PROGRESS REPORT

on the project sponsored by the UC Pierce's Disease Research Grants Program
Submitted February 27, 2008 by Serguei Triapitsyn, PI

I. Project Title. Identify the Species of Mymaridae Reared in Argentina and Mexico for Potential Introduction to California against Glassy-winged Sharpshooter and Prepare and Submit for Publication a Pictorial, Annotated Key to the *ater*-group species of *Gonatocerus* – Egg Parasitoids of the Proconiine Sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the Neotropical Region.

II. Principal Investigator. Dr. Serguei Triapitsyn, Principal Museum Scientist, Department of Entomology, University of California, Riverside, CA 92521. Phone: 951-827-7817; E-mail: serguei.triapitsyn@ucr.edu

Cooperators. Dr. Guillermo Logarzo, USDA-ARS South American Biological Control Laboratory, Hurlingham, Buenos Aires, Argentina; E-mail: glogarzo@speedy.com.ar. Dr. John T. Huber, Canadian Forest Service, c/o Canadian National Collection of Insects, Ottawa, Ontario, K1A OC6, Canada. E-mail HUBERJH@AGR.GC.CA. Dr. Jesse H. de León, Beneficial Insects Research Unit, Kika de la Garza Subtropical Agricultural Research Center, USDA-ARS, 2413 E. Highway 83, Weslaco, Texas, 78596, USA, Email: jhleon@weslaco.ars.usda.gov. Dr. Eduardo G. Virla, CONICET-PROIMI, Avenida Belgrano y Pasaje Caseros, San Miguel de Tucumán, Argentina, E-mail: evirla@hotmail.com.

III. Objectives and Description of Activities.

Objective 1 (mostly Year 1, partly Year 2). Identification of the numerous species of *Gonatocerus* reared by USDA researchers (G. Logarzo) in Argentina, Chile, and Peru, colonies of some of which were established in the quarantine facilities in California and Texas, and also of several species reared in Mexico from eggs of *Homalodisca* and other proconiine sharpshooters.

Activities:

Types. All the available types of the *Gonatocerus* species (70 species), described from the Neotropical region, were located and examined, and digital photographs were taken from them and arranged in plates. All but one (lost) holotypes of the species described by A. A. Ogloblin from Ecuador were remounted into Canada balsam because the original mounting medium was so dark that the specimens were not visible.

Specimen preparation. Due to the enormous volume of the material of *Gonatocerus* from Argentina, Chile, and Mexico (more than 2,500 specimens have already been point-mounted in the course of this project), work on point-and slide-mounting of the specimens, which began in October 2006, will continue till the end of the project.

Specimen identification. Morphologically, we recognized at least three more unidentified species among altogether at least 18 species of *Gonatocerus* reared in Argentina by G. Logarzo from eggs of the proconiine sharpshooters. *Gonatocerus* sp. #12, previously misidentified by A. A. Ogloblin as *G. nigriflagellum* (Girault), turned out to be a new species. *Gonatocerus* sp. #1 from Argentina and a similar, yet clearly different species, reared from eggs of *Homalodisca* sp. or *Oncometopia* sp. in Veracruz, Mexico, are also new, undescribed species. *Gonatocerus* sp. #6 from Argentina was described taxonomically in the course of this study (Triapitsyn et al. 2007). Also described was a new species of *Gonatocerus* from Sonora, Mexico, an egg parasitoid of

Homalodisca liturata Ball (Triapitsyn & Bernal in press). The identities of other species were also figured out, particularly of seemingly the most promising neoclassical biological control agent, *G. tuberculifemur* (Ogloblin) [sp. #7], which turned out to be a complex of at least two different species and several molecular clades, one of which was described taxonomically as new (Triapitsyn et al. in preparation). Because *G. tuberculifemur* was originally described from a single, poorly preserved female specimen, G. Logarzo, S. Triapitsyn, and E. Virla made in February 2007 a collecting trip to its type locality in Pucará, at the shore of Lago Lácar in Neuquén Province, Argentina, where *G. tuberculifemur* was collected using sentinel eggs of *Tapajosa rubromarginata* (Signoret) on leaves of a citrus plant. The collected specimens were used to initiate a laboratory colony in Argentina (G. Logarzo) and also for molecular (J. de León) and morphological (S. Triapitsyn) analyses in the USA. The *G. tuberculifemur* complex also includes several morphological forms and molecular clades (de León et al. 2006a,b,c; 2007, 2008), such as *Gonatocerus* sp. #3 from Argentina. *Gonatocerus metanotalis* (Ogloblin) also turned out to be a complex of several molecular clades (de León et al. 2006d), which, however, were not found to differ morphologically. Altogether, more than 2,500 specimens of *Gonatocerus* from South America and Mexico were sorted to morphospecies, compared with the types of the described species, and identified, many as undescribed taxa. The newly collected specimens have been matched with the types of more than 30 described species of *Gonatocerus* from South America, and also more than 30 undescribed species have been recognized, many of which were reared from eggs of the proconiine sharpshooters and thus are of interest to this project as potential neoclassical biological control agents against the GWSS in California. At least 10 of those are being described as new taxa, mostly the species that have known host records as egg parasitoids of Proconiini.

