Progress Report for PD Project 2009-214
March 1, 2010

I. Project title: Development of Effective Monitoring Techniques for Sharpshooters and their Parasitoids

II. Principal investigators and cooperators:

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III. List of objectives and description of activities conducted to accomplish each objective:

A method is proposed that can identify the species of host, GWSS or Smoke Tree Sharpshooter (STSS), and its parasitoids within half a day of collection rather than two weeks. A further benefit would be that old egg masses can be used after wasp eclosion as the pupal and sharpshooter egg casing can be analyzed. This method uses a multiplex high resolution melting curve analysis real-time PCR, to identify DNA fragments specific to the hosts and their parasitoids simultaneously. The specific objectives are:

1. Develop primer pairs that can be used in a multiplex high resolution melting curve analysis real-time PCR system for each species of sharpshooter and parasitoid. Several primer pairs have been identified for the *Gonatocerus* and sharpshooter species COI gene sequences. (Figures 1 and 2).
2. Clone the target genes from those species of parasitoid for which there is no sequence data available. This will be accomplished through the use of published primers or the development of degenerate primers.
3. Determine the limits of detection of each species of sharpshooter and parasitoid. Based on other studies, we are confident we will be able to detect developing parasitoid embryos in sharpshooter eggs. We hope to be able to determine the both the host and parasitoid species from sharpshooter egg cases from which the parasitoids have eclosed by amplifying the layer of cells which remain in the parasitoid egg.

IV. Summary of major research accomplishments and results for each objective:

Changes in Methodology to Accomplish Objectives. Overall, the project has changed from development of a mutliplex PCR system to development of a multiplex high resolution melting curve analysis system to allow better differentiation of closely-related parasitoid species. The methodology changes and progress are as follows:

DNA Extraction:
Genomic DNA is being extracted using the Qiagen MagAttract 96 DNA Plant Core Kit (Qiagen, Valencia, CA.) either in individual 1.5 ml microfuge tubes or in 1 ml 96 well plates (USA Scientific, Ocala, FL.) sealed with TPE Capcluster mats (Micronic, Leltstad, The Netherlands). Samples are homogenized by shaking for 6 min. on a Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK) with glass and stainless steel beads. Quantification is performed using the PicoGreen assay (Invitrogen Corporation, Carlsbad, CA.) and the DTX 880 Multimode Detector (Beckman Coulter, Brea, CA.).

Primer development and amplification:
Sets of primers for COI gene amplification have been developed (Figures 1 and 2) for high resolution melt analysis amplification using the MegAlign and Primer Select programs of the Lasergene suite of programs (DNASTAR Inc., Madison, WI.) and the Primer Express program (Applied Biosystems, Inc., Foster City, CA.). Amplification parameters are being worked out using MeltDoctor High Resolution Melt Reagents and the 7500 Fast Real-Time PCR System (Applied Biosystems, Inc., Foster City, CA.)

V. Publications or reports resulting from the project:

Cooksey, D. A. 2009. Development of Effective Monitoring Techniques for Sharpshooters and their Parasitoids. Page 72 In: Pierce's Disease Research Symposium, 2009, CDFA.

VI. Presentations on research:

Poster and oral presentations at CDFA Pierce's Disease Research Symposium, Sacramento, CA, December 9-11, 2009.

VII. Research relevance statement:

The suppression of Glassy-winged Sharpshooter (GWSS) populations is accomplished in part by biological control agents. An accurate and rapid method for identification of the eggs of sharpshooter species, determining whether eggs are parasitized, and by which parasitoid species, is essential for estimating success. Post-release monitoring is a keystone of biological control agent evaluation. The only methodology currently available involves the collection and incubation of field-collected GWSS eggs. Eggs are removed from the field before development has been completed and so are removed from the possibility of further parasitism. Parasitism rates are underestimated and significant developmental mortality occurs during the two-week incubation period needed for wasps and GWSS to develop. Optimal incubation conditions vary for each parasitoid species so certain species are underreported as they die in imperfect

incubation conditions. No economical method for identifying whether eggs are from GWSS or the native STSS is possible. A more efficient method for monitoring biological control activity is essential as it will result in more accurate, timely, and economic reporting of GWSS parasitism. Development of a single-step multiplex high resolution melting curve real-time PCR assay for sharpshooters and their parasitoids will allow for accurate reporting of GWSS occurrences and facilitate development of effective control agents. This method can identify the species of host, GWSS or Smoke Tree Sharpshooter (STSS), and its parasitoids within half a day of collection rather than two weeks. A further benefit is that old egg masses should be able to be used after wasp eclosion, as the pupal and sharpshooter egg casing can be analyzed. This method, multiplex high resolution melting curve real-time PCR, uses real-time PCR techniques to identify specific sequence differences between the parasitoid species and their hosts simultaneously.

