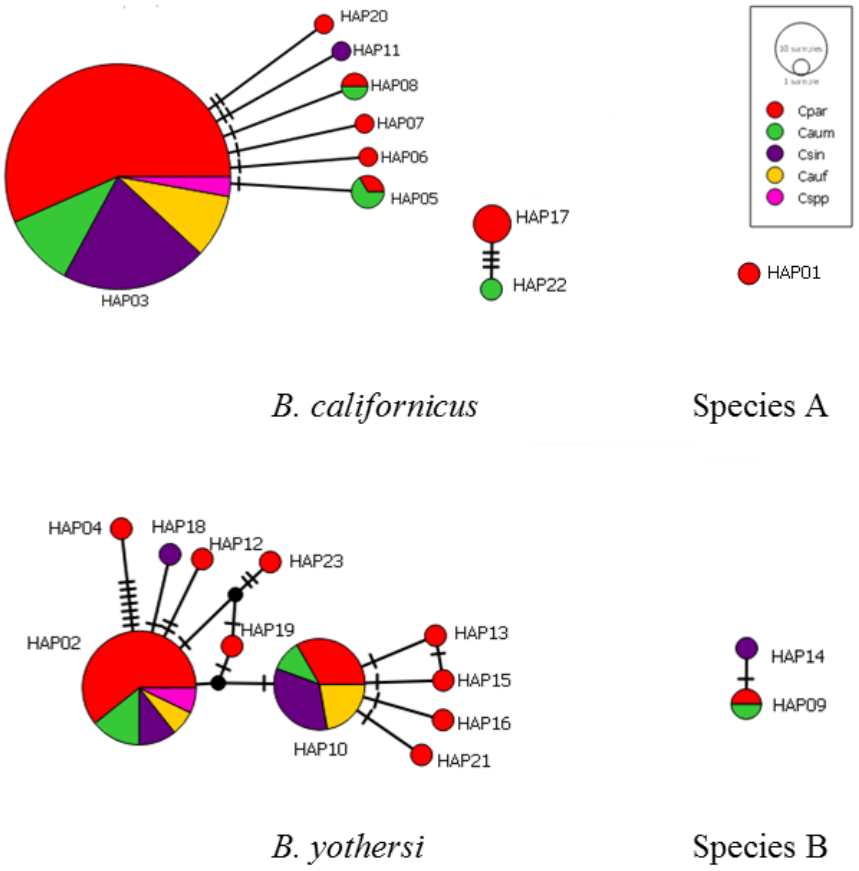

Figure 2.1. Haplotype TCS network showing the genetic relationship of 23 haplotypes recovered from this study. Circles represent haplotypes and connecting lines represent patterns of relationship. Hash marks represent mutational events. Colored circles represent haplotypes observed in the host plant associated with their color. Size of each colored circle is proportional to the observed frequency of the haplotype and proportion of samples from host plant is indicated by the proportion of color associated with the citrus host plant (Cpar – *C. paradisi*, Caum – *C. aurantium*, Csin – *C. sinensis*, Cauf – *C. aurantifolia*, Cspp – other citrus species). Black circles represent inferred haplotypes.

Table 2.3. Haplotype distribution of *Brevipalpus* mites among citrus host plants in South Texas. N = total number of specimens.

HAP	Mite species	N	Host plant species
HAP01	Species "A"	1	*C. paradisi* (1)
HAP02	*B. yothersi*	29	*C. paradisi* (16). *C. sinensis* (4), *C. aurantium* (4), *C. aurantifolia* (4), other citrus (1)
HAP03	*B. californicus*	143	*C. paradisi* (81). *C. sinensis* (30), *C. aurantium* (15), *C. aurantifolia* (13), other citrus (4)
HAP04	*B. yothersi*	1	*C. paradisi* (1)
HAP05	*B. californicus*	3	*C. paradisi* (1), *C. aurantium* (2)
HAP06	*B. californicus*	1	*C. paradisi* (1)
HAP07	*B. californicus*	1	*C. paradisi* (1)
HAP08	*B. californicus*	2	*C. paradisi* (1), *C. aurantium* (1)
HAP09	Species "B"	2	*C. paradisi* (1), *C. aurantium* (1)
HAP10	*B. yothersi*	18	*C. paradisi* (5). *C. sinensis* (6), *C. aurantium* (2), *C. aurantifolia* (4)
HAP11	*B. californicus*	1	*C. sinensis* (1)
HAP12	*B. yothersi*	1	*C. paradisi* (1)
HAP13	*B. yothersi*	1	*C. paradisi* (1)
HAP14	Species "B"	1	*C. sinensis* (1)
HAP15	*B. yothersi*	1	*C. paradisi* (1)
HAP16	*B. yothersi*	1	*C. paradisi* (1)
HAP17	*B. californicus*	2	*C. paradisi* (2)
HAP18	*B. yothersi*	2	*C. sinensis* (2)
HAP19	*B. yothersi*	1	*C. paradisi* (1)
HAP20	*B. californicus*	1	*C. paradisi* (1)
HAP21	*B. yothersi*	1	*C. paradisi* (1)
HAP22	*B. californicus*	1	*C. aurantium* (1)
HAP23	*B. yothersi*	1	*C. paradisi (1)*

Table 2.4. Genetic diversity of *Brevipalpus* populations among citrus host plants analyzed in this study. (Citrus host plants: *C. paradisi*, *C. sinensis*, *C. aurantium*, *C. aurantifolia*). No statistical significance was observed (pvalue<0.0000).

Source of variation	Sum of squares	Variance components	Variation (%)
Amongst pop. (citrus host)	3.712	-0.1487	-1.97097
Within pop.	1508.409	7.28797	101.97097
Total	1512.121	7.14711	
F_{ST}	-0.01971		

The AMOVA analysis showed that variation cannot be explained by the host plant species (Table 2.4). Similarly, the mantel test of IBD did not show a significant correlation pattern between genetic variation and geographic distance (r = 0.0174, P = 0.1510 for *B. californicus*, r = 2.494^{-03}, P =0.2170 for *B. yothersi*). The geographic distribution of *B. californicus* and *B. yothersi* haplotypes in South Texas is shown in Figure 2.2, and Figure 2.3, respectively.

Genetic Variation among *B. californicus* geographic collections from South Texas, Mexico, and Brazil

The phylogenetic analysis revealed different clades, each corresponding to different *Brevipalpus* species (Figure 2.4). For *B. californicus*, 185 sequences were included and the final alignment was of 425bp. A total of 25 variable sites and 12 parsimoniously informative sites were observed. *B. californicus* was identified as the most common species among South Texas (155/216) collections. It was also recovered from some collections in Mexico (30/169). *B. californicus* has not been reported to be present in Brazil; Sanchez-Velazquez et al. (2016) sampled 22 mites and did not recover *B. californicus*.

In South Texas, nine haplotypes were recovered from *B. californicus* samples. For clarity while comparing these samples to Mexico and Brazil, a new nomenclature for haplotypes was created (Table 2.5). Haplotype B.calH1 was the most common haplotype, seen among 143/155 (92.24%) of the samples. This haplotype was also the most common among Mexico samples. B.calH2 was recovered from 3/155 (1.94%). B.calH5 and B.calH7 were recovered from two individuals each. The remaining five haplotypes were each recovered from a single individual.

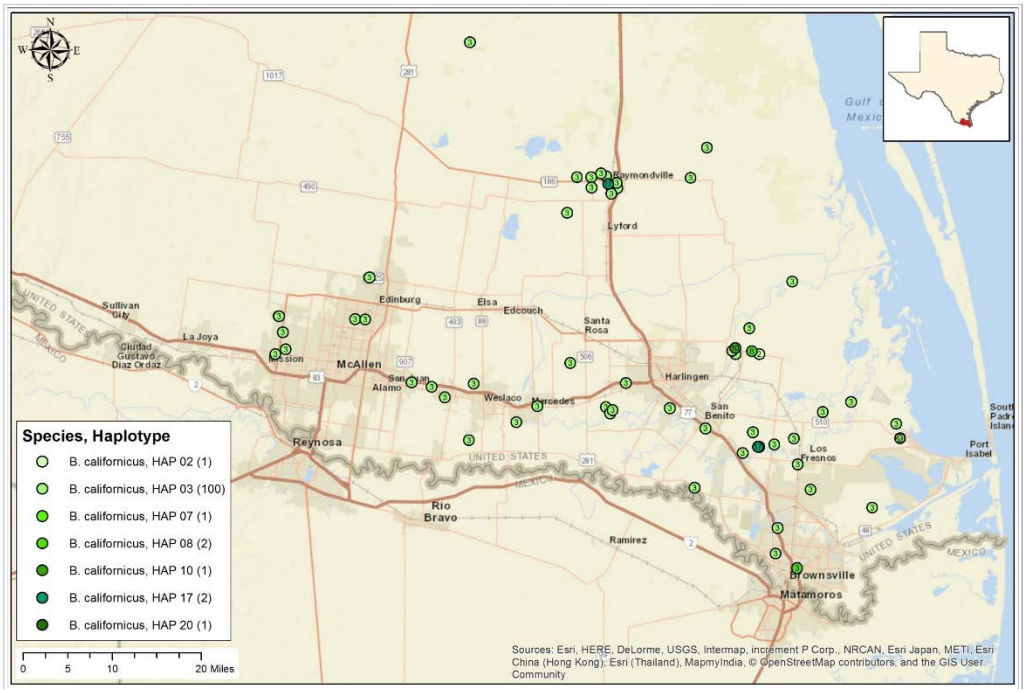

Figure 2.2. Geographic distribution of *B. californicus* haplotypes in South Texas.

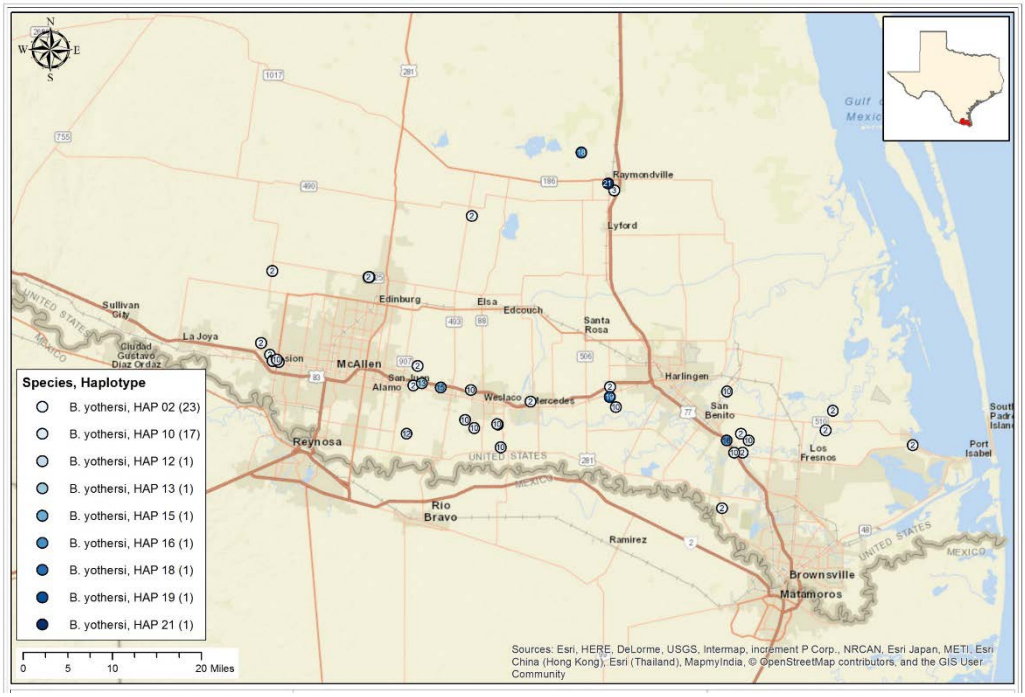

Figure 2.3. Geographic distribution of *B. yothersi* haplotypes in South Texas.

In Mexico three haplotypes were recovered, B.calH1, B.calH10, and B.calH11. *B. californicus* in Mexico was collected in Nuevo Leon (Northeastern Mexico), Veracruz (Eastern Mexico), and Yucatan (Southeast Mexico), by Salinas-Vargas et al. (2016). The most common haplotype for Mexican collections was B.calH1. It was recovered from 26/30 (86.87%) of the Mexican samples analyzed. This haplotype was found in the states of Nuevo Leon and Veracruz only. B.calH10 was recovered from one individual, and B.calH10 was recovered from three. The distribution of *B. californicus* haplotypes in South Texas and Mexico is shown in Table 2.5.

Table 2.5. Haplotype distribution of *B. californicus* in South Texas and Mexico.