Objective 2 (Year 2). Preparation and submission for publication of a pictorial, annotated key to the *ater* species group of *Gonatocerus*, egg parasitoids of proconiine sharpshooters in the Neotropical region, with emphasis on the species targeted for introduction into California (Year 2). **Activities:** Almost all the illustrations (about 95%) have already been prepared by the hired professional illustrator, who took high quality Automontage images of the type specimens of the available species of *Gonatocerus* from the Neotropical region and assembled them in plates. The most important morphological features of each species were illustrated, usually the female antenna, body, propodeum, and forewing as well as the male antenna, forewing, and genitalia. Additionally, scanning electron micrographs were taken from some of them to facilitate their recognition and to illustrate some key morphological features. Currently, species descriptions and re-descriptions and taxonomic keys are ready for the smaller *sulphuripes* species group (12 recognized species in the Neotropics) and the *litoralis* species group (5 recognized species in the Neotropics). In addition, several species from the *ater* species group were redescribed. It is estimated that by the end of the current fiscal year, up to 50% of the already described species will be redescribed. During the next fiscal year (as a no-cost extension of the current budget allocation), this work will be completed, about 10 new species will be described, and the manuscript (now ready about by 30%) will be submitted for publication to Zootaxa and published by 6/30/2009.

IV. Summary of Major Research Accomplishments and Results. Progress on Objective 1.
Types. Type specimens of all but one species were located, examined, and curated.

Identification. Morphologically, we recognized at least 18 species parasitizing eggs of Proconiini in Argentina, at least 5 such species (2 of them new) from Mexico, and 2 new species from Costa Rica. In addition, almost 2,500 specimens were sorted to morphospecies and another 500 to species group. 10 new species are being described.

Specimen preparation. Numerous samples were processed, about 3,000 specimens of *Gonatocerus* were dried, labeled, and point-mounted.

Preparation of the illustrations (mostly Objective 2). High quality digital photographs (later arranged in plates) were taken, using the Automontage system, of all but a few *Gonatocerus* spp. Additionally, scanning electron micrographs were taken from some of them to facilitate their recognition and to illustrate some key morphological features.

Publications and reports. The project has already resulted in 13 scientific papers and reports listed below (including 6 scientific articles in peer reviewed journals) that either have been published, submitted, or are ready to be submitted.

V. Publications and Reports Resulting from the Project.

de León, J. H., Logarzo, G. A. Triapitsyn, S. V. 2006a. Genetic characterization of *Gonatocerus tuberculifemur* from South America uncovers divergent clades: Prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California. In: Proceedings of the 2006 Pierce's Disease Research Symposium, November 27-29, 2006, The Westin Horton Plaza Hotel, San Diego, California, organized by California Department of Food and Agriculture (T. Esser, Chief Ed.). Copeland Printing, Sacramento, California, pp. 40-43.

de León, J. H., Logarzo, G. A. Triapitsyn, S. V. 2006b. Preliminary evidence from reproductive compatibility studies suggests that *Gonatocerus tuberculifemur* exists as a cryptic species complex, or a new species is identified: Development and utility of molecular diagnostic markers. In: Proceedings of the 2006 Pierce's Disease Research Symposium, pp. 44-47.

de León, J. H., Logarzo, G. A. Triapitsyn, S. V. 2006c. ISSR-PCR DNA fingerprinting uncovers distinct banding patterns in *Gonatocerus* species 3 (*G.* sp. 3) individuals emerging from different host tribes: A prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California. In: Proceedings of the 2006 Pierce's Disease Research Symposium, pp. 48-51.

de León, J. H., Logarzo, G. A. Triapitsyn, S. V. 2006d. Genetic studies of *Gonatocerus metanotalis* populations from Argentina uncover divergent clades: A prospective egg parasitoid candidate agent for the glassy-winged sharpshooter in California. In: Proceedings of the 2006 Pierce's Disease Research Symposium, pp. 52-55.

de León, J .H., Logarzo, G. A. & Triapitsyn, S. V. 2007. Progress on resolving the *Gonatocerus tuberculifemur* complex: Neither COI nor ITS2 sequence data alone can discriminate all the species within the complex, whereas, ISSR-PCR DNA fingerprinting can. *In*: *Proceedings of the 2007 Pierce's Disease Research Symposium, December 12–14, 2007, The Westin Horton Plaza Hotel, San Diego, California, organized by California Department of Food and Agriculture* (Esser, T., Chief Ed., compiled by Blincoe, P., West, D., Mochel, M. & Veling, S.). PIP Printing and Document Services, Sacramento, California, pp. 73–76. Available online at http://www.cdfa.ca.gov/pdcp/Research_Symposium_Index.html.

de León, J. H., Logarzo, G. A. & Triapitsyn, S. V. 2008. Molecular characterization of *Gonatocerus tuberculifemur* (Ogloblin) (Hymenoptera: Mymaridae), a prospective

Homalodisca vitripennis (Germar) (Hemiptera: Cicadellidae) biological control candidate from South America: Divergent clades. Bulletin of Entomological Research 98: 97-108.

Logarzo, G. A., J. H. de León, S. V. Triapitsyn, R. H. González & E. G. Virla. 2006. First report of a proconiine sharpshooter, *Anacuerna centrolinea* (Hemiptera: Cicadellidae), in Chile, with notes on its biology, host plants, and egg parasitoids. Annals of the Entomological Society of America 99 (5): 879-883.

Triapitsyn, S. V., G. A. Logarzo, E. G. Virla & J. H. de León. 2007. A new species of *Gonatocerus* (Hymenoptera: Mymaridae) from Argentina, an egg parasitoid of *Tapajosa rubromarginata* (Hemiptera: Cicadellidae). Zootaxa 1619: 61-68.

Triapitsyn, S.V. 2006. Identify the species of Mymaridae reared in Argentina and Mexico for potential introduction to California against the glassy-winged sharpshooter and prepare and submit for publication a pictorial, annotated key to the *ater*-group species of *Gonatocerus* - egg parasitoids of the proconiine sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the Neotropical region. *In*: *Proceedings of the 2