VIII. Lay summary of current year's results:

Funding for the project was received in late Fall of 2009. Since that time, we redesigned the methodology for the project from the development of a simple mutliplex PCR system to development of a multiplex high resolution melting curve analysis system that should allow better distinction between closely-related parasitoid species. Primers were developed as shown in Figures 1 and 2 below.

IX. Status of funds:

As of 1/31/2010 (the most recent financial statement from UCR), there was a balance of $31,448.40 from the appropriated amount of $33,465.

X. Summary and status of intellectual property produced during this research project:

No intellectual property issues are anticipated.

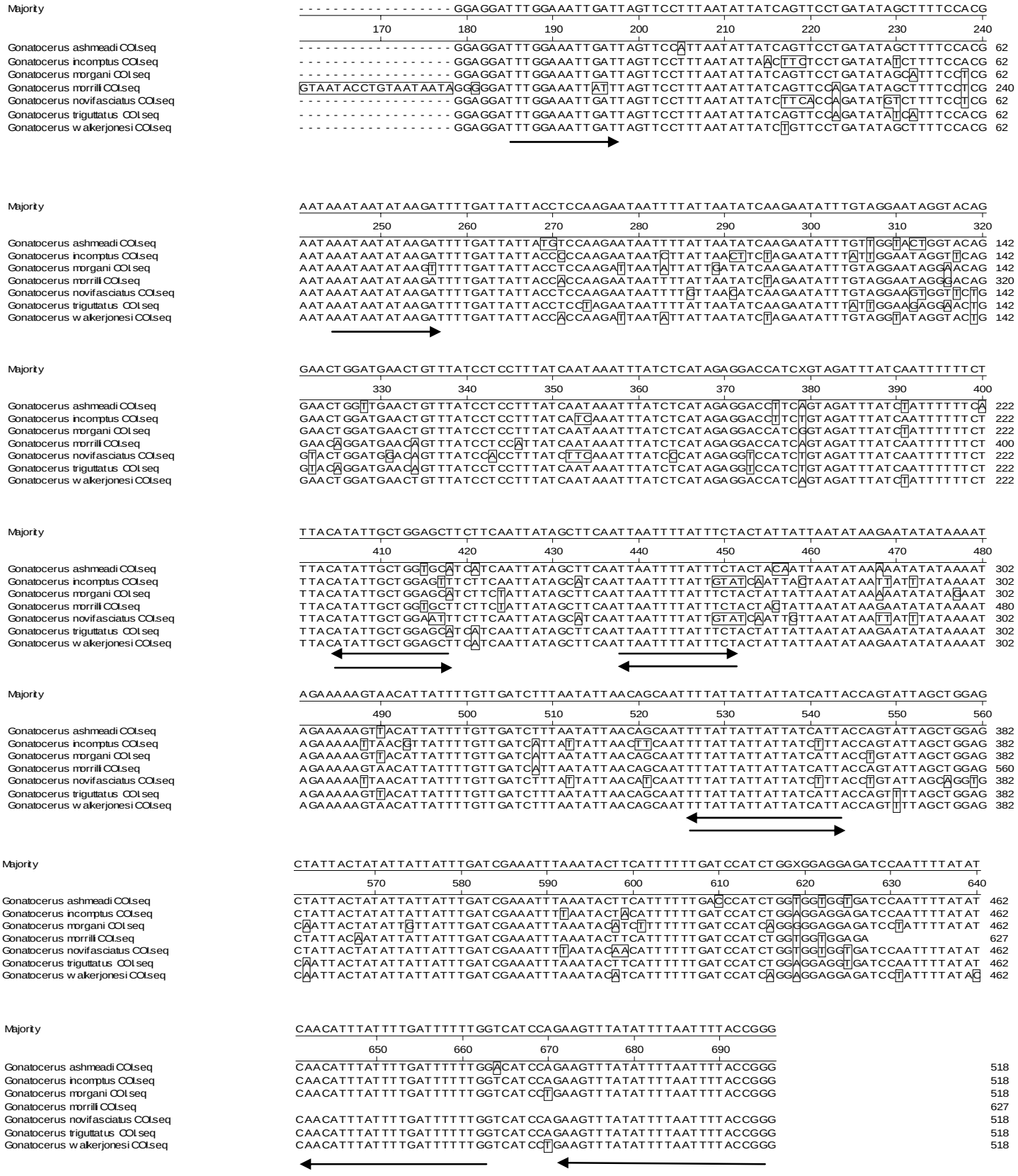

Figure 1. Alignment of all Gonatocerus COI sequences using ClustalW (Slow/Accurate, IUB) with sequence differences boxed and primers indicated by arrows:

Figure 2. Alignment of sharpshooter COI sequences using ClustalW (Slow/Accurate, IUB) with sequence differences boxed and primers indicated by arrows: