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Mitochondrial DNA variation among populations of the glassy-winged sharpshooter, *Homalodisca coagulata*

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Abstract

The glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae), is a highly polyphagous insect species that is distributed throughout most of the southern regions of the United States. In the last 10 years, *H. coagualta* has become established in California and represents a significant threat to the state's $35 billion wine and table grape industries. DNA sequencing analysis was used to characterize a portion of the mitochondrial cytochrome oxidase I gene from a single population of the smoke tree sharpshooter, *Homalodisca liturata,* in California and from 20 natural populations of *H. coagulata* distributed in Tahiti, California, Texas, Louisiana, Mississippi, Alabama, and Florida. The results indicate that *H. liturata* and *H. coagulata* are genetically distinct, suggesting that they do not hybridize. Populations of *H. coagulata* are geographically structured into two groups of haplotypes; a group of populations from east of the Mississippi River including Louisiana, Mississippi, Alabama and Florida and a group comprised of populations west of the Mississippi River from Texas and California, and from Tahiti. There was no genetic structure among haplotypes within the eastern and western groups, respectively. The data also indicates that *H. coagulata* in California most likely originated from a source in Texas and not from any of the populations east of the Mississippi River.

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Introduction

An invasive pest species of agricultural importance has wide economic impact because of decreased yield, and costs associated with implementing pest control strategies. In the last 10 years, the glassy-winged sharpshooter, *Homalodisca coagulata*, has become established in California and represents one of the most costly invasive species threatening California agriculture due to the insect's wide host range and ability to vector *Xylella fastidiosa*, the pathogenic bacterium that causes Pierce's disease in grapes (Sorensen and Gill, 1996; Redak et al., 2004). *H. coagulata* is native to south/southeastern United States with a range extending from Texas, east to Florida (Turner and Pollard, 1959). Of the 19 described species of *Homalodisca*, only *H. coagulata* and the native smoke tree sharpshooter, *Homalodisca liturata* (formerly *H. lacerta*, see Burks and Redak, 2003), are currently established in California (Young, 1958, 1968). The presence of *H. liturata* most likely delayed the initial recognition of *H. coagulata* in California, as the two species are morphologically similar and are sympatric throughout a portion of their ranges. Adult *H. coagulata* can be distinguished from adult *H. liturata* by a series of white spots (versus white sinuous marks in *H. liturata*) on the dorsal aspect of the thorax. In contrast, immature stages of both species are much more difficult to distinguish.

Various molecular genetic techniques (e.g., PCR-RFLP, AFLPs, microsatellites and DNA sequencing) have been successfully utilized to study the population genetics of invasive species (e.g., Gasparich et al., 1997; Bohonak et al., 2001). Population genetic studies of an invasive species can have important implications for its management. For example, the extent of within-population variation may be important with respect to the uniformity of response of a pest population to a particular pest management tactic, as well as a major element in the long-term evolutionary response of a pest population to that tactic. Furthermore, the success of a biological control effort against a pest species may benefit considerably through knowledge of geographic origins. In this regard, if genetic analysis can shed some light on the geographic origin of an invasive species, then biological control workers can concentrate their search efforts for suitable natural enemies in the same geographic area.

de León et al. (2004) were the first to investigate genetic variation in *H. coagulata* populations using inter-simple sequence repeat (ISSR) analysis. ISSRs are a class of anonymous multilocus dominant markers that are scattered throughout the genome and are very useful for assessing whole genome genetic variation. de León et al. (2004) showed that there may have been more than one founding event in California and that at least a subset of the California population may have originated from Texas. Comparative DNA sequence analysis, although more costly than ISSR analysis, remains one of the most powerful, and widely used, tools for inferring phylogenetic relationships among taxa, uncovering phylogeographic patterns and examining genetic structure among populations, provided that suitable variation exists in the marker employed (e.g., Smith et al., 2002, 2003; Mun et al., 2003).

