EXTENSIVE SEQUENCE DIVERGENCE IN THE ITS2 RDNA FRAGMENT IN A POPULATION OF *GONATOCERUS ASHMEADI* FROM FLORIDA: PHYLOGENETIC RELATIONSHIPS OF *GONATOCERUS* SPECIES

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ABSTRACT

The aim of the present study was to resolve the genetic relationships of geographic populations of *Gonatocerus ashmeadi*, a primary egg parasitoid of the glassy-winged sharpshooter. A phylogenetic approach was implemented by sequencing the Internal Transcribed Spacer-2 (ITS2) region of several individuals per population. In addition, the phylogenetic relationships of several *Gonatocerus* species were also determined. Six geographic populations of *G. ashmeadi* were analyszed: Quincy, FL (QFL), two populations from Weslaco, TX (WTXa and WTXb), Louisiana (LA), San Antonio, TX (SATX), and California (CA). The percentage divergence (%D) of the ITS2 sequences, as measured by genetic distance, was small among LA, SATX, and CA (0.10-1.10%); whereas, the %D for QFL vs these populations was extremely high (65.9-69.8%). A Nieghbor-Joining distance tree separated the QFL population into a separate clade supported by very high bootstrap values (100%). When the Weslaco populations were included in the anaylsis, they clustered into two distinctive clades, WTXb clustered with QFL and WTXa clusterd with the rest of the populations; again very high bootstrap values (100%) supported the topology of the distance tree. These results indicate the present of sympatric strains in Weslaco. The phylogenetic analysis of several *Gonatocerus* species clustered the respective species into North and South American clades. The %D of the QFL population fell within the range (75.4-87.2%) of the South American *Gonatocerus* species and clustered within the South American clade. The present molecular phylogenetics results provide strong evidence that *G. ashmeadi* from Florida may be a different species. In addition, the data is suggestive that the origin of *G. ashmeadi* in California is the Texas region, including the closely located Louisiana. The findings of the present study are important to the Glassy-winged Sharpshooter/Pierce's Disease biological control program in California.

INTRODUCTION

Gonatocerus ashmeadi (Girault) (Hymenoptera: Mymaridae) is a primary egg parasitoid of *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), the Glassy-winged Sharpshooter (Huber 1998). A biological control program is currently in progress in California against *H. coagulata* because this xylem feeding leafhopper is a serious economic pest that vectors a strain of *Xylella fastidiosa* (Wells), a bacterium that causes Pierce's Disease in grapevines. 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). The Internal Transcribed Spacer regions (ITS-1 and –2) have been used extensively to examine the taxonomic status of species and for diagnostic purposes, and success with this approach has been reviewed by Collins and Paskewitz (1996).

OBJECTIVES

1. Determine the phylogenetic relationships of geographic populations of *G. ashmeadi*.
2. Determine the phylogenetic relationships of several *Gonatocerus* species, including candidate species from South America (Argentina).

RESULTS AND CONCLUSIONS

Genetic Relatedness Among Geographic Populations Of G. ashmeadi

Levels of genetic divergence in the ITS2 rDNA fragment among populations were determined by calculating the pairwise estimates for genetic distance (Table 1). Recently, we determined by ISSR-PCR DNA fingerprinting that *G. ashmeadi* geographic populations were highly differentiated (de León and Jones 2004). The data demonstrated that the Quincy, FL (QFL) population had the highest gene diversity value. In addition, the data indicated that two Welsaco, TX populations collected at different times of the year were divergence or differentiated from each other and gave a first clue as to the presence of sympatric strains in Weslaco. As seen on Table 1, the sequence percentage divergence (%D) between the QFL population and the rest of the *G. ashmeadi* geographic populations (LA, SATX, WTXa, and CA) was extremely high, ranging from 65.9 to 69.8%. The %D between QFL and the outgroup population (*G. morrilli*) ranged from 77.8-81.2%, whereas LA, SATX, WTXa, and CA ranged from 31.4 to 37.0% compared to the outgroup. The %D among LA, SATX, WTXa,,and CA populations was extremely low, 0.10 to 1.10%, indicating the very close genetic similarity among these geographic populations. This range is within the intra-populational variation found within each of these populations. A phylogenetic anaylsis (Fig. 1A) demonstrated that the QFL and the LA, SATX, WTXa, and CA populations formed two distinct clades supported by extremely high bootstrap support values; in most case they were at 100%. Our second goal was to confirm whether sympatric strains of *G. ashmeadi* indeed existed in Weslaco. Table 1 shows that the %D between QLF