Hap name	Present Study	Salinas-Vargas et al. 2016	Distribution
B.calH1	HAP03	H2	Texas (143), Nuevo Leon and Veracruz Mexico (26)
B.calH2	HAP05		Texas (3)
B.calH3	HAP06		Texas (1)
B.calH4	HAP07		Texas (1)
B.calH5	HAP08		Texas (2)
B.calH6	HAP11		Texas (1)
B.calH7	HAP17		Texas (2)
B.calH8	HAP20		Texas (1)
B.calH9	HAP22		Texas (1)
B.calH10		H1	Yucatan, Mexico (1)
B.calH11		H3	Nuevo Leon, Mexico (3)

Genetic Variation among *B. yothersi* Geographic Collections from South Texas, Mexico, and Brazil

For *B. yothersi*, a total of 212 sequences were recovered representing 29 of haplotypes from collections in South Texas, Mexico and Brazil. The final alignment for these sequences was made up of a total of 352 bases for the COI gene fragment that contained 64 variable sites and 24

parsimoniously informative sites. As previously reported by Sanchez-Velazquez et al. (2015), *B. yothersi* was the most common species for Brazil and Mexico. Sequences specific to this species were recovered from 57/216 (26.38%) individuals from South Texas, 139/169 (82.25%) of the sequences from Mexico, and16/22 (72.72%) of the sequences from Brazil. Phylogenetic analysis shows that all *B. yothersi* samples cluster to form a monophyletic arrangement (Figure 2.4). A new haplotype nomenclature was created for *B. yothersi* from South Texas, to compare with the samples from Mexico and Brazil. A total of 29 haplotypes from Brazil (14), Mexico (12) and South Texas (7) were observed (Table 2.6).

The phylogenetic analyses were concordant with previous reports, showing that *B. yothersi* samples can be further divided in two major groups (Group 1 and Group 2). Group 1 contained 24/29 (82.76%) haplotypes gathered from the two previous reports by Sanchez-Velazquez et al. (2015) and Salinas-Vargas et al. (2016), and the samples collected in South Texas from the present study. Group 1 included all the samples from Mexico (139), all the samples from South Texas (57), and 11 of the samples from Brazil. Group 2 included five haplotypes only, and included only the previously described sequences collected in Brazil from Sanchez-Velazquez et al. (2015).

Among the mites analyzed from South Texas collections, seven haplotypes were observed. A total of four were unique to this region. The nucleotide (π) and haplotype diversity (Hd) was of .00208 and 0.594 respectively. Two haplotypes were predominant 51/57 (89.47%) among the mites from this region. The most common haplotype was B.yotH1. It was recovered from 30/57 (52.63%) individuals. The second most common haplotype was B.yotH3 and was recovered from 21/57 (36.84%) individuals. Haplotype B.yotH5 was recovered from 2 individuals.

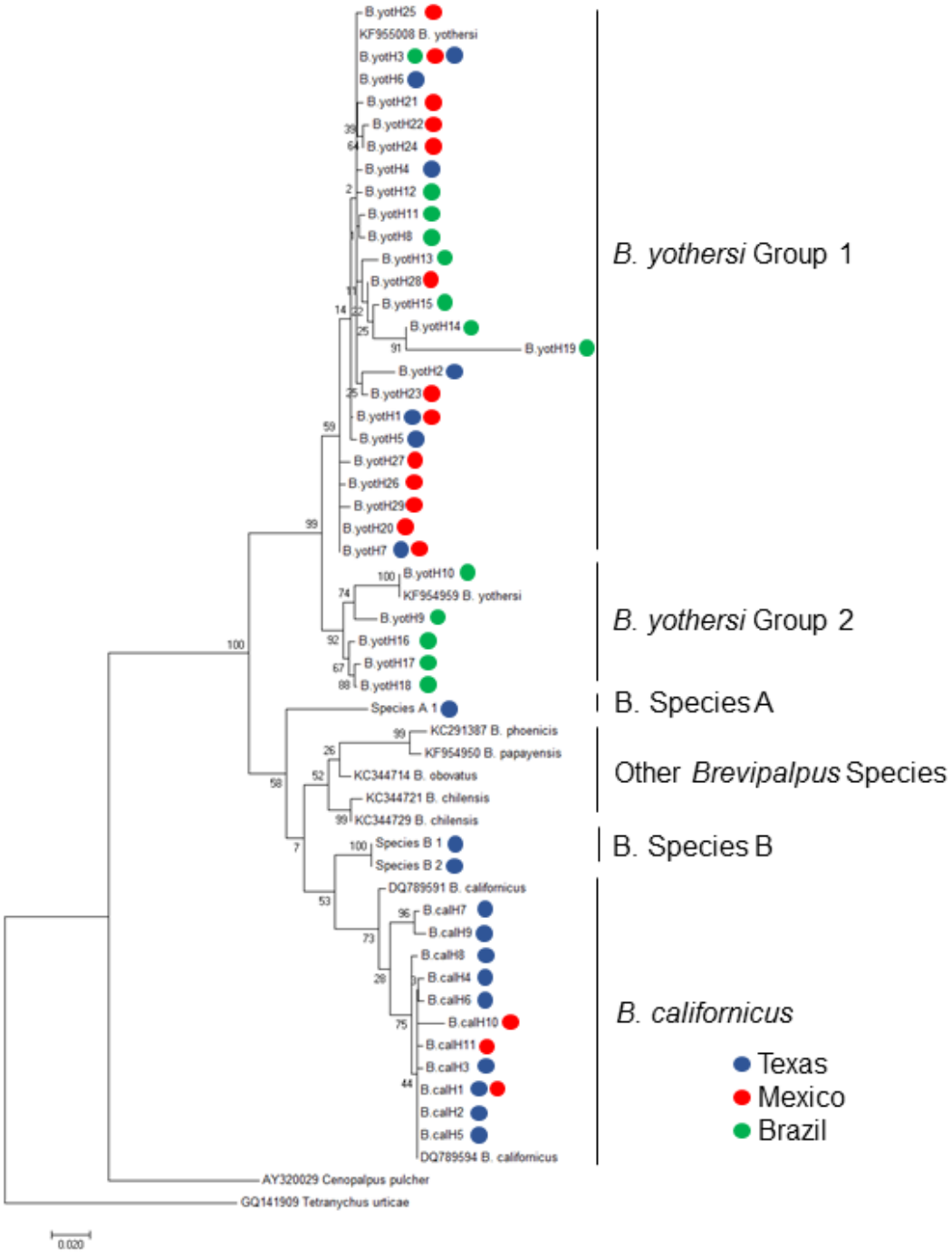

Figure 2.4. Phylogenetic analyses of *B. californicus* and *B. yothersi* from South Texas, Mexico, and Brazil. Haplotypes found in Texas (blue), Mexico (red), and Brazil (green) are indicated by colored circles.

Haplotype B.yotH7 was recovered from a single individual but it was a common haplotype in samples from Mexico. Both B.yotH2 and B.yotH4 haplotypes were each recovered from a single individual (Table 2.6). Demographic analyses for South Texas samples did not deviate significantly from neutrality for Tajima's D (-1.08544), Fu and Li's D (-2.35273), and Fu and Li's F (-2.28689) (Table 2.7).