In multilocus studies, such as ISSR analysis, there is usually a trade-off between practicality and accuracy/repeatability (Sunnucks, 2000). DNA sequence analysis facilitates direct comparison of data among different/future studies, whereas there is limited ability to compare data among studies that utilize multilocus markers. In other words, the data generated by multilocus techniques allow only superficial comparisons between different studies, as pairwise data comparisons have meaning that is only relative to the study at hand. The data generated via DNA sequence analysis, on the other hand, have applicability to future studies as the sequences can be directly compared and are readily accessible to other workers via GenBank. Establishment of a mitochondrial DNA haplotype database for *H. coagulata* will facilitate the monitoring of genetic changes that may occur in California populations over time. Thus, in the present study, DNA sequence data from the mitochondrial cytochome oxidase I (COI) gene was used to examine the level of genetic variability between *H. liturata* and *H. coagulata* and among natural populations of *H. coagulata*. The data were also used to test for the existence of genetically distinct populations and to identify the geographic source for *H. coagulata* in California.

Materials and Methods

A list of samples analyzed in this study is presented in Table 1. DNA was extracted from thoracic muscle using the DNAeasy Tissue Kit (Qiagen, www.qiagen.com) according to the manufacturer's

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instructions. Voucher specimens and/or genomic extracts are located in the Insect Genetics Laboratory, Department of Biology, California State University, Bakersfield. The mitochondrial cytochrome oxidase I primers used in this investigation were; (forward, SSF: 5'-CACATATTATTACTCAGG-3'), (reverse, SSR: 5'-GAGGGAAAAATGTTAAGTT-3'). These primers were designed from a single *H. coagulata* sequence that was amplified using C1-J-2183 and TL2-N-3014 from Simon et al. (1994). SSF and SSR were used to amplify a ~500 bp fragment from 57 individuals representing 20 populations (Table 1). The smoke tree sharpshooter, *H. liturata*, was used as an outgroup for two reasons: (1) it is the only other known *Homalodisca* species present in California and (2) since *H. liturata* and *H. coagulata* are morphologically very similar it was of interest to examine the genetic variation between the two species as it is not known if *H. liturata* and *H. coagulata* are able to hybridize in the field (B. Stone-Smith, pers. comm.).

Table 1. List of samples analyzed along with their mitochondrial cytochrome oxidase I haplotype designation(s).

Species	Locality	N	Haplotype
Homalodisca liturata	Riverside, CA	3	
H. coagulata	Tahiti, FP	3	A
H. coagulata	Bakersfield, CA	2	B, C
H. coagulata	Ventura, CA	1	A
H. coagulata	Mentone, CA	3	B, D
H. coagulata	Temecula, CA	2	B
H. coagulata	Riverside, CA	3	B, C
H. coagulata	Indio, CA	2	C
H. coagulata	College Station, TX	3	F, G
H. coagulata	Tow, TX	3	A, E
H. coagulata	Bexar Co., TX	3	B, C
H. coagulata	Springtown, TX	3	A
H. coagulata	Weslaco, TX	3	B
H. coagulata	Pharr, TX	3	B
H. coagulata	Pearl River, LA	3	H, I
H. coagulata	Baton Rouge, LA	3	H, J
H. coagulata	Martinville, MS	2	H
H. coagulata	Dothan, AL	3	H, L, M
H. coagulata	Quincy, FL	3	M, N
H. coagulata	Crestview, FL	3	J, K
H. coagulata	Tallahassee, FL	3	M, N

Polymerase chain reaction (PCR) amplifications were performed in 50µl volume as described by Kambhampati et al. (1992). The temperature profile for the amplification of the COI gene fragment included an initial denaturation step of 95° C for 3 min followed by 40 cycles of 95° C for 30 sec, 48° C for 45 sec and 72° C for 45 sec. PCR products were purified using the Wizard PCR-Preps DNA Purification System (Promega, www.promega.com). Sequencing was conducted on an ABI 377 automated sequencer (DNA Sequencing Core Facility, Univ. of Florida), using the d-Rhodamine Dye Cycle Sequencing Ready Reaction Kit FS (Perkin-Elmer, www.perkinelmer.com) according to the manufacturer's specifications. Both strands of the PCR product were sequenced for all samples. CO1 sequences are available from GenBank under accession numbers DQ168883-DQ168916.

