SEQUENCE DIVERGENCE IN TWO MITOCHONDRIAL GENES (COI AND COII) AND IN THE ITS2 RDNA FRAGMENT IN GEOGRAPHIC POPULATIONS OF *GONATOCERUS MORRILLI*, A PRIMARY EGG PARASITIOD OF THE GLASSY-WINGED SHARPSHOOTER

Project Leader:
Jesse H. de León
USDA, ARS
BIRU
Weslaco, TX 78596

Cooperators:
Walker A. Jones
USDA, ARS
BIRU
Weslaco, TX 78596

David J. W. Morgan
CDFA
Mount Rubidoux Field Station
Riverside, CA 92501

Russell F. Mizell, III
University of Florida
Quincy, FL 32351

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ABSTRACT

The aim of the present study was to resolve the genetic relationships of geographic populations of *Gonatocerus morrilli*, a primary egg parasitoid of the Glassy-winged Sharpshooter. A phylogenetic approach was implemented by sequencing two mitochondrial genes (COI and COII) and the Internal Transcribed Spacer-2 (ITS2) region of several individuals per population. Two populations from Weslaco, TX (WTX) (collected at different times), one from Quincy, FL (QFL), two from California (CA) (Orange and San Diego counties), and an outgroup (*G. ashmeadi*) were analyzed. For all three sequence fragments, percentage sequence divergence (%D) (as measured by genetic distance), the results demonstrated that both the WTX and QFL populations were closely related; in constrast, the %D between WTX and CA fell within the range of the outgroup, *G. ashmeadi*. For all three sequence fragments, Nieghbor-Joining distance trees separated the CA and WTX and QFL populations into two distinctive clades (A and B). The topology of the clades in each case was supported by very strong bootstrap values, 100% in the three sequence fragments (COI, COII, and ITS2). The present molecular phylogenetics results provide strong evidence that *G. morrilli* from California may be a different species. The findings of the present study are important to the Glassy-winged Sharpshooter/Pierce's disease biological control program in California.

INTRODUCTION

Gonatocerus morrilli (Howard) (Hymenoptera: Mymaridae) is an egg parasitoid of *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), the Glassy-winged Sharpshooter (Turner and Pollard 1959; Triapitsyn *et al*. 1998). This primary egg parasitoid species is common in the southern United States and Mexico (Huber 1988). A biological control program is currently in progress in California against *H. coagulata*, a xylem feeding leafhopper, which is a serious economic pest that transmits a strain of *Xylella fastidiosa* (Wells), a bacterium that causes Pierce's disease in grapevines (*Vitis vinifera* L. and *V. labrusca* L.) (Hopkins and Mollenhauer 1973). Accurate identification of natural enemies is critical to the success of classical biological control programs. Lack of proper identification procedures has affected the early stages of several projects (Messing and Aliniazee 1988; Löhr *et al*. 1990).

OBJECTIVES

Determine the phylogenetic relationships of geographic populations of *G. morrilli* by sequencing two mitochondrial genes (COI and COII) and one rDNA spacer region (ITS2).

RESULTS AND CONCLUSIONS

Sequence divergence in the mitochondrial COI gene in G. morrilli geographic populations. Levels of genetic divergence in the mtCOI gene among populations were determined by calculating the pairwise estimates for genetic distance. Recently, we determined that populations of *G. morrilli* from California and Texas shared no ISSR-PCR banding patterns, indicating that these populations were reproductively isolated. In addition, we demonstrated that the ITS2 rDNA fragments varied in size between these geographic populations (de León *et al*. 2004). The percentage sequence divergence (%D) for mtCOI is shown on Table 1. In general, the intra-populational variation (0.0-0.6%) was small within each population and species, with the exception of the Quincy, FL population (QFL) (2.0-4.8%). The %D between Weslaco, TX (WTX) and QFL is 0.0-4.8%, which falls within the intra-populational variation of these populations; these results indicate that these geographic populations are genetically similar. In constrast, the %D of WTX and CA is 5.4-5.6%, falling within the range (5.4-6.9%) of the outgroup (*G. ashmeadi*). The Nieghbor-Joining distance tree in Fig. 1 demonstrates that the CA and WTX and QFL populations cluster into two distinctive clades (A and B). These clades are supported by very strong bootstrap values (100%).

