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Assessing Genetic Diversity in Four Stink Bug Species, *Chinavia hilaris, Chlorochroa uhleri, Chlorochroa sayi*, and *Thyanta pallidovirens* (Hemiptera: Pentatomidae), Using DNA Barcodes

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Abstract

Stink bugs (Hemiptera: Pentatomidae) are an economically important group of insects that attack numerous crops in the central valley of California. Management of these pests using pheromones or biological control can be species specific, and proper identification of insect species is essential for effective management. The objective was to examine genetic variability in four species of stink bugs, which included *Chinavia hilaris* (Say) (= *Acrosternum hilare*) (Hemiptera: Pentatomidae) , *Chlorochroa uhleri* (Stål) (Hemiptera: Pentatomidae) , *Chlorochroa sayi* (Stål) (Hemiptera: Pentatomidae), and *Thyanta pallidovirens* (Stål) (Hemiptera: Pentatomidae) and to determine whether there may be cryptic species present. Stink bugs were collected in pistachios or on adjacent vegetation when abundant in the central valley of California. The mitochondrial DNA cytochrome oxidase I (COI) gene region (i.e., the barcode) was sequenced for each individual. Data were combined with available GenBank accessions for each species and used to construct a phylogenetic tree. Divergence between genera ranged from 11.2 to 15.7%, whereas divergence between the two *Chlorochroa* spp. was 4.6%. Genetic variation within *Chinavia hilaris* collections was up to 4.7%, which suggests the presence of a cryptic species. Genetic divergence was highest between individuals of *Chinavia hilaris* from the west coast and the east coast of the United States. In contrast, genetic variation within individuals of *C. uhleri* and *Ch. sayi* was less than 1%. Nine haplotypes were found for *Chinavia hilaris*, five for *C. uhleri,* three for *Ch. sayi*, and five for *T. pallidovirens*. The relevance of correct species identification and genetic diversity to stink bug management practices was discussed.

Key words: Stink bugs, DNA barcode, genetic diversity, pistachio, cryptic species

Stink bugs (Hemiptera: Pentatomidae) are a major concern for growers in agricultural production regions around the world, including in the central valley of California (Zalom et al. 1997). One of the major nut crops grown in this region is pistachio, which accounts for 98% of the production in the nation. The stink bug complex in pistachios includes a number of agriculturally important species (McPherson and McPherson 2000, Panizzi et al. 2000). In nut crops, both immatures and adults feed by probing their needle-like mouthparts into the developing nuts and can cause necrotic lesions, deformities, and premature fruit drop (Rice et al. 1985, McPherson and McPherson 2000). Following feeding injury, secondary infection by pathogenic fungi and bacteria is common in pistachio (Michailides and Morgan 1990). Stink bugs are among the important insect pests of pistachios and can result in considerable economic losses, and the damage potential of each of the species varies (Daane et al. 2005). Therefore, correct identification of the stink bug species, documenting their genetic diversity, and determining whether cryptic species are present will help inform management decisions for the grower.

In the central valley of California, a number of species of stink bugs occur in pistachio, including *Chinavia hilaris* (Say) (=*Acrosternum hilare*), *Chlorochroa uhleri* (Stål), *Chlorochroa sayi* (Stål) and *Thyanta pallidovirens* (Stål) (Holtz 2002). *Chinavia hilaris* is characterized by a deep green color and black lines on the edges of abdomen, whereas *T. pallidovirens* is smaller with a red strip across the pronotum. Two other species that can be found are *C. uhleri* and *Ch. sayi*, which can be difficult to distinguish (Buxton et al. 1983, Holtz 2002) (Fig.1). Both are similar in size and have few apparent morphological differences. *Ch. sayi* typically has a faint orange band around the abdomen, which *C. uhleri* lacks (Fig. 1). The confusion in accurate identification of these stink bugs also may arise due to the presence of a color polymorphism in this group (Vivan and Panizzi 2002, Esquivel et al. 2015).

Fig. 1. Adults of the species of stink bugs collected and sequenced in this study (A) *Chinavia hilaris,* (B) *Ch. sayi,* (C) *C. uhleri,* and (D) *T. pallidovirens* (Photo Credit: Jack Kelly Clark, courtesy University of California Statewide IPM Program.).

Among the four species, *Chinavia hilaris* has the widest distribution that ranges from Quebec and New England in the east of the United States, and extends west to the Pacific Coast, including throughout most of the United States (McPherson and McPherson 2000). The other two species, *C. uhleri* and *Ch. sayi*, are primarily distributed throughout the western United States (Caffrey and Barber 1919). *C. uhleri* occurs from southern Canada and into part of the great plains, whereas *Ch. sayi* is more restricted to the western and central part of the United States (Buxton et al. 1983, Scudder and Thomas 1987, Prado and Almeida 2009). *T. pallidovirens* is distributed in the western part of the United States and southern British Columbia (Schotzko and O'Keeffe 1990). These stink bug species are polyphagous and reported to cause damage to many agricultural crops (Caffrey and Barber 1919, Jacobson 1965).