DNA sequences were read from electropherograms into a computer using the Sequence Navigator program and aligned manually. DNA sequences were analyzed using parsimony and neighbor-joining (NJ) analysis (Saitou and Nei, 1987) based on the Tajima-Nei distance (Tajima and Nei, 1984) in PAUP* version 4.0b10 (Swofford, 2001). Parsimony analysis was conducted using the multiple equally parsimonious heuristic search option with tree bisection-reconnection and 100 random addition sequence replicates. Support for specific nodes on the parsimony and NJ tree was estimated by bootstrap analysis (Felsenstein, 1985). The sequence alignment file is available from the author.

Results and Discussion

The size of the amplified mitochondrial cytochrome oxidase I (COI) gene fragment was invariant among the included samples and the base composition was biased toward adenine and thymine, which together constituted an estimated 71% of the total. Sequence alignment was straightforward and did not necessitate the insertion of any gaps. The alignment of the COI fragment resulted in 486 characters. Of these, 35 (7.2%) characters were variable and 34 characters (6.9%) were parsimony informative. The *H. liturata* sequences were invariant and exhibited 25-29 (5.1-5.9%) base pair differences relative to all *H. coagulata* sequences, suggesting that despite strong morphological similarity, *H. liturata* and *H. coagulata* are distinct species. A comparison of the amino acid sequence revealed only a single substitution that was unique between *H. liturata* and *H. coagulata* (75th amino acid of the alignment = serine in *H. liturata* and asparagine in *H. coagulata*), thus the majority of genetic variation exhibited between the two species is neutral, but could still be useful for the development of a molecular diagnostic tool to discriminate between nymphs of *H. liturata* and *H. coagulata*. The only other amino acid difference was in the 76th position of the alignment where all *H. liturata* samples and *H. coagulata* samples from Florida, Mississippi, Alabama, Louisiana, and

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College Station, TX exhibited glycine instead of aspartic acid. Pairwise differences among the *H. coagulata* sequences was small (0-1.8%), suggesting that *H. coagulata* is most likely a single species and not a species-complex. An examination of the *H. coagulata* sequences revealed 14 unique haplotypes (A-N) (Table 1). A list of diagnostic nucleotide sites that distinguish between the 14 haplotypes is shown in Table 2.

Table 2. List of diagnostic nucleotide sites that distinguish between the 14 mitochondrial cytochrome oxidase I haplotypes (A-N).

	Nucleotide Position of Alignment						
Haplotype	**58**	**67**	**142**	**205**	**231**	**289**	**322**
A	C	G	T	A	A	T	C
B	C	G	T	A	A	T	C
C	C	A	T	A	A	T	C
D	C	G	T	A	A	T	T
E	T	G	T	A	A	T	C
F	C	G	T	A	G	T	C
G	C	G	T	A	G	T	C
H	C	G	G	A	G	G	T
I	C	A	G	A	G	G	T
J	C	A	G	A	G	G	T
K	C	G	G	G	G	G	T
L	C	G	G	A	G	G	T
M	C	G	G	A	G	G	T
N	C	A	G	A	G	G	T