and WTXb is very low (0.00-0.40%) and falls within the range of the inra-populational variation. In contrast, the %D between WTXb and the rest of the populations falls within the same range that the QFL population (65.9-69.8%) fell in. The phylogenetic analysis of all populations (Fig. 1B), including the two Weslaco populations (WTXa and WTXb) demonstrated that these two populations fell on separate clades, confirming the existence of sympatric strains in Weslaco. WTXb clustered with QFL and WTXa clustered with the rest of the *G. ashmeadi* populations. Again, the distance tree is supported by extremely high bootstrap support values (100%). The very high %D values indicate that the QFL and WTXb complex diverged some time ago. The earliest record of *G. ashmeadi* in California was from 1979 (Vickerman et al. 2004) and recently, we showed that a subset of glassy-winged sharpshooters in California had their origin in central Texas (de León et al. 2004). The present results lend support to the idea that *G. ashmeadi* may have its origins in central Texas (SATX) (including the very closely located Louisiana). So it is possible that *G. ashmeadi* was transported to California along with the Glassy-winged Sharpshooter from central Texas.

Phylogenetic Relationships Among Gonatocerus Species

Resolution of relationships requires information about variability not only at the level of populations within a species but also between species (Narang et al. 1993; Unruh and Woolley 1999); therefore, a molecular systematic approach was undertaken with various *Gonatocerus* species, including candidates from South America (Argentina). For the pairwise sequence distance analyses, the *G. ashmeadi* populations (LA, SATX, WTXa, and CA) that formed one clade in fig. 1 were pooled (Ga*, Table 2) and compared to the rest of the *Gonatocerus* species. The %D values among these populations were very low (0.10-0.90%), falling within the range of the intra-specific variation seen within each individual species. The %D of *G. triguttutas* (Gt) and *G. morrilli* (Gm) vs Ga* is 15.8-17.9 and 35.0-38.9%, respectively. In contrast, the %D of *G. ashmeadi* from Florida [Ga(FL)] vs Ga* is 75.4-79.8%, these values fall within the %D range of all South American species (Table 2). This is demonstrated visually on the phenogram in Fig. 2 with very strong bootstrap values supporting the topology of the Nieghbor-Joining distance tree. As seen from the phenogram, the North and South American *Gonatocerus* species are separated into their perspective clades. It is interesting to note that Ga(FL) is more closely related to *G. metanotalis* (Gmet) (8.30-9.00%), a South American species than it is to any North America species (Fig. 2). The *Gonatocerus* species more closely related to Ga* is Gt (15.8-17.9%). The present results showing extensive sequence divergence at the ITS2 rDNA fragment in a population of *G. ashmeadi* from Florida lends strong support to the fact that these individuals may actually be another species or rather *G. ashmeadi* exists in nature as a species-complex. Our results are in contrast with those of Vickerman et al. (2004). In our studies we performed a phylogenetic analyses of the ITS2 rDNA sequences. In addition, Vickerman et al. (2004) demonstrated that populations of *G. ashmeadi* from Florida vs other geographic regions were able to hybridize. We have not yet performed these types of studies, but it may be necessary to extend these crossing studies to the F2 generation to seen a negative effect or as demonstrated by Wu et al. (2004) a negative effect was not seen until backcrosses were performed. The findings of the present study are important to the Glassy-winged Sharpshooter/Pierce's Disease biological control program in California.

Table 1. Pairwise sequence distances (range) of ITS-2 rDNA fragments from geographic populations of *G. ashmeadi* 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 (3-4) were included. QFL, Quincy, Florida; WTXb, Weslaco, TX; LA, Louisiana; SATX, San Antonio, TX; WTXa, Weslaco, TX; CA, California; Gm, *G. morrilli* (outgroup). Relate to figure 1B.