Accurate identification of insect species is important for effective pest management. In particular, using insect pheromones and classical biological controls can be species specific. Male-produced aggregation pheromones have been characterized for these four green stink bug species (McBrien et al. 2001, 2002; Ho and Millar 2001a, b). To effectively use these pheromones to monitor or for mass trapping, correct identification of the stink bug species in orchards or fields is vital. Similarly, success of biological control is dependent on the use of species-specific natural enemies such as parasitoid wasps. In California, there are 10 native parasitoid species that have the ability to supress stink bug populations, and these parasitoids are often host specific (Hoffman et al. 1991, Herlihy et al. 2016). For example, the parasitoid *Trissolocus utahensis* (Ashmead) parasitizes more eggs of *C. uhleri* than that of *Ch. sayi* (Jubb and Watson 1971). Thus, biological control of stink bugs and many other insect species depends on both the correct identification of the pest and its parasitoids. Similarly, a pheromone designed for a specific insect pest species may not work effectively in an area if genetically different subpopulations of the same species or cryptic species exist (Groot et al. 2014).

Molecular markers are helpful in identifying cryptic species or polymorphic species, which may not be possible to distinguish using traditional taxonomy (Hebert et al. 2004, Xiao et al. 2010). Arrays of molecular markers such as amplified fragment length polymorphisms (AFLPs), microsatellites, and DNA sequences including mitochondrial DNA cytochrome oxidase I (COI) (i.e., barcodes) are available for characterization of insect species or populations (Hebert et al. 2004, Behura 2006, Alessandrini et al. 2008, Park et al. 2011). Mitochondrial DNA sequencing can determine both interspecific and intraspecific variation in organisms including insects (Wang et al. 2011). In particular, DNA barcoding has been used extensively to identify or confirm new and existing species and cryptic species, establish phylogenetic relationships among related taxa, and has even been used to understand species interactions such as predation and parasitism (Ball et al. 2005, Monaghan et al. 2005, Schindel and Miller 2005, Burns et al. 2008, Jurado-Rivera et al. 2009). Genetic divergence of 2% or more among individuals within several insect groups suggests that a cryptic species may be present (Hebert et al. 2004). In the case of Hemiptera, an intraspecific divergence of 3–5% has been found to suggest cryptic species or delineate species in populations (Jung et al. 2011, Park et al. 2011, Kaur and Sharma 2017).

The objectives of this study were to sequence the COI gene region of mtDNA from stink bugs collected from pistachio orchards and adjacent vegetation, and to combine these sequences with existing publically available sequences of the same species to explore the genetic diversity within each species, and to determine whether there was evidence for any cryptic species. The results of this study will contribute to pest management of these stink bug species within their distribution ranges and in the central valley of California.

Materials and Methods

Insect Collections

Stink bugs were collected from several pistachio orchards and adjacent vegetation when abundant, primarily from locations in the southern San Joaquin Valley in Kern County, California from May 2013 to February 2014 (Fig. 2, Table 1). Adult insects were examined under the dissecting microscope and were putatively assigned to one of the following four species: *Chinavia hilaris*, *C. uhleri*, *Ch. sayi*, or *T. pallidovirens*. The assignment of insect samples to a particular species was based on external morphological features for those species described in literature (Buxton et al. 1983, Rider and Chapin 1992).

DNA Extraction and Polymerase Chain Reaction

The thorax of each stink bug was removed and used for extraction of genomic DNA. The Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) was used for DNA extraction, following manufacturer's protocol for tissue extraction and using a 1 h incubation period at 55°C (Qiagen 2006). The DNA quantity was measured using the Qubit dsDNA HS Assay kit (Life Technologies, Carlsbad, CA). The quantity of DNA in samples averaged 20–40 ng/µl. Both male and female adults were used for DNA extraction. Two sets of universal primers were used: LCO1490 (5′-GGTCAACAAA TCATAAAGATATTGG-3′) and reverse primer HCO2198 (5′-TAAACTTCAGGGTGACCA AAAAATCA-3′) (Folmer et al. 1994) and LepF2_t1, 5′-ATTCAACCAATCATAAAGATAT-3′; and LepR1, 5′-TAAACTTCTGGATGTCCAAAAA-3′ (Hebert et al.