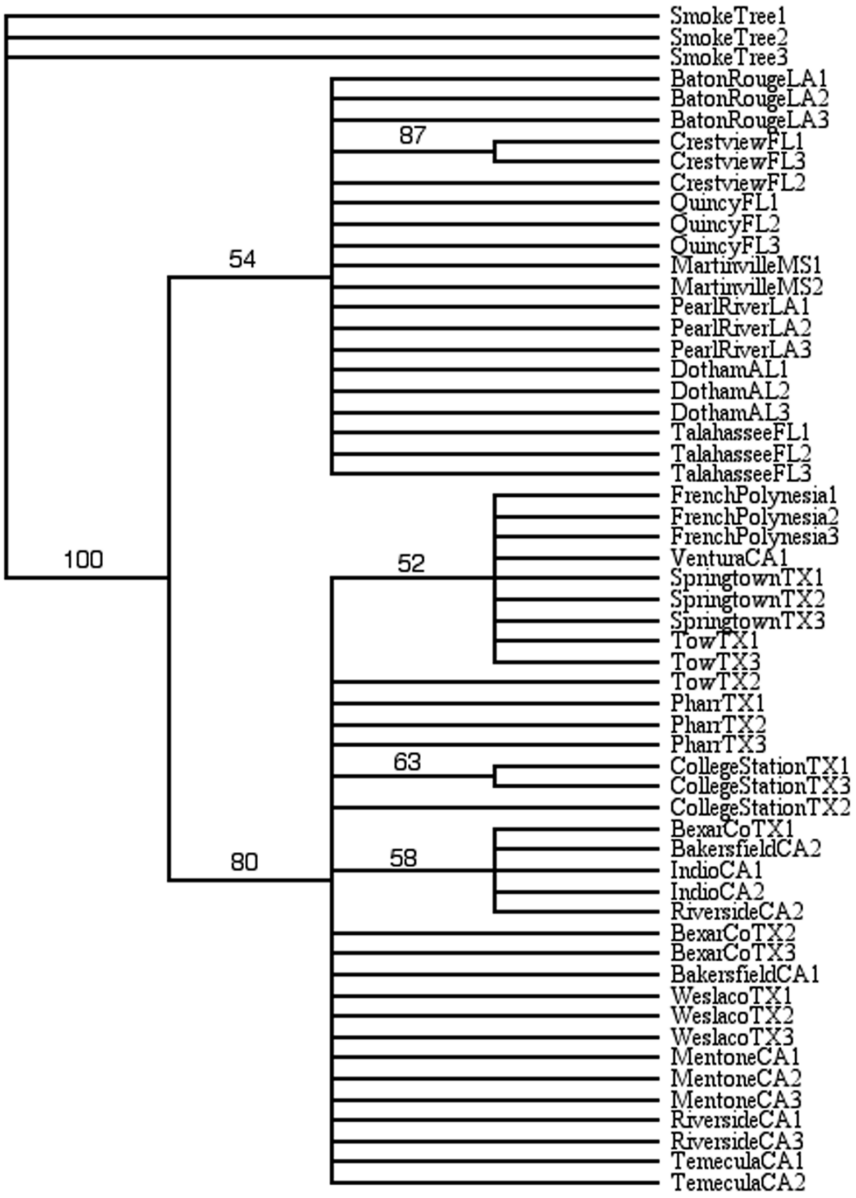
Parsimony analysis recovered 135 equally parsimonious trees (length = 41, CI = 0.83, RI = 0.96) (Figure 1). The most notable result of the phylogenetic analyses was the partitioning of haplotypes into two distinct clades that geographically correspond to groups of populations that appear to be distributed east and west of the Mississippi River (Figures 1, 2, 3). Haplotypes A-G, represented in samples from Texas, California, and Tahiti, grouped together to form a monophyletic lineage (bootstrap = 80%), whereas all of the populations east of the Mississippi River, represented by haplotypes H-N, formed a monophyletic lineage (bootstrap = 54%) (Figure 1). The topology of the neighbor-joining tree (Figure 2) was congruent with the results based on parsimony analysis. Figure 3 shows the geographic distribution of populations analyzed and their respective haplotype distribution. The presence of genetic variability, but lack of genetic structure among Texas and California samples, supports the hypothesis that *H. coagulata* has been distributed randomly by humans, most likely through nursery and/or produce shipments. Among the 7 haplotypes distributed in Tahiti, California, and Texas, one was unique to California (haplotype D; Mentone, CA), and two (F and G) were unique to Texas. *H. coagulata* is not native to Tahiti so its presence there is also likely due to human activity. There was also no haplotype structure observed among populations east of the Mississippi River.

The results of the present study (i.e., partitioning of *H. coagulata* populations into two groups) are congruent with those of de León et al. (2004). One area of interest concerns the placement of the Bakersfield/Edison samples within the western group. In the study of de León et al. (2004), the Bakersfield/Edison samples fell at the base (i.e., were the most differentiated) of the western clade. This is of interest because if there was a single founding event in California then the Bakersfield/Edison population represents that population; however, detection of *H. coagulata* in Edison/Bakersfield, CA came no less that 2-5 years after *H. coagulata* was first detected in California (L. Wendel, personal communication). Thus, it is certainly possible that there may have been more than one founding event in California as suggested by de León et al. (2004). Nursery and produce shipments would facilitate multiple introductions of *H. coagulata* into California. Data from the present study were unable to identify which population analyzed, if any, constitutes the founding population, there simply was not enough genetic variation exhibited in the mitochondrial cytochome oxidase I gene to shed light on this one issue.

H. coagulata is native to the southeastern United States from Texas to Florida (Turner and Pollard, 1959). However, the results of the present study suggest that the 'native' populations in Texas are separate and distinct from the 'native' populations that exist from Louisiana to Florida. Several scenarios may explain the current distribution of COI haplotypes in the U.S. First, it appears that *H. coagulata* populations east and west of the Mississippi River may be reproductively isolated, and the Mississippi River may be a geographic barrier for this species. More intensive sampling in and around Louisiana is required to test this hypothesis. Second, it is possible that *H. coagulata* populations in Texas originated from a source east of Texas that was followed by reproductive isolation and subsequent molecular evolution as there was no geographic overlap of mitochondrial haplotypes between the eastern and western groups. Third, it is possible that *H. coagulata* in Texas represents a separate and distinct colonization event from an unknown geographic source. Mexico would be a

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Figure 1. Strict consensus tree based on parsimony analysis of 57 mitochondrial cytochrome oxidase I sequences representing 20 *Homalodisca coagulata* populations. Numbers above branches are bootstap values (%).