Sequence divergence in the mitochondrial COII gene in G. morrilli geographic populations. The percentage sequence divergence (%D) for mtCOII is shown on Table 2. Intra-populational variation is seen in both the WTX (0.0-4.5%) and QFL (0.0-3.2%) populations. The %D between WTX and QFL is 0.3-4.7%, these values fall within the intra-populational variation range and therefore these populations would be considered closely related. On the other hand, the %D between WTX and CA is 7.4-11.1%, these values fall within the range (7.4-11.5%) of the outgroup (*G. ashmeadi*). The Nieghbor-Joining distance tree in Fig. 2 demonstrates that the CA and WTX and QFL populations cluster into two distinctive clades (A and B). These clades are supported by very strong bootstrap values (100%).

Sequence divergence in ITS rDNA fragment in G. morrilli geographic populations. The percentage sequence divergence (%D) for ITS2 is shown on Table 3. The %D between WTX and QFL is 0.0-1.40%, this falls within the intra-populational range of both populations and therefore shows that these populations are closely related. In contrast, the %D between WTX and CA is 6.2-10.7%, falling within the range (7.9-13.3%) of the outgroup (*G. ashmeadi*). The Nieghbor-Joining distance tree in Fig. 3 demonstrates that the CA and WTX and QFL populations cluster into two distinctive clades (A and B). These clades are supported by very strong bootstrap values (100%).

Table 1 (COI) and Table 2 (COII). Pairwise sequence distances (range) of mitochondrial COI and II genes from geographic populations of *G. morrili* showing percentage divergence. The alignment program ClutstalW (Thomas *et al.* 1994) from DNAStar was utilized for these analyses. To account for intra- and inter-populational variation, several individuals (3-6) were included. WTX, Weslaco, TX (two populations from Hidalgo Co; 5-6 total individuals); QFL, Quincy, FL (3 individuals); CA, California (two populations, Orange Co. and San Diego Co.; 6 total individuals); Ga, *G. ashmeadi* (outgroup) (3 individuals).

Table 1. COI.

Pop	WTX	QFL	CA	Ga
WTX	0.0-0.6			
QFL	*0.0-4.8*	*2.0-4.8*		
CA	*5.4-5.6*	*5.4-8.6*	*0.0-0.2*	
Ga	5.4-6.9	5.4-10.8	6.7-7.1	0.0-0.2

Table 2. COII.

Pop	WTX	QFL	CA	Ga
WTX	0.3-4.50			
QFL	*0.3-4.70*	*0.2-0.6*		
CA	*7.4-11.1*	*7.6-8.9*	*0.0-3.2*	
Ga	7.4-10.5	7.1-7.8	6.9-8.0	0.0-0.2