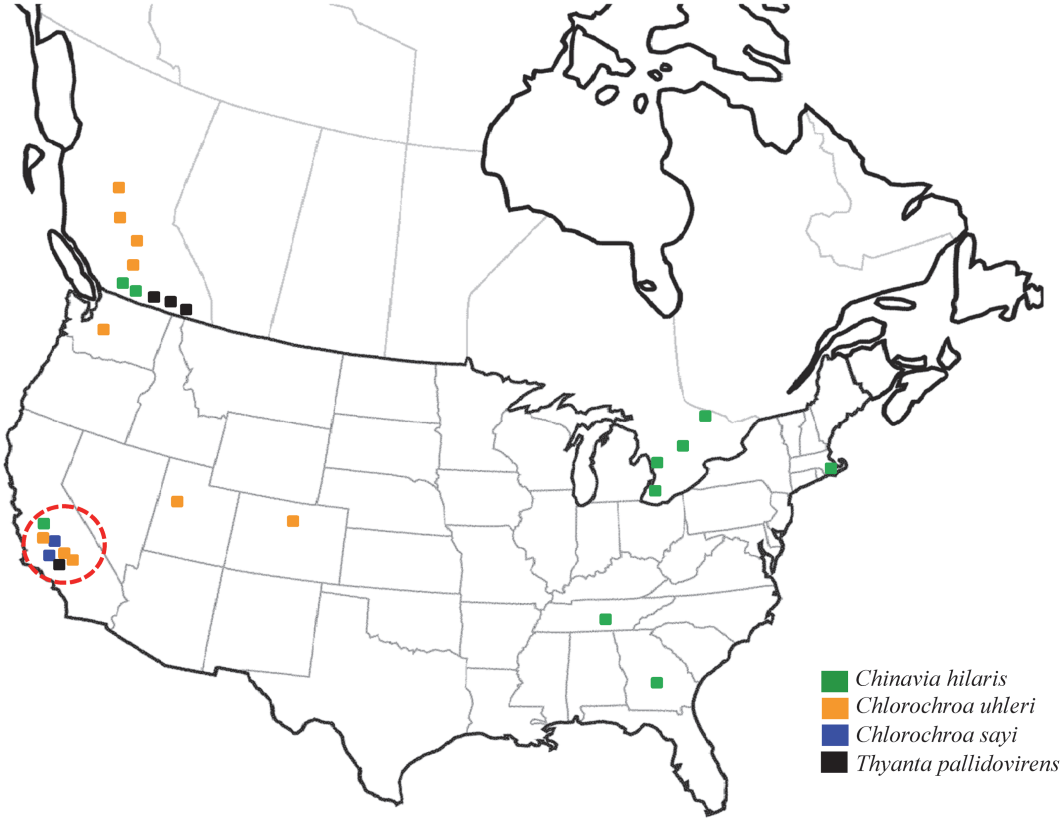


Fig. 2. Map of collection areas of *Chinavia hilaris, C. uhleri, Ch. sayi,* and *T. pallidovirens.* Samples from California are within the red dotted circle. Locations of other individuals obtained from GenBank are indicated on map. See Tables 1 and 2 for coordinates associated with collections.

Table 1. Collection locations for stink bugs collected in California and sequenced in this study

Species	Stink bug sample #	Latitude	Longitude
Chinavia hilaris	Ch03, Ch51,Ch57, Ch61, Ch67, Ch81, Ch82, Ch83, Ch96, Ch99	37.192570 N	−120.27006 W
Chlorochroa uhleri	Cu126, Cu140, Cu141, Cu144, Cu145, Cu147, Cu149, Cu152, Cu154, Cu158, Cu159, Cu160, Cu176, Cu177, Cu180	35.492748 N	−119.727729 W
	Cu303, Cu307	35.491866 N	−119.716268 W
	Cu342, Cu343	35.621033 N	−119.928974 W
Chlorochroa sayi	Cs341, Cs351, Cs352, Cs353, Cs354, Cs355, Cs356, Cs357, Cs358	35.621033 N	−119.928974 W
	Cs 305	35.491866 N	−119.716268 W
Thyanta pallidovirens	Tp197	35.492748 N	−119.727729 W

Ch, *Chinavia hilaris*; Cu, *Chlorochroa uhleri*; Cs, *Chlorochroa sayi*; Tp, *Thyanta pallidovirens*.

2004). Polymerase chain reaction (PCR) was performed using a mixture of 6µl of DNA, 1 µl of each forward and reverse primer at 10 µM concentration, 0.5 µl of Taq polymerase (Takara-Clonetech Bio USA Inc., Mountain View, CA), 5 µl of buffer, 4 µl of dNTPs (2.5 µM) and 32.5 µl of sterile water to make the reaction volume of 50 µl. The PCR program was the following: 1 min at 95°C, followed by 40 cycles of the following: 92°C for 30 s, 43°C for 30 s, and 72°C for 1 min each. There was a final hold of 10 min at 68°C and samples were then maintained at 4°C until refrigeration. The PCR products were verified by visualizing samples on a 1.5% agarose gel and then cleaned up by the USB Exo-sap-it (Affymetrix Inc., Santa Clara, CA) PCR cleanup kit. Each forward and reverse sequence reaction was prepared using 1 µM primer and analyzed on a Applied Biosystems 3730xl genetic sequencer using BigDye Terminator v.3.1 Kit (Applied Biosystems, Foster City, CA) at the UC Berkeley DNA Sequencing Facility.

Sequence Analysis

The DNA sequences were edited using Geneious 7.0 (Biomatters, Aukland, New Zealand) (Kearse et al. 2012). Consensus sequences for each individual were generated by aligning forward and reverse sequences and were then subjected to multiple sequence alignment algorithms using ClustalW with default settings in MEGA 7.0 (Kumar et al. 2016). In addition to the sequences generated in this study, reference sequences from GenBank were included from *Chinavia hilaris*, *C. uhleri*, and *T. pallidovirens* for phylogenetic analysis (Table 2). The aligned sequences were used for generating a neighbor-joining (NJ) tree in MEGA 7.0 (Kumar et al. 2016) with the K-2 parameter model and 1000 bootstrap replications. In addition to the NJ tree based on K2P model, which is a widely used model in DNA barcode literature, we also generated a phylogenetic tree using model-based maximum likelihood (ML) analysis for the same