In Mexico, using the sequences available from previous reports, twelve haplotypes were observed. A total of nine were unique to this region, the nucleotide (π) and haplotype (Hd) diversity was of 0.0565 and 0.621 respectively. The most common haplotype was B.yotH3, and it was observed in 79/139 (56.84%) individuals from previous reports (Sanchez-Velazquez et al. 2015; Salinas-Vargas et al. 2016). B.yotH3 was also recovered from a 21/57 (36.84%) individuals from Texas, and in two individuals from Brazil. The second most common haplotype in this country was B.yotH7, and it was recovered from 66/139 (47.48%) individuals. B.yotH7 was also recovered from 30 individuals from South Texas. B.yotH1 was recovered from 13/139 (9.35%) individuals. This haplotype was also recovered from a single individual from South Texas. The rest of the haplotypes (9) were limited to Mexico and were each recovered from a single individual (Table 2.6). Demographic analyses for Mexican samples were significantly different from neutral expectations for Tajima's D (-2.59529, p=0.01), Fu and Li's D (-8.56997, p=0.02), and Fu and Li's F (-7.14151, p=0.02) (Table 2.7).

Table 2.6. Haplotype distribution of *B. yothersi* in South Texas, Mexico, and Brazil.

Hap name	Texas	Salinas-Vargas et al. 2016	Sanchez-Velazquez et al. 2015	Geographic Distribution
B.yotH1	HAP02, HAP12	H10		Texas (30) Mexico (13)
B.yotH2	HAP04			Texas (1)
B.yotH3	HAP10, HAP13, HAP15, HAP19	H1, H6	H8	Texas (21) Brazil (2) Mexico (79)
B.yotH4	HAP16			Texas (1)
B.yotH5	HAP18			Texas (2)
B.yotH6	HAP21			Texas (1)
B.yotH7	HAP23	H4, H5		Texas (1) Mexico (66)
B.yotH8			H5	Brazil (2)
B.yotH9			H6	Brazil (1)
B.yotH10			H7	Brazil (1)
B.yotH11			H9	Brazil (1)
B.yotH12			H10	Brazil (1)
B.yotH13			H11	Brazil (1)
B.yotH14			H12	Brazil (1)
B.yotH15			H13	Brazil (1)
B.yotH16			H14	Brazil (1)
B.yotH17			H15	Brazil (1)
B.yotH18			H16	Brazil (1)
B.yotH19			H17	Brazil (1)
B.yotH20			H4	Brazil (1)
B.yotH21			H18	Mexico (1)
B.yotH22			H19	Mexico (1)
B.yotH23			H20	Mexico (1)
B.yotH24		H2		Mexico (1)
B.yotH25		H3		Mexico (1)
B.yotH26		H7		Mexico (1)
B.yotH27		H8		Mexico (1)
B.yotH28		H9		Mexico (1)
B.yotH29		H11		Mexico (1)

Table 2.7. Genetic diversity of *B. yothersi* populations from South Texas, Mexico, and Brazil. The values on the columns represents Tajima's D (D_1), Fu and Li's D (D_2), Fu and Li's F (F), sample size (*n*), average number of nucleotide differences (k), segregating sites (S), nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (*H*d). Values in **bold** are significantly different from zero (P<0.01, *P < 0.02).

Population	*n*	D_1	D_2	*F*	k	S	π	h	*H*d
1. Texas	57	-1.08544	-2.35273	-2.28689	0.73183	6	0.00208	7	0.594
2. Mexico	169	**-2.19529**	**-8.56997***	**-7.14151***	1.98832	42	0.00565	12	0.621
3. Brazil	16	-1.27762	-2.19529	-1.62691	11.59167	51	0.03293	14	0.983

Among the 14 of sequences from Brazil, 14 haplotypes were observed from the previous report by Sanchez-Velazquez et al. (2015). A total of 13 were unique to this country and the nucleotide (π) and haplotype (Hd) diversity was of 0.03293 and 0.983 respectively. Relative to South Texas and Mexico populations, Brazil showed the highest haplotype diversity. B.yotH3 was recovered from two individuals, and this haplotype was also found in 21/57 of the samples from South Texas, and 79/139 of the samples from Mexico. Haplotype B.yotH8 was also recovered from two individuals from Brazil. The rest of the haplotypes from this country were recovered from a single individual each. Demographic analyses for Brazil samples did not deviate significantly from neutral expectations for Tajima's D (-1.27762), Fu and Li's D (-2.19529), and Fu and Li's F (-1.62691) (Table 2.7).

To test for population structure, among the populations from South Texas, Mexico, and Brazil, an AMOVA test was performed. For *B. yothersi*, the AMOVA results showed that the highest percent of variation is found within populations (80.73%). The variation among populations was relatively low (19.27%) (Table 2.8).

Table 2.8. AMOVA test for *B. yothersi* populations from South Texas, Mexico, and Brazil. No statistical significance was observed (pvalue<0.0000).

Source of variation	Sum of squares	Variance components	Variation (%)
Amongst pop. (origin)	32.292	0.27395	19.27
Within pop.	274.381	1.14804	80.73
Total			
F_{ST}	0.19265		

Discussion

Species Diversity and Population Structure on *Citrus* Host Plants

The results confirm the presence of four different species of *Brevipalpus* mites in South Texas citrus. The most common species observed for this region was *B. californicus.* This species represented 155/216 (71.76%) of the samples. This result contradicts the conclusion of Mata et al. (2010) that *B. yothersi*, a former synonym of *B. phoenicis* s. I, is the dominant species in South Texas. The results from Mata et al. (2010) were based on sampling of Sweet orange and Grapefruit only, and used a different molecular technique for identification (RFLP). However, an earlier work by Dean (1959) supports our finding that *B. californicus* is more abundant in this region, based on morphological identification. Disagreement between Mata et al. (2010), Dean et al. (1959), and the present study could be due to differences in the number of samples analyzed, the number of citrus host plants sampled, the recent re-description of some *Brevipalpus* species, and the use of different identification techniques.

The second most abundant species in South Texas was *B. yothersi*, and represented 57/216 (26.38%) of the samples analyzed for this study. In addition, two other species, not previously reported were found. In fact, these two species (Species A and Species B), have not

been described yet. The phylogenetic analysis shows strong support for a monophyletic arrangement, clearly separating these individuals from previously described *Brevipalpus* species. Additional samples of these two species and a detailed morphological analysis is needed to confirm the existence of Species A and Species B, and to further understand the diversity of *Brevipalpus* mites in South Texas citrus.

The use of mtDNA sequencing allowed us to further analyze genetic diversity within species. For *B. californicus*, the haplotype network revealed the existence of nine different haplotypes that were divided in two different haplotype networks (Fig. 2.1). For *B. yothersi*, eleven haplotypes were found in the samples collected for this study. However, even though *B. californicus* was more abundant, *B. yothersi* populations appear to be more genetically diverse since a greater number of haplotypes were found for this species.

To examine the association between genetic distance and geographic distance, an IBD analysis was performed. For both species, *B. californicus* and *B. yothersi* there is no significant correlation between genetic distance and geographic distance, according to the IBD analysis. Similarly, analyses to examine the association between genetic variation and citrus host plant, according to AMOVA methods, showed non-significant relationships for *B. californicus* and *B. yothersi* (Table 2.4). This is in accordance with previous reports on citrus host association for *B. californicus* and *B. yothersi* in Mexico (Salinas-Vargas et al. 2016). Salinas-Vargas et al. (2016) included orange, mandarin, grapefruit and limes as host plants, and no association of haplotype diversity and citrus host plant was observed for *B. yothersi*. *B. californicus*, was found mostly on orange and mandarin. But because of their limited sampling, it was not possible to determine an association of *B. californicus* with citrus host plant. In the present study, *B. californicus* was

collected from the four different citrus host plant studied, suggesting no association to citrus host plant, demonstrating similar results as *B. yothersi*.

Although host-associated differentiation (HAD) was not detected among *Brevipalpus* on *Citrus* in South Texas, the pattern is common among phytophagous insects (Stireman et al. 2005), and it has been reported for some *Brevipalpus* species including *B. phoenicis* (Groot et al. 2005) and *B. lewisi* (Hao et al. 2016). Given the wide host range of these mite species, it is possible that haplotype diversity among host plants can be detected for *B. californicus* and *B. yothersi* if the host plants are from different genera or families.

Genetic Variation among populations from South Texas, Mexico, and Brazil

Based on those COI sequencing data, four species of *Brevipalpus* mites in South Texas (*B. californicus*, *B. yothersi*, Species A, and Species B) showed distinct haplotypes. Previous reports showed three species to be present on citrus plants in Mexico: *B. yothersi*, *B. californicus*, and *B. papayensis*; and two species on citrus in Brazil: *B. yothersi*, and *B. papayensis* (Sanchez-Velazquez et al. 2015).

For *B. californicus*, 11 haplotypes were recovered from the collections gathered from South Texas and Mexico. In South Texas, *B. californicus* was the most abundant species and nine haplotypes were found for this species in this region. In Mexico, only three haplotypes were reported for *B. californicus* (Salinas-Vargas et al. 2016). However, only 30 samples of *B. californicus* were included in the study for Mexican populations. It is possible that *B. californicus* in Mexico is more diverse than previously reported. But because of limited sampling, a relatively low diversity was detected. The most abundant haplotype of *B. californicus* in South Texas was B.calH1which was also the most abundant haplotype in Mexico.

The presence of this haplotype in both South Texas and Mexico suggests that there is gene flow between these two regions.

Previously, it has been suggested that the distribution of *B. californicus* and *B. yothersi* in Mexico was affected by temperature and elevation. It was suggested that *B. californicus* prefers temperate localities at high elevations, while *B. yothersi* is more prevalent in warm regions with low elevations (Roy et al. 2015). *B. californicus* was found mostly in the Northeastern and Eastern areas of Mexico, while *B. yothersi* was more abundant in the Southeastern regions (Salinas-Vargas et al. 2016). For South Texas, species composition appears to be similar to the Northeastern areas of Mexico, because both *B. californicus* and *B. yothersi* are present, but *B. californicus* is more abundant. It is possible that the distribution of these two species, as previously suggested, is affected by elevation, temperature, and other environmental factors. Information on the distribution of *Brevipalpus* species can provide a better understanding on the dispersal and spreading of citrus leprosis.

For *B. yothersi*, a total of 29 COI haplotypes were found in South Texas, Mexico, and Brazil. Based on these data, a total of seven haplotypes were found in South Texas, twelve in Mexico, and fourteen in Brazil. One haplotype was shared between South Texas, Mexico and Brazil (B.yotH3), and two haplotypes were found in both, Mexico and South Texas (B.yotH1 and B.yotH7). *B. yothersi* was previously reported as the most abundant species on citrus plants in Southern areas in Mexico and Brazil. The most common haplotype in South Texas was B.yotH1, which was also recovered from thirteen individuals from Mexico. The most common haplotype in Mexico was B.yotH3, and was recovered from 79 of the samples. This haplotype was also found in 21 individuals from South Texas, and two individuals from Brazil. These results suggest that mitochondrial gene flow is possibly occurring between the three countries.

Since citrus leprosis is currently affecting Mexico and Brazil, the US could be at risk of infection if *B. yothersi* mites are moving between the three countries.

The AMOVA analysis revealed no population structure for these three populations (South Texas, Mexico, and Brazil) (Table 2.8). The demographic analyses for these three populations only revealed significant values for Tajima's D (-2.19529), Fu and Li's (-8.56997) and F (-7.14151) for populations from Mexico (Table 2.7). However, the higher nucleotide and haplotype diversity was reported for individuals from Brazil. Even though only sixteen samples from Brazil were included, a total of 14 haplotypes were found with a nucleotide and haplotype diversity of 0.3293 and 0.983, respectively. These results suggest that Brazil could be the center of diversity for *B. yothersi*. A reduction of genetic diversity for this species is observed as we move north.

B. yothersi was more genetically diverse than *B. californicus* among citrus hosts plants in South Texas and it is the most abundant and diverse *Brevipalpus* species on citrus plants in Mexico and Brazil (Salinas-Vargas et al. 2016; Sanchez-Velazquez et al. 2015). It has been suggested that the higher genetic diversity in other mite species might be caused by the use of acaricides in some regions (Pascual-Ruiz et al. 2014). Acaricides or miticides are widely used in Brazil and Mexico to control *Brevipalpus* mites populations, specifically *B. yothersi*, which is the most common species in these areas. Intensive use of acaricides can produce greater selection pressures on these populations, possibly resulting in an increased genetic diversity. However, more sampling from Brazil and northern populations is needed to further understand the diversity and distribution of *Brevipalpus* mites.

REFERENCES

Chapter I

Aris-Brosou, S. Excoffier, L. (1996). The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. *Molecular Biology and Evolution*, 13(3): 494-504.

Avise, J. C. (2000). Phylogeography: the history and formation of species. *Harvard University Press*, Cambridge.

Ayres, A. J. Massari, C. A. Lopes, S. A. Bassanezi, R. B. Belasque, J. Yamamoto, P. T. Teixeira, D. C. Wulff, N. A. Gimenes-Fernandes, N. Bergamaschi, O. A. (2005). Proceedings of 2nd International Citrus Canker and Huanglongbing Research Workshop, *Florida Citrus Mutual*, Orlando 2005, H-4.

Barr, N. B. Hall, D. G. Weathersbee, A. A. Nguyen, R. Stansly, P. Qureshi, A. Flores, D. (2009). Comparison of laboratory colonies and field populations of *Tamarixia radiata*, an ectoparasitoid of the Asian citrus psyllid, using internal transcribed spacer and cytochrome oxidase subunit I DNA sequences. *Journal of Economic Entomology*, 102(6): 2325-2332.

Beattie, G. A. C., Holford, P. Mabberley, D. J. Haigh, A. M. & Broadbent, P. (2008). *IRCHLB Proceedings*, December 2008. Plant Management Network. http://www.plantmanagementnetwork.org/proceedings/irchlb/2008/presentations/IRCHLB.K.2.pdf. Accessed 19 Dec 2016

Bove, J. M. (2006). Huanglongbing; a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, 88, 30.

Boykin, L. M., De Barro, P., Hall, D. G., Hunter, W. B., McKenzie, C. L., Powell, C. A., & Shatters, R. G., Jr. (2012). Overview of worldwide diversity of *Diaphorina citri* Kuwayama mitochondrial cytochrome oxidase 1 haplotypes: two Old World lineages and a New World invasion. *Bulletin of Entomological Research*, 102(5): 573-582.

Chien, C. C. Chu, Y. I. (1996). Biological control of citrus psyllid, *Diaphorina citri* in Taiwan. *International Journal of Pest Management*, 34: 93-105.

Chu, C. C., Gill, T. A., Hoffmann, M., & Pelz-Stelinski, K. S. (2016). Inter-Population Variability of Endosymbiont Densities in the Asian Citrus Psyllid (*Diaphorina citri* Kuwayama). *Microbial Ecology*, 71(4): 999-1007.

Clement, M. P., D. Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9(10): 1657-1659.

Costa Lima, A. M. (1942). Insectos do Brazil: Homoptera. Escola Nacional de Argonomia, Rio de Janeiro, 3:141.

da Graca, J. V. Korsten, L. (2004). Citrus huanglonging review, present status and future strategies. Pp 229-245. *In Diseases of Fruits and Vegetables* Vol. I (S. A. M. H. Naqvi, ed). Kluwer Academic Publishers, Dordrecht, Netherland.

Darriba, D. Taboada, G. L. Doallo, R. Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8): 772.

De Leon, J. Setamou, M. (2010). Molecular evidence suggests that populations of the Asian Citrus Psyllid parasitoid *Tamarixia radiata* from Texas, Florida, and Mexico, represent a single species. *Annals Entomological Society of America*, 10.

De Leon, J. Setamou, M. Gastaminza, G. Buenahora, J. Caceres, S. Yamamoto, P. Bouvet, J. Logarzo, G. (2011). Two separate introductions of Asian citrus psyllid populations found in the American continents. *Annals Entomological Society of America*, 104(6), 1392-1398.

Etienne J, Aubert, B. (1980). Biological control of psyllid vectors of greening disease on Reunion Island, pp. 118-121 *In Proceeding of the 8th Conference of the International Organization of Citrus Virologists*. Sidney, Australia.

Excoffier, L. Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564-567.

French, J. V., Kahlke, C. J. de Graca, J. V. (2001). First record of the Asian citrus psylla *Diaphprina citri* Kuwayama (Homoptera: Psyllidae), in Texas. *Subtropical Plant Science*, 53: 14-15.

Fu, Y. X. Li, W. H. (1993). Statistical tests of neutrality of mutations. *Genetics*. 133: 693-709.

Guidolin, A. S. Fresia, P. Consoli, F. L. (2014). The genetic structure of an invasive pest, the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae). *PLoS One*, 9(12).

Guillemaud, T., Ciosi, M., Lombaert, E., & Estoup, A. (2011). Biological invasions in agricultural settings: insights from evolutionary biology and population genetics. *Comptes Rendus Biologies*, 334(3): 237-246.

Guindon, S. D., J. Lefort, V. Anisimova, M. Hordijk, W. Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biollogy*, 59(3): 307-21.

Halbert, S. E. Manjunath, K. L. (2004). Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida. *Florida Entomologist*, 87(3): 330-353.

Halbert, S. E. Núñez, C. A. (2004). Distribution of the Asian Citrus Psyllid, *Diaphorina Citri* Kuwayama (Rhynchota: Psyllidae) in the Caribbean Basin. *Florida Entomologist*, 87(3): 401-402. 2

Hall, D. G. (2008). Biology, history and world status of *Diaphorina citri*. North American Plant Protection Organization, *in Proceedings of the Taller Internacional Sobre Huanglongbing y el Psillido Asiatico de los Citricos*, del 7 al 9 de Mayo de 2008, Hermosillo, Sonora, p. 11. Available at http://www. concitver.com/huanglongbingYPsilidoAsiatico/Memor_ıa-2% 20Hall.pdf. Accessed 19 Dec 2016.

Hall, D. G. McCollum, G. (2011). Survival of Adult Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), on Harvested Citrus Fruit and Leaves. *Florida Entomologist*, 94(4): 1094-1096.

Hall, D. G. Richardson, M. L. Ammar, E. Halbert, S. (2013). Asian citus psyllid, *Diaphorina citri*, vetor of citrus huanglongbing disease. *Entomologia Experimentalis et Applicata*, 146: 207-223.

Hilgenboecker, K. Hammerstein, P. Schlattmann, P. Telschow, A. Werren, J. H. (2008). How many species are infected with *Wolbachia*? - a statistical analysis of current data. *FEMS Microbiology Letters*, 281: 215-220.

Hodges, A. W. Spreen, T. H. (2012). Economic impacts of citrus greening (HLB) in Florida, 2006/2007 to 2010/2011. Institute of Food and Agricultural Sciences, University of Florida, p. 5. Document FE903 http://edis.ifas.ufl.edu/fe903.

Hollis, D. 1987. A new citrus-feeding psyllid from the Comoro Islands, with a review of the *Diaphorina amoena* species group (Homoptera). *Systematic Entomology*, 12: 47-61.

Hoy, M. A. Nguyen, R. (2001). Classical biological control of Asian citrus psylla. *Citrus industry*, 81: 48-50.

Johnson, E. Wu, J. Bright, D. Graham, J. (2013). Association of '*Candidatus* Liberibacter asiaticus' root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. *Plant Pathology*, (63): 290-298.

Jourdie, V. A., N. Molina-Ochoa, J. Williams, T. Bergvinson, F. (2010). Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidoptera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): to what extent is the host plant important? *Molecular Ecology*, 19: 2168-2179.

Lashkari, M., Manzari, S., Sahragard, A., Malagnini, V., Boykin, L. M., & Hosseini, R. (2014). Global genetic variation in the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae) and the endosymbiont *Wolbachia*: links between Iran and the USA detected. *Pest Management Science*, 70(7): 1033-1040.

Leigh, J. W. Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6: 1110-1116.

López-Collado, J., Isabel López-Arroyo, J., Robles-García, P. L., & Márquez-Santos, M. (2013). Geographic Distribution of Habitat, Development, and Population Growth Rates of the Asian Citrus Psyllid, *Diaphorina citri*, in Mexico. *Journal of Insect Science*, 13: 114.

McClean, A. P. D., & Schwarz, R. E. (1970). Greening or blotchy-mottle disease of citrus. *Phytophylactica*, 2(3): 177-194.

Mead, F. (1977). The Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Florida Department of Agriculture Conservation Service, *Division of Plant Industry Entomology Circular 180*. Florida.

Michaud, J. P. (2004). Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in Central Florida. Biological Control, 29: 260-269.

Miller, M. A. W. Pfeiffer, W. Schwartz, T, (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In: Proceedings of the Gateway Computing Environments Workshop* (GCE), Louisiana.

Pluke, R. W. Qureshi, J. Stansly, P. (2008). Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. *Florida Entomologist*, 91: 36-42.

Qureshi, J. A. Rogers, M. E. Hall, D. G. Stansly, P. A. (2009). Incidence of invasive *Diaphorina citri* (Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. *Journal of Economic Entomology*, 102: 247-256.

Remais, J. V. Xiao, N. Akullian, A. Qiu D. Blair, D. (2011). Genetic assignment methods for gaining insight into the management of infectious disease by understanding pathogen, vector, and host movement. *PloS Pathogens*, (7) 4.

Riegler, M. Stauffer, C. (2002). W*olbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diperta, Tephritidae). *Molecular Ecology*, (11): 2425-2434.

Ronquist, F. Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed model. *Bioinformatics*, 19: 1572-1574.

Rozas, J. Sanchez-DelBarrio, J. C. Messeguer, X. and Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19: 2496-2497.

Saha, S. Hunter, W. Reese, J. Morgan, K. Marutani-Hert, M. Huang, H. Lindeberg, M. (2012). Survey of endosymbtionts in the *Diaphorina citri* metagenome and assembly of a *Wolbachia* wDi draft genome. *PLoS One*, (7)11.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, 105: 437-460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123: 585-595.

Tamura, K. Stecher, G. Peterson, D. Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30(12): 2725-2729.

Thompson, J. D. Higgins, D. G. Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22): 4673-4680.

Tsai, J. H. Liu, Y. H. (2000). Biology of *Diaphorina citri* (Homoptera: Psyllidae) on Four Host Plants. *Journal of Economic Entomology*, 93: 1721-1725.

Chapter II

Bastaniel, M. Oliveira, A. C. de Cristofanil, M. Guerreiro-Filho, O. Freitas-Astua, J. Rodrigues, V. Astua-Mongue, G. Machado, M. A. (2006). Inheritance and heritability of resistance to citrus leprosis. *Phytopahtology*, 96(10): 1092-1096.

Beard, J. Ochoa, R. Braswell, E. Bauchan, G. (2015). *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae) – a closer look. *Zootaxa*, 1: 001-067.

Caceres, S. Aguirre, A. Costa, N. de Coll, O. Gonzales-Segnana, L. Farina, N. Tassi, A. Calegario, R. F. de Moraes, G. J. Freitas-Astua, J. Pereira, J. A. Salaroli, R. B. Kitajima, E. W. (2013). Present status of citrus leprosis in Argentina and Paraguay. *Tropical Plant Pathology*, 38(4): 282-294.

Chen, T. French, V. Liu, T. da Graca, L. (2006). Predation of *Galendromus helveolus* (Acari: Phytoseiidae), on *Brevipalpus californicus* (Acari: Tenuipalpidae). *Biocontrol Science and Technology*, 16: 753-759.

Childers, C. Rodrigues, J. Welbourn, W. (2003). Host plants of *Brevipalpus californicus*, *B. obovatus* and *B. phoenicis* (Acari: Tenuipalpidae) and their potential involvement in the spread of viral diseases vectored by these mites. *Experimental and Applied Acarology*, 30: 29-115.

Clement, M. P., D. Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9(10): 1657-1659.

Dean, H. 1959. Seasonal distribution of mites on Texas grapefruit. *Journal of Economic Entomology*, 52(2): 228-232.

Excoffier, L. Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567.

French, J, V. Rakha, M. A. (1994). False spider mite damage and control on Texas citrus. *Subtropical Plant Science*, 46:16-19.

Fu, Y. X. Li, W. H. (1993). Statistical tests of neutrality of mutations. *Genetics*, 133: 693-709.

Gahi, S. Shenhmar, M. 1984. A review of the world fauna of Tenuipalpidae (Acarina: Tetranychoidea). *Oriental Insects*, 18: 99-172.

Gomez, E. Vargas, R. Rivadameria, E. Locali,A. Fretias, G. Astua, J. Rodrigues, J. Mesa, C. Kitajima, E. (2005). First report of citrus leprosis virus on citrus in Santa Cruz, Bolivia. *Plant Disease*, 89(6): 5686.

Groot, T, Janseen, A. Pallini, A. Breeuwer, J. (2005). Adaptation in the asexual false spider mite *Brevipalpus phoenicis*: evidence for frozen niche variation. *Experimental and Applied Acarology*, 36: 165-176.

Hao, D. Su, P. Pfammatter, J. Liu, Q. Fan, B. Q. Wang, Y. Gu, Z. T. (2016). Morphological and genetic characteristics of *Brevipalpus lewisi* (Acari: Tenuipalpidae) and comparison with other three *Brevipalpus* species. *International Journal of Acarology*, 80: 34-40.

Helle, W. Bolland, H. R. Heitmans, W. R. B. (1980). Chromosomes and types of parthenogenesis in false spider mites (Acari: Tenuipalpidae). *Genetica*, 54: 45-50.