Pop	QFL	WTXb	LA	SATX	WTXa	CA	Gm
QFL	0.10-0.40						
WTXb	0.00-0.40	0.00-0.10					
LA	68.0-69.8	68.1-70.4	0.60-0.90				
SATX	68.2-69.8	67.9-70.8	0.30-0.80	0.20-0.90			
WTXa	67.1-69.5	66.6-70.1	0.20-0.70	0.20-0.90	0.10-0.90		
CA	65.9-67.6	66.0-67.9	0.80-1.00	0.60-1.10	0.30-1.00	0.20-0.80	
Gm	77.8-81.2	77.6-82.3	32.3-36.3	31.4-37.0	31.8-40.6	36.3-36.7	0.00-0.30

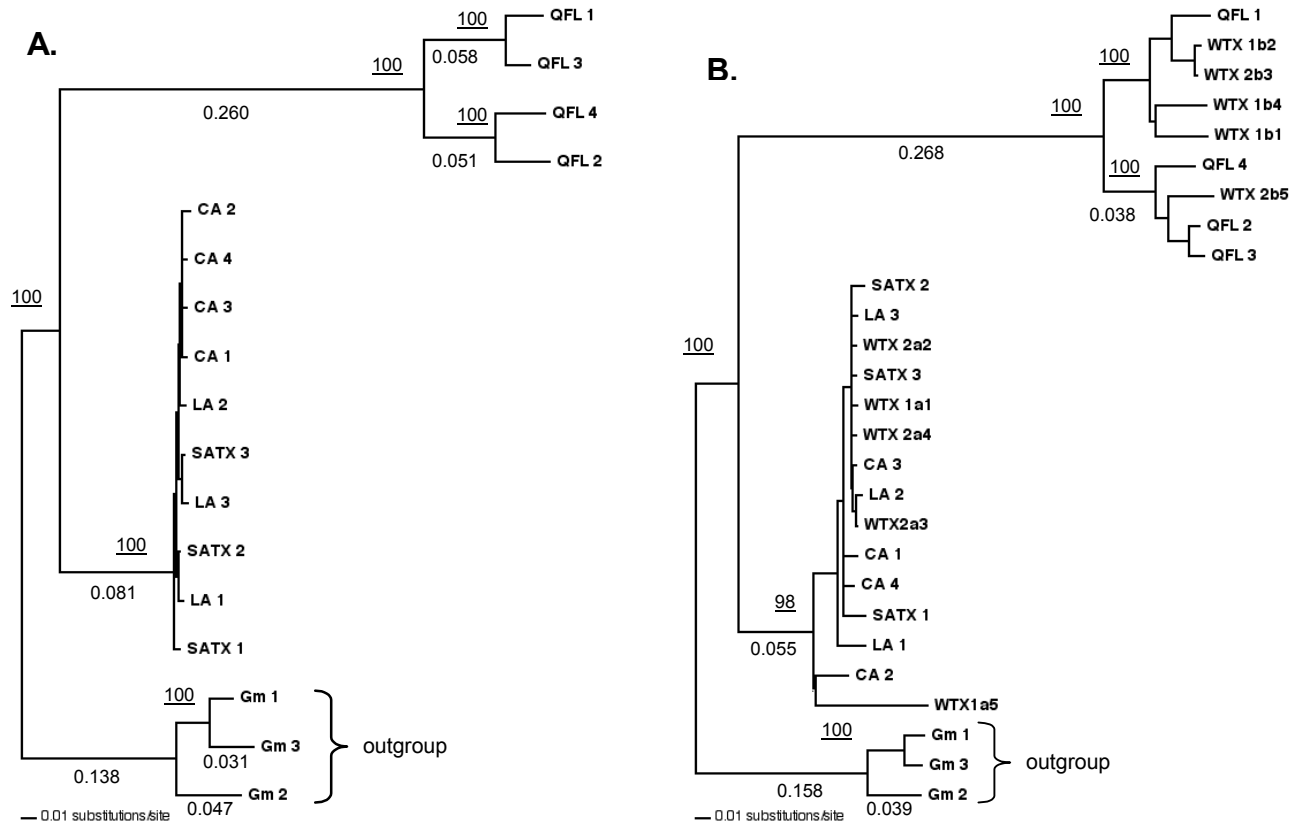

Figure 1. Phenograms of ITS2 rDNA sequence fragments from geographic populations of *G. ashmeadi*. Analyses were performed with the alignment program ClustalX (Thompson *et. al.* 1997) and the Nieghbor-Joining trees were created with the phylogenetic program PAUP 4.0 (Swofford 2002). In the genetic distance trees *G. morrilli* are included as an outgroup, displaying branch lengths (below branches) and bootstrap values (above branches underlined), as percentage of 1000 replications. Trees are presented both without Weslaco, TX populations **(A)** and with Weslaco, TX populations **(B)**. To account for intra- and inter-populational variation, several randomly chosen individuals (3-4) were included.

Table 2. Pairwise sequence distances (range) of ITS-2 rDNA fragments from *Gonatocerus* species showing percentage divergence. The alignment program ClutstalW (Thomas *et al.* 1994) from DNAStar was utilized for this analysis. To account for intra- and inter-specific variation, several individuals (2-3) were included. Ga*, *G. ashmeadi* (California, San Antonio, TX, and Louisiana were pooled for a total of 10 individuals); Gt, *G. triguttutas* (TX); Gm, *G. morrilli* (TX); and candidate South American (Argentina) species: Gann, *G. annulicornis*; nGt, near *G. triguttutas*; Gtub, *G. tuberculifermur*; Ga(FL), *G. ashmeadi* (Quincy, FL USA); Gmet, *G. metanotalis*; and Tb, *Trichogramma bourarachae* (outgroup).

G species	***Ga****	***Gt***	***Gm***	***Gann***	***nGt***	***Gtub***	***Ga(FL)***	***Gmet***	***Tb***
Ga*	0.10-0.90								
Gt	15.8-17.9	0.10-0.20							
Gm	35.0-38.9	41.7-45.5	1.80-1.80						
Gann	82.4-87.2	97.5-101	87.0-88.1	0.00-0.10					
nGt	80.0-83.5	94.8-97.3	82.7-84.2	3.40-3.60	0.10-0.10				
Gtub	*78.0-82.0*	*90.8-92.0*	*81.4-84.1*	*11.5-12.1*	*11.6-11.8*	*0.10-0.50*			
Ga(FL)	75.4-79.8	88.4-90.2	84.3-87.0	37.7-39.3	36.7-38.1	35.9-36.4	0.10-1.00		
Gmet	76.2-80.4	87.6-89.4	85.5-88.2	35.4-36.4	34.7-35.3	34.9-36.1	8.30-9.00	0.10-.040	
Tb	84.8-92.5	87.2-91.5	88.4-90.4	66.1-67.6	69.0-70.3	68.5-70.5	77.3-79.6	74.2-76.2	0.20-0.90