Table 2. Information about the taxa and their GenBank accession numbers for samples included in this study

Family	Subfamily	Tribe	Species	Accession ID	Location	Latitude	Longitude
Pentatomidae	Pentatominae	Nezarini	*Chinavia hilaris*	HQ105391.1	BC, Canada	49.15 N	119.533 W
				HQ105392.1	BC, Canada	49.05 N	119.517 W
				HQ105390.1	BC, Canada	49.05 N	119.517 W
				JX548471.1	GA, USA	31.5417 N	83.2949 W
				JX548472.1	GA, USA	31.5417 N	83.2949 W
				JX548473.1	GA, USA	31.5417 N	83.2949 W
				JX548474.1	GA, USA	31.5417 N	83.2949 W
				KJ642001.1	TN, USA	N/A	N/A
				HQ978858.1	MA, USA	41.5298 N	70.6547 W
				KJ167924.1	ON, Canada	41.939 N	82.516 W
				KR032745.1	ON, Canada	45.6334 N	77.0697 W
				KR037751.1	ON, Canada	45.6334 N	77.0697 W
				KR039634.1	ON, Canada	45.6334 N	77.0697 W
				KR043989.1	ON, Canada	45.6334 N	77.0697 W
				KR577823.1	ON, Canada	44.38 N	80.58 W
				KR578242.1	ON, Canada	44.564 N	78.505 W
		Nezarini	*Chlorochroa uhleri*	KR042273.1	BC, Canada	54.686 N	124.937 W
				KR570598.1	BC, Canada	50.643 N	120.517 W
				KR035090.1	UT, USA	39.078 N	111.753 W
				KR035441.1	WA, USA	47.263 N	120.322 W
				KR038367.1	CO, USA	38.702 N	105.425 W
				KR037941.1	BC, Canada	54.686 N	124.937 W
				HQ105553.1	BC, Canada	51.4667 N	122.267 W
		Pentatomini	*Thyanta pallidovirens*	HQ106418.1	BC, Canada	49.15 N	119.533 W
				HQ106419.1	BC, Canada	49.40 N	119.55 W
				HQ106420.1	BC, Canada	49.15 N	119.533 W
				KR035886.1	BC, Canada	49.2714 N	119.606 W

dataset. Using the model selection option in MEGA 7.0, we found that the Tamura 3-parameter with discrete Gamma distribution (T92 + G) was the best-fit model to our dataset based on the lowest BIC (Bayesian Information Criterion) value. ML analysis was done using the best-fit model and clade support was assessed via 1000 bootstrap replicates. Pairwise genetic distances among the individual sequences were calculated using MEGA 7.0 (Kumar et al. 2016). A separate phylogenetic analysis was conducted to compare the genetic diversity between sequences from the California population of *Chinavia hilaris* with others (n = 16) from GenBank in MEGA 7.0 (Kumar et al. 2016). The number of haplotypes and haplotype diversity within each species was calculated using DnaSP v.5 (Librado and Rozas 2009), for insects collected in California combined with those from GenBank. A haplotype network for *Chinavia hilaris* individuals both from this study and 16 individuals from GenBank was created using the TCS software package (Clement et al. 2000). All the sequences generated in this study were submitted to GenBank.

Results

A total of 40 barcode sequences were generated for four stink bug species (Table 1). The length of the sequences for individuals varied (average 636 bp), but after trimming both ends of the final alignment, 574 bp of the original sequences were retained for further analysis. Another 27 sequences were obtained for comparison from GenBank including *Chinavia hilaris* (n = 16), *C. uhleri* (n = 7), and *T. pallidovirens* (n = 4) (Fig. 2, Table 2). No sequences for *Ch. sayi* were available in GenBank. A NJ tree was produced that included all individuals (n = 67) from this study and individuals from GenBank (Fig. 3). Similarly, a ML tree was also generated for the same dataset (Fig. 4). The tree was rooted to the group representing *T. pallidovirens*. Both NJ tree and ML tree showed similar topologies with well-resolved clusters representing individuals of four different species. There were two primary branches, one representing the two *Chlorochroa* species, *C. uhleri* and *Ch. sayi*; another branch represented individuals of *Chinavia hilaris*. The two species, *C. uhleri* and *Ch. sayi*, were well separated into two clades with high bootstrap value.

The individuals of most species clustered well within their respective branches without any deep intraspecific divergence (>2.0%, Hebert et al. 2003, Huemer et al. 2014), with the exception of *Chinavia hilaris* (Fig. 3). The *Chinavia hilaris* cluster further separated into two branches: one represented all the individuals from California and three individuals from British Columbia (HQ105390.1, HQ105391.1, and HQ105392.1) (Figs 3 and 4), whereas the second branch consists of individuals from Georgia, Tennessee, Massachusetts, and Ontario Canada (Fig. 5). No previous sequences were available for *Ch. sayi* in GenBank for comparison; however, the morphological descriptions, cohesive clustering of individuals within the branch, and the phylogenetic distance from *C. uhleri* (4.6% divergence) provide support for the identification of *Ch. sayi* individuals in this study. Genetic divergence was high for pairs of species in different genera; 15.2% between *Chinavia hilaris* and *C. uhleri*, 15.0% between *Chinavia hilaris* and *Ch. sayi*, 11.2% between *Chinavia hilaris* and *T. pallidovirens*, 14.4% between *T. pallidovirens* and *C. uhleri*, and 15.7% between *T. pallidovirens* and *Ch. sayi* (Fig. 3, Table 3).

The numbers of haplotypes within each species were 9, 5, 3, and 5 for *Chinavia hilaris, C. uhleri*, *Ch. sayi*, and *T. pallidovirens*, respectively (Table 4). The haplotype network analysis for *Chinavia hilaris* individuals indicated that all the individuals from

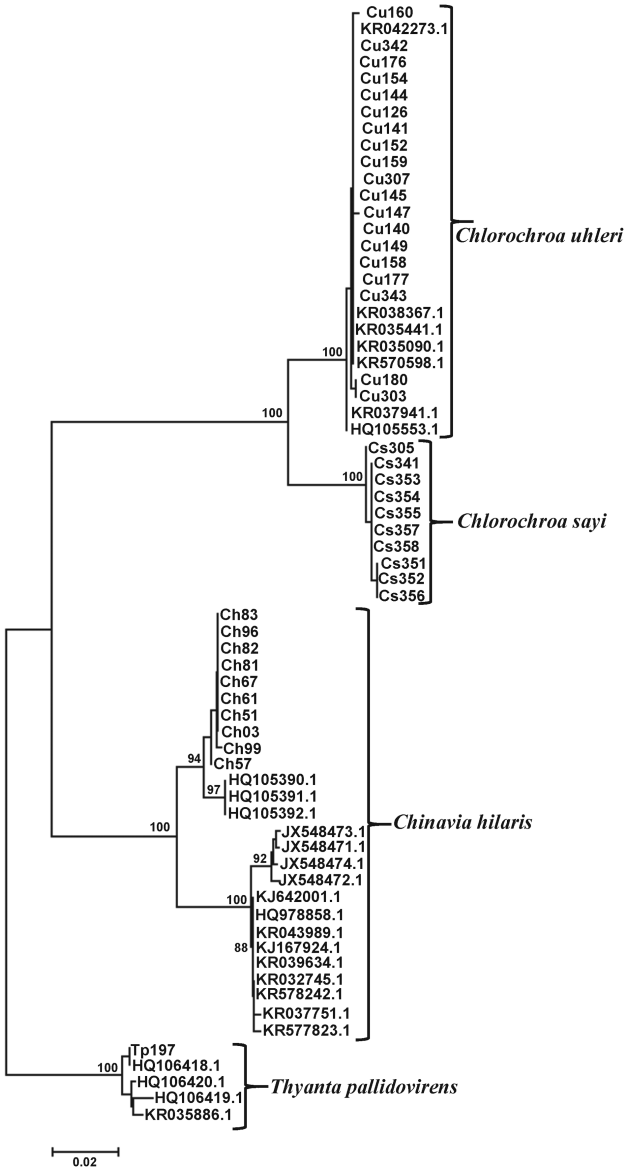


Fig. 3. Neighbor-joining tree of stink bugs based on the mtDNA CO1 gene region. The tree is based on the K-2 parameter model and 1,000 bootstrap replications. Branches with >90% consensus support are shown, and the genetic distance is shown in the scale below.

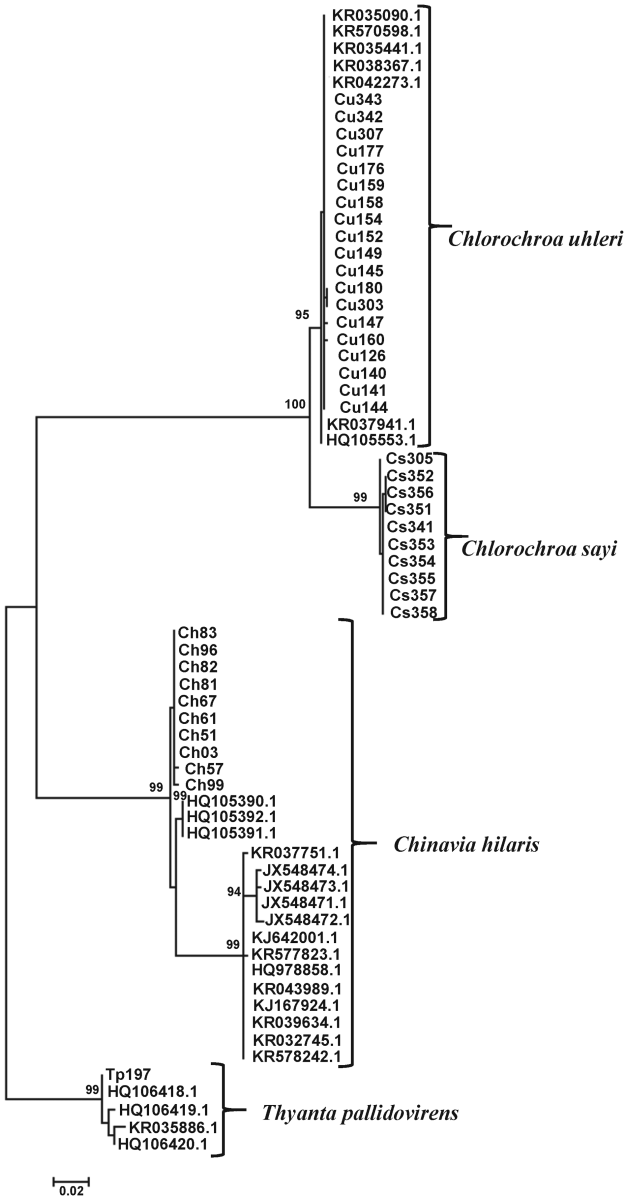


Fig. 4. Maximum likelihood tree of stink bug species based on mtDNA COI gene region. The tree is based on Tamura-3 parameters with discrete Gamma distribution (T92 + G) and 1,000 bootstrap replications. Bootstrap support values are given on the nodes.

California represented a unique haplotype (Fig. 6). The closest unique haplotype to the California individuals consists of the individuals from British Columbia. All the individuals from California were separated by 0.9% divergence from individuals from British Columbia, Canada (Fig. 5). However, the individuals from Georgia (JX548471.1, JX548472.1, JX548473.1, and JX548474.1) were the most distant in the network from the California population (4.7% divergent) and were 4.0% divergent from individuals from Ontario Canada (Fig. 5).

The intraspecific divergence was calculated for all the individuals within each species. The highest intraspecific divergence of 4.7% was recorded within *Chinavia hilaris*, between individuals from California and Georgia (Ch03 and JX548474.1) (Supp Table 1 [online only]). Mean intraspecific diversity among the individuals was lowest for *C. uhleri* (0.1%) and ranged between 0.0 and 0.5%. The mean intraspecific divergence was low for the other species as well: 0.2 and 0.6% for *Ch. sayi* and *T. pallidovirens*, respectively (Supp Table 1 [online only]).

Discussion

The 648 bp region of the 5′ end of the mitochondrial COI gene known as the DNA barcode, has played a significant role in identifying insect species and other organisms, tracing the origin of introduced species, uncovering the presence of cryptic species, and examining genetic variability (Hebert et al. 2004, Park et al. 2011). In this study, barcode sequences were generated for four stink bug species, *Chinavia hilaris*, *C. uhleri*, *Ch. sayi*, and *T. pallidovirens*, from California specimens. No previous public sequences for *Ch. sayi* were available, and the sequences in this study were the first produced from these stink bugs from California. The sequences

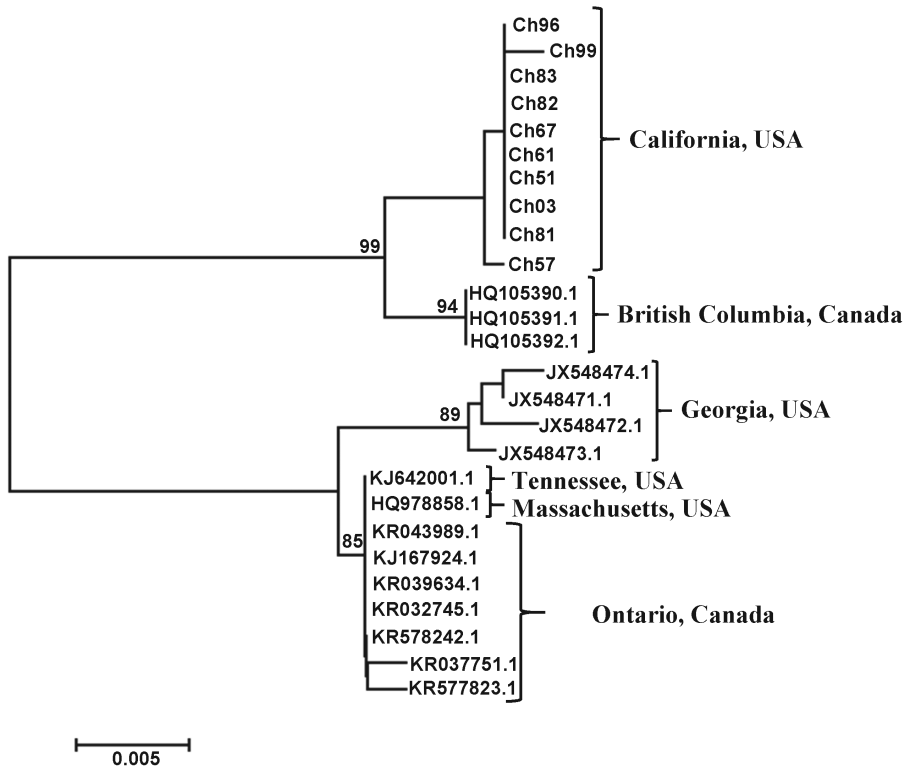


Fig. 5. Neighbor-joining tree based on the mtDNA CO1 region of *Chinavia hilaris* from California combined with GenBank accessions from different geographic locations. The tree is based on the K-2 parameter model and 1,000 bootstrap replications. Branches with >80% support are shown above each branch.