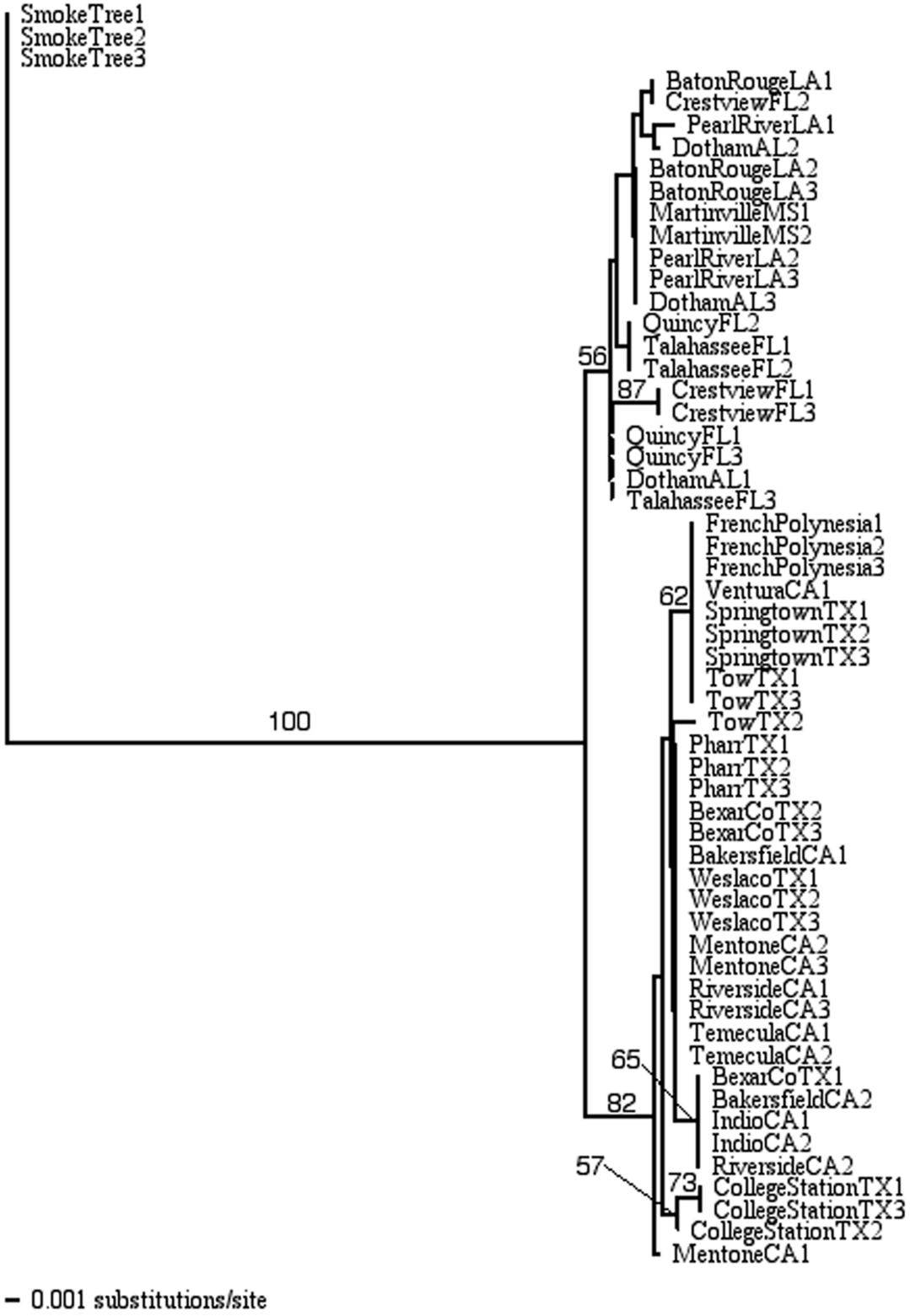


likely candidate based on the proximity of the border and various ports of entry. However, specimens from multiple populations within Mexico are needed to test this hypothesis and were unavailable for the present study. Finally, the data strongly suggest that *H. coagulata* in California did not originate from any of the populations east of Texas, but rather, most likely originated from Texas itself. This finding supports the results of de León et al. (2004) using ISSR analysis. This is important information for biological control workers as the best candidate natural enemy for *H. coagulata* in California most likely will be discovered in Texas.

The scenarios outlined above are tentative and subject to further verification. However, the utility of the mitochondrial COI gene to study the genetic structure of natural populations of *H. coagulata* has been demonstrated. Investigating the genetic variation of the mitochondrial COI gene from additional populations (including Mexico), larger sample sizes within each population, and over time

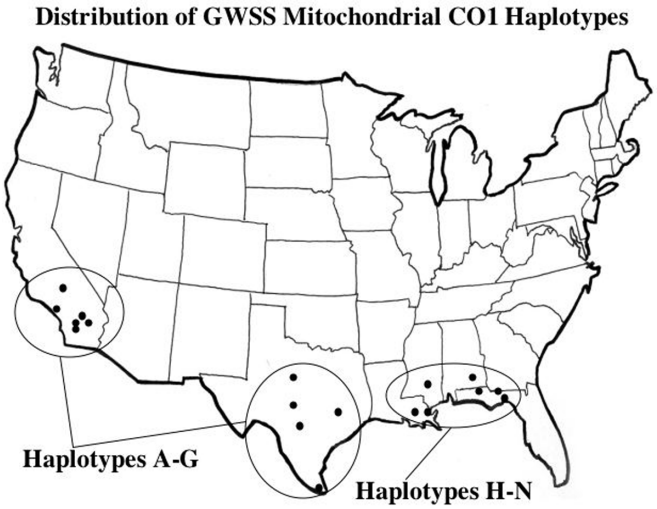
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Figure 2. Neighbor-joining tree based on the Tajima-Nei distance. Numbers above branches are bootstap values (%).



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Figure 3. Distribution of mitochondrial cytochrome oxidase I haplotypes for *Homalodisca coagulata* in the United States.



will provide valuable information regarding *H. coagulata* evolution and colonization history.

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