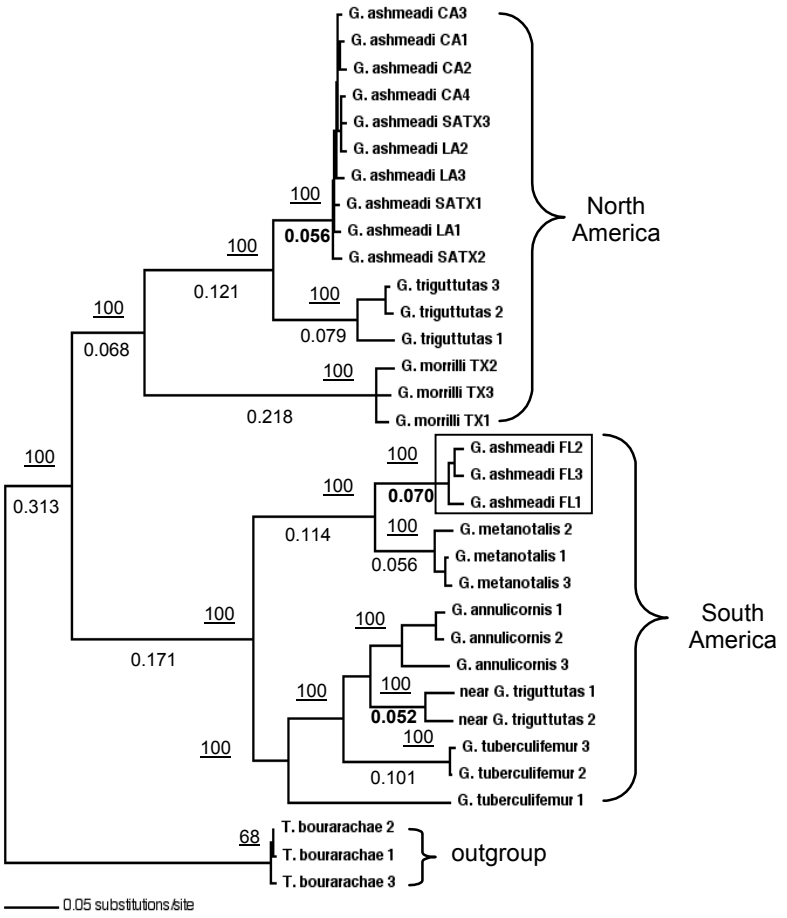

Figure 2. Phenograms of ITS2 rDNA sequence fragments from *Gonatocerus* egg parasitoid species, including candidate species from South America (Argentina). Analysis was performed with the alignment program ClustalX (Thompson *et. al.* 1997) and the Nieghbor-Joining trees were created with the phylogenetic program PAUP 4.0 (Swofford 2002). In the genetic distance trees *Trichogramma bourarachae* (1, AF043624; 2, AF043625; 3, AF043626) are included are an outgroup, displaying branch lengths (below branches) and bootstrap values (above branches underlined), as percentage of 1000 replications. To account for intra- and inter-specific variation, several randomly chosen individuals (2-4) were included.

REFERENCES

de León JH, Jones WA. 2004. Genetic differentiation among geographic populations of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), the predominant egg parasitoid of *Homalodisca coagulata* (Homoptera: Cicadellidae). Journal of Insect Science (in press).

de León JH, Jones WA, Morgan DJW. 2004. Population genetic structure of *Homalodisca coagulata* (Homoptera: Cicadellidae), the vector of the bacterium *Xylella fastidiosa* causing Pierce's disease in grapevines. *Annals of the Entomological Society of America* 97: 809-818.

Huber JT. 1998. The species groups of *Gonatocerus* Nees in North America with a revision of the *sulphuripes* an *ater* groups (Hymenoptera: Mymaridae). *Memoirs of the Entomological Society of Canada* 141: 1-109.

Löhr BA, Varela M, Santos B. 1990. Exploration for natural enemies of the cassava mealybug, *Phenococcus manihoti* (Homoptera: Pseudococcidae), in South America for the biological control of this introduced pest in Africa. *Bulletin of Entomological Research* 80: 417-425.

Messing RH, Aliniazee MT. 1988. Hybridization and host suitability of two biotypes of *Trioxys pallidus* (Hymenoptera: Aphidiidae). *Annuals of the Entomological Society of America* 81: 6-9.

Narang SK, Tabachnick WJ, Faust RM. 1993. Complexities of population genetic structure and implications for biological control programs. In: Narang SK, Barlett AC, Faust RM, editors. Applications of Genetics to Arthropods of Biological Control Significance, 19-52. CRC Press Inc, Boca Raton, Florida.

Porter CH, Collins FH. 1991. Species-diagnostic difference in a ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* (Diptera: Culicidae). *American Journal of Tropical Medicine and Hygiene* 45, 271-279.

Thompson JD, Gibson TJ, Plewniak F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876-4882.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Vickerman DB, Hoddle MS, Triapitsyn S, Stouthamer R. 2004. Species identity of geographically distinct populations of the glassy-winged sharpshooter parasitoid *Gonatocerus ashmeadi*: morphology, DNA sequences, and reproductive compatibility. Biological Control 31: 338-345.

Unruh TR, Woolley JB. 1999. Molecular Methods in Classical Biological Control. In: Van Driesche RG, Bellows TS, Jr., editors. Biological Control, 57-85. Chapman and Hall, NY.

Wu Z, Hopper KR, O'Neil RJ, Voegtlin DJ, Prokrym DR, Heimpel GE. 2004. Reproductive compatibility and genetic variation between two strains of *Aphelinus albipodus* (Hymenoptera: Aphelinidae), a parasitoid of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). Biological Control 31: 331-319.

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