Table 3. Pairwise mean genetic distance (K2P) between species calculated using MEGA 7.0

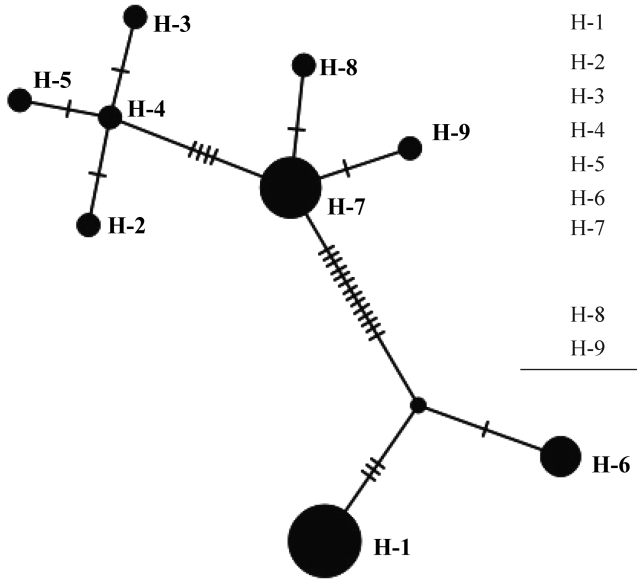
	Chinavia hilaris	*Chlorochroa uhleri*	*Chlorochroa sayi*	*Thyanta pallidovirens*
Chinavia hilaris		0.015	0.016	0.012
Chlorochora uhleri	0.152		0.009	0.014
Chlorochroa sayi	0.150	0.046		0.014
T. pallidovirens	0.112	0.144	0.157	

Below the diagonal is the genetic distance and above the diagonal represents the SEM.

Table 4. Number of haplotypes and haplotype diversity, and the intraspecifc genetic divergence for each species

Species	No. of individuals (*n*)	No. of haplotypes (h)	Haplotype diversity (H_d)	Intraspecific divergence (mean)	Intraspecific divergence (SE)
Chinavia hilaris	26	9	0.788	0.024	0.005
Chlorochroa uhleri	26	5	0.409	0.001	0.000
Chlorochroa sayi	10	3	0.600	0.002	0.001
Thyanta pallidovirens	5	5	1.000	0.006	0.003

confirmed the identity of the four insect species with existing sequences available for comparison. Barcode sequences are useful to distinguish species, which are morphologically similar, such as *C. uhleri* and *Ch. sayi*. *Ch. sayi* was previously believed to damage wheat crops in Canada, but it was later determined that the species responsible for crop damage was actually *C. uhleri* (Jacobson 1965). Closer examination of the distribution of *Ch. sayi* found it is restricted to the western part of the United States and absent in Canada (Buxton et al. 1983, Scudder and Thomas 1987). Insects can now be rapidly barcoded to confirm their identification and prevent misidentification of species. However, presence of nuclear mitochondrial pseudogenes or infection by *Wolbachia* can limit the success of DNA barcode in species identification (Whitworth et al. 2007, Song et al. 2008). We believe that our study was unaffected by these limitations as all the sequences were manually checked and we did not find any double peaks in chromatograms, indels or gaps, or stop codons in the translated sequences. In addition, species identity of the all the individuals identified by the barcode were also



Haplotype	No. (n)	Individuals
H-1	10	Ch03, Ch51, Ch57, Ch81, Ch82, Ch96, Ch99
H-2	1	JX548474.1
H-3	1	JX548473.1
H-4	1	JX548471.1
H-5	1	JX548472.1
H-6	3	HQ105391.1, HQ105392.1, HQ105390.1
H-7	7	KJ642001.1, HQ978858.1, KJ167924.1, KR032745.1, KR039634.1, KR043989.1, KR578242.1
H-8	1	KR037751.1
H-9	1	KR577823.1

Fig. 6. A haplotype network for *Chinavia hilaris* based on the barcode sequences from the current study combined with 16 individuals from GenBank. Circle size represents the number of individuals comprising each haplotype; hash marks represent the number of nucleotide base changes between haplotypes. Information about the individuals belonging to each haplotype is presented in the adjacent table.

confirmed through their morphological descriptions available in the literature.

Comparing the stink bug individuals sequenced from the central valley of California with the publicly available sequences of the same species from the United States and Canada provided a more complete picture of genetic variability within each species. The genetic divergence within collections of *Chinavia hilaris* ranged up to 4.7%. Notable was the divergence between the populations from the west coast and east coast of the United States. Individuals from British Columbia, Canada were less divergent from California individuals, whereas individuals from Georgia (United States) and Ontario (Canada) were 4.7% and 4.0% divergent from the California individuals, respectively (Fig. 5). The intraspecific genetic divergence value of 4.7% suggests the presence of cryptic species within populations (Park et al. 2011). In contrast, the genetic divergence among individuals within the other species in this study was in the range of 1%. In the case of *Nezara viridula* (L.) (Hemiptera: Pentatomidae), intraspecific divergence of >2.0% was reported from populations in India, possibly due to the distribution of this species across a wide geographic range (Tembe et al. 2014). Geographic distance between populations is one of the reasons for genetic divergence among populations.

Genetic divergence in this study among genera, congeners, and within species was comparable to results for Hemiptera in previous studies. For example, interspecific genetic divergence in this study was in the range of 11.2–15.7%, divergence of congeneric species was in the range of 4–5%, and intraspecific divergence for species other than *Chinavia hilaris* was <1% (Table 3). A previous study of 39 species in the family Anthocoridae (Hemiptera) found interspecific genetic distances ranged from 12.6 to 19.9%, with an average intraspecific genetic distance of 0.8% (Jung et al. 2011). Similarly, a study of 380 species belonging to 191 genera of true bugs found divergences of 10.67 and 19.81% for species within the same and different genera, respectively, whereas the mean intraspecific distance was 0.75%, respectively (Park et al. 2011). The sequence divergence between *C. uhleri* and *Ch. sayi* in this study was 4.6%, similar to the 5.1% found for two Hemipteran congeners, *N. viridula* (L.) and *N. antennata* Scott (Kaur and Sharma 2017), and the 4.5% genetic divergence in mitochondrial COI sequences of two mirid bugs (Hemiptera: Miridae), *Lygus hesperus* Knight and *Lygus lineolaris* (Palisot de Beauvois) (Zhou et al. 2012).

Genetic variation in insects is influenced by factors such as an insect's dispersal ability, host plant range, adaptation to habitats, and geographic distance among populations (Bush 1969, Roderick 1996, Nosil et al. 2007, Egan and Funk 2009). High genetic variation is observed between insect populations, which are geographically distant or isolated such as observed for *Chinavia hilaris* in this study (Roderick 1996, Medina et al. 2010, Barman et al. 2013, Joyce et al. 2014). For example, genetic variation of the mitochondrial COI region for the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), was lower when samples were from one state region but variation increased when samples were included from geographically distant populations including Florida (Joyce et al. 2014). Specialization on host plants can also significantly reduce the gene flow among the individuals, as documented in apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Feder et al. 1988, 1994), leading to increased genetic divergence among the populations. The higher genetic variation for *Chinavia hilaris* in this study could be due to geographic isolation among the distant populations, where gene flow between populations may be restricted. Although considered polyphagous, *Chinavia hilaris* may prefer the most abundant host plants in a region, or could be locally adapted to available host plants.

Genetic diversity within the species may translate into variation in insect behavior, response to pheromones, natural enemies or to insecticides (Monneart et al. 2006, Hartfield et al. 2010, Palacio Cortes et al. 2010). For example, the genetic variation among several populations of sugarcane borer, *D. saccharalis* (Fabricius), was found to relate to variation in the sex pheromone blends for the insect. Variation in pheromone blends from distant populations suggests that the trapping efficiency of one particular pheromone blend could be more in some regions than the others within the range of

the insect. It would be useful to determine whether the available pheromones for a pest insect species such as *Chinavia hilaris* are effective across the geographic range of the species. Similarly, insect populations from different geographic regions can vary in their level of susceptibility to a particular insecticide. Rice stem borer populations of *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) in China varied in response to the insecticide Flubendiamide (Wu et al. 2014); similarly, the toxicity of *Bacillus thuringiensis* to *Spodoptera frugiperda* (J. E. Smith) populations varied for insect populations from different countries in Latin America (Monneart et al. 2006). Pheromones have been characterized for *Chinavia hilaris*, *C. uhleri*, *Ch. sayi*, and *T. pallidovirens*. The higher genetic diversity observed in *Chinavia hilaris* (~5%) from the west coast of the United States and the east coast of the United States and Canada suggests that geographically distant populations of this species could have different responses to available pheromones. In contrast, the lower genetic diversity in *C. uhleri* populations might suggest that populations would have similar responses to a pheromone blend or other control methods. Laboratory or field experiments would be needed to evaluate the efficiency of existing pheromone lures against genetically diverse stink bug populations to further substantiate this idea.

This study found that stink bug species grouped into distinct phylogenetic clades using DNA barcode sequences. Genetic variability was higher within *Chinavia hilaris* than within *C. uhleri*. Accurate identification of a species is essential for effective pest management. Consideration of the genetic variability within a species and the variation among regional populations could improve the use of pest management tools, such as pheromones and biological control. Comprehensive sampling and barcoding of stink bugs through the range of each species and across diverse habitats to uncover genetic diversity within and between populations could benefit pest management programs.

Data Availability

Ch03 (MF679607), Ch51 (MF679608), Ch57 (MF679609), Ch61 (MF679610), Ch67 (MF679611), Ch81 (MF679612), Ch82 (MF679613), Ch83 (MF679614), Ch96 (MF679615), Ch99 (MF679616), Cs305 (MF679617), Cs341 (MF679618), Cs351 (MF679619), Cs352 (MF679620), Cs353 (MF679621), Cs354 (MF679622), Cs355 (MF679623), Cs356 (MF679624), Cs357 (MF679625), Cs358 (MF679626), Cu126 (MF679627), Cu140 (MF679628), Cu141 (MF679629), Cu144 (MF679630), Cu145 (MF679631), Cu147 (MF679632), Cu149 (MF679633), Cu152 (MF679634), Cu154 (MF679635), Cu158 (MF679636), Cu159 (MF679637), Cu160 (MF679638), Cu176 (MF679639), Cu177 (MF679640), Cu180 (MF679641), Cu303 (MF679642), Cu307 (MF679643), Cu342 (MF679644), Cu343 (MF679645), Tp197 (MF679646)

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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