Izquierdo-Castillo, I. Zermeno-Diaz, L. F. Mendez, W. Otero-Colina, G. Freitas-Astua, J. Locali-Fabris, E. C. de Moraes, G. J. Calgario, R. F. Tassi, A. D. Kitajima, E. W. (2011). Confirmation of the presence of the *Citrus leprosis* virus C (CiLV-C) in Southern Mexico. *Tropical Plant Pathology*, 36(6): 400-403.

Jensen, J. L. Bohonak, A. Kelley, S. (2005). Isolation by distance, web service. *BMC Genetics*, 6:13.

Kearse, M. Moir, R. Wilso, A. Stones-Havas, S. Cheung, M. Sturrock, S. Buxton, S. Cooper, A. Markowitz, S. Duran, C. Thierer, T. Ashton, B. Meintjes, P. Drummond, A. (2012). Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatic*s, 28(12): 1647-1649.

Kitajima, E. Verle-Rodrigues, J. Freitas-Astua, J. (2010). An annotated list of ornamentals naturally found by infected *Brevipalpus* mite-transmitted viruses. *Scientia Agricola*, 67: 348-371.

Knorr, L. C. Price, W. C. (1958). Leprosis, *In:* RM. Ed. Florida guide to citrus insects, diseases and nutritional disorders (in color). Univ. Florida Agric. Exp. Sta. Gainesville. p. 112-114.

Knorr, L. C. Studies on the etiology of leprosis in citrus. Proc. Conf. Int. Org. Citrus Virol. Univ. Florida Press. Gainesville, 4: 332-341.

Leigh, J. W. Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6: 1110-1116.

Leon, G. Kitajima, E. Freitas, A. Childers, C. Archor, D. Salarolli, R. Mesa, C. (2006). Occurrence of citrus leprosis virus in Llanos Orientales, Colombia. *Plant Disease*, 90(5): 682.

Locali, E. C. Freitas-Astua, J. Souza, A. A. Takita, M. A. Astua-Mongue, G. Antonili-Luizon, Kitajima, E. W. Machado, M. A. (2003). Development of a molecular tool for the diagnosis of leprosis, a major threat to citrus production in the Americas. *Plant Disease,* 87: 1317-1321.

Mata, J. Setamou, M, French, J. Louzada, E. (2010). Molecular fingerprinting and population dynamics of *Brevipalpus* mites on Texas Citrus. *Entomological Society of America*, 103(6): 898-907.

Mejia, L. Paniagua, N. Cruz, N. Porras, N. Palmieri, M. (2005). Citrus leprosis, disease that endangers plantations in Guatemala. *Annals Annual Meeting of American Phytopathological Society,* Caribbean Division, San Jose.

Musumeci, M. R. Rossetti, V. (1963). Transmissao dos sintomas da leprose dos citros pelo acaro *Brevipalpus phoenicis*. *Ciencia e Cultura,* 15:228.

Navia, D. Mendoca, R. Ferragut, F. Miranda, L. Trincado, R. Michaux, J. Navajas, M. (2013). Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). *Zoologica Scripta*, 42: 406-426.

Pazcual-Ruiz, S. Gomez-Martinez, M. A. Ansaloni, T. Segarra-Moragues, J. G. Sebater-Munoz, B. Jacas J. A. (2014) Genetic structure of a phytophagous mite species affected by crop practices: the case of *Tetranychus urticae* in clementine mandarins. *Experimental and Applied Acarology*, 62: 477-498.

Pijnacker, L. P. Ferwerda, M. A. Bolland, H. R. Helle, W. (1980). Haploid female parthenogenesis in the false spider mite Brevipalpus obovatus. (Acari: Tenuipalpidae). *Genetica*, 21: 221-214.

Rodrigues, J. (2000). Relacoes patogeno-vector-planta no sistema leprose dos citrus. Thesis PhD. Piracicaba, Brasil, Centro de Energia Nuclear na Agricultura, da Universidade de Sao Paulo.

Rodrigues, J. Kitajima, E. Childers, C. C. Chagas. (2003). Citrus leprosis virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) on citrus in Brazil. *Experimental and Applied Acarology*, 30: 161-179.

Roy, A. Hartung, J. Schneider, W. L. Shao, J. Leon, G. Melzer, M. J. Beard, J. J. Otero-Colina, G. Bauchan, G. R. Ochoa, R. Briansky, R. H. (2015). Role bending: complex

relationships between viruses, hosts, and vectors related to citrus leprosis, an emerging disease. *Phytopathology*, 105(7):1013-1025.

Rozas, J. Sanchez-DelBarrio, J. C. Messeguer, X. and Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19: 2496-2497.

Sanchez-Velazquez, E. J. Santillan-Galicia, M. T. Novelli, V. M. Nunes, M A. Mora-Aguilera, G. Valdez-Carrasco, J. M. et al. (2015). Diversity and genetic variation among *Brevipalpus* populations from Brazil and Mexico. *PLoS ONE*, 10(7):e0133861.

Salinas-Vargas, D. Santillan-Galicia, M. Guzman-Franco, A. Hernandez-Lopez, A. Ortega-Arenas, L. Mora-Aguilera, G. (2016). Analysis of genetic variation in *Brevipalpus yothersi* (Acari: Tenuipalpidae) populations from four species of citrus host plants. *PLoS ONE*, 11(10): e0164552.

Stireman, J. O. Nason, J. D. Heard, S. O. (2005). Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *The society for the study of evolution*, 59(12): 2573-2587.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, 105: 437-460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123: 585-595.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 7.0. *Molecular Biology and Evol*ution *(submitted)*, 30(12): 2725-2729.

Thompson, J. D. Higgins, D. G. Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22): 4673-4680.

BIOGRAPHICAL SKETCH

Alejandra Fuentes was born on March 14, 1992 in Monterrey, Nuevo Leon, Mexico. In 2010, she started her undergraduate education at the Universidad Autonoma de Nuevo Leon, with a major in Biotechnology and Genomics. In 2013, she transferred to the University of Texas Pan-American (UTPA). As an undergraduate student she had the opportunity to participate in a research internship at the USDA-APHIS Mission Laboratory, in Edinburg, TX. In the fall of 2014, she received a Bachelor of Science in Biology from UTPA. Her research experiences as an undergraduate intern led her to pursue a graduate degree to further develop her knowledge and skills in science.

Alejandra received her Master of Science in Biology from the University of Texas Rio Grande Valley (UTRGV) in the spring of 2017. Her graduate research was a collaboration between the UTRGV and the USDA-APHIS Mission Laboratory. Her project focused on the genetic variation of two agriculturally important arthropod vectors of citrus plants diseases. Prior to graduation, she obtained a position in the mentioned USDA-APHIS laboratory, where she works as a Biological Science Laboratory Technician. Alejandra currently resides at 3404 Tyler Ave. McAllen, Texas 78503.