Figure 1. OI

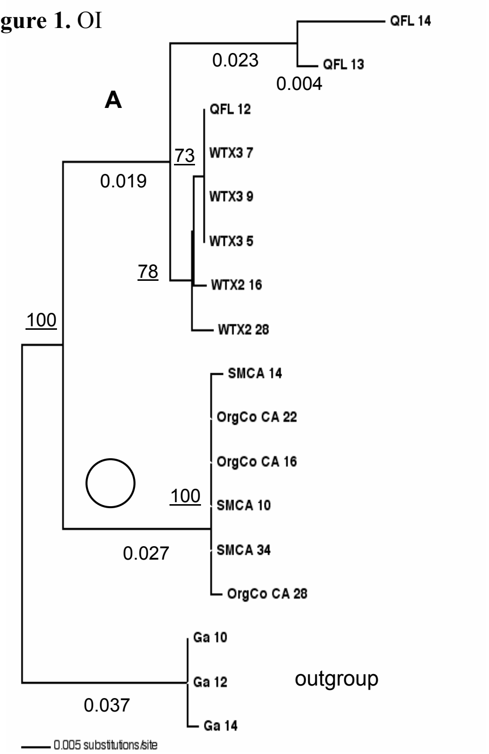

Figure 2 COII

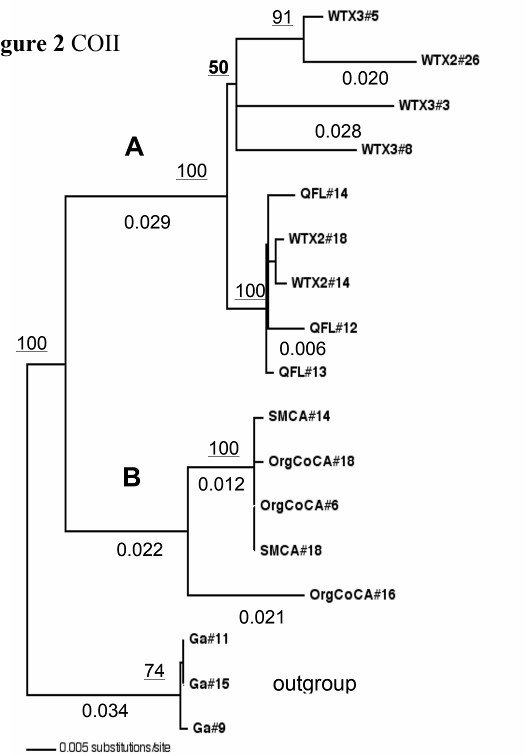

Figure 1 (COI) and Figure 2 (COII). Phenograms of mitochondrial COI and COII genes from geographic populations of *G. morrilli*. Analyses were performed with the alignment program ClustalX (Thompson *et. al.* 1997) and the Nieghbor-Joining trees were created with the phylogenetic program PAUP 4.0 (Swofford 2002). In the genetic distance trees *G. ashmeadi* are included as an outgroup, displaying branch lengths (below branches) and bootstrap values (above branches underlined), as percentage of 1000 replications. To account for intra- and inter-populational variation, several randomly chosen individuals (3-6) were included. SMCA, San Marcos, CA; OrgCo CA; Orange county, California.

Table 3. Pairwise sequence distances (range) of ITS-2 rDNA fragments from geographic populations of *G. morrilli* showing percentage divergence. The alignment program ClutstalW (Thomas *et al.* 1994) from DNAStar was utilized for this analysis. To account for intra- and inter-populational variation, several individuals (2-7) were included. WTX, Weslaco, TX (two populations from Hidalgo Co; 7 total individuals); QFL, Quincy, Florida (2 individuals); CA, California (two populations, Orange Co. and San Diego Co; 5 total individuals) Ga, *G. ashmeadi* (outgroup) (4 individuals).

Pop	WTX	QFL	CA	Ga
WTX	0.0-1.70			
QFL	0.0-1.40	0.0-0.30		
CA	6.2-10.7	6.3-7.70	0.0-0.60	
Ga	7.9-13.3	8.4-12.4	7.8-12.0	0.5-0.9

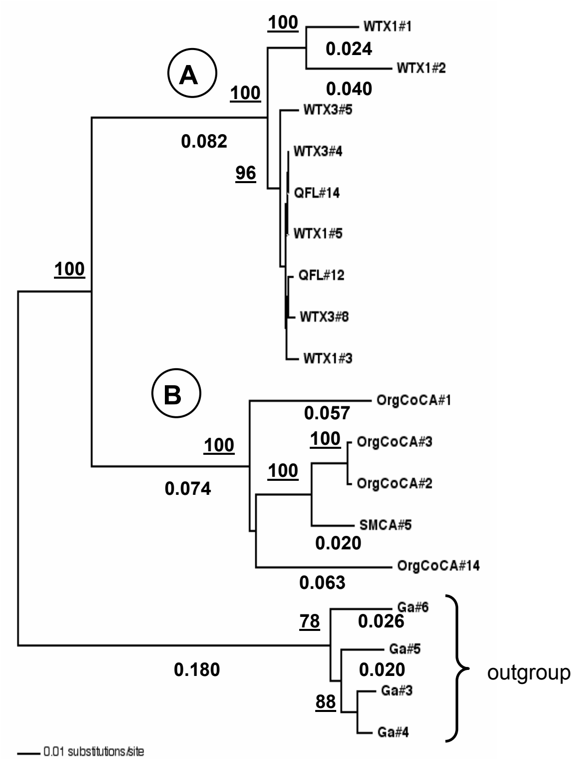

Figure 3. Phenogram of ITS2 rDNA sequence fragment from geographic populations of *G. morrilli*. Analysis was performed with the alignment program ClustalX (Thompson *et. al.* 1997) and the Nieghbor-Joining tree was created with the phylogenetic program PAUP 4.0 (Swofford 2002). In the genetic distance trees *G. ashmeadi* are included as an outgroup, displaying branch lengths (below branches) and bootstrap values (above branches underlined), as percentage of 1000 replications. To account for intra- and inter-populational variation, several randomly chosen individuals (2-7) were included. SMCA, San Marcos, CA; OrgCo CA; Orange county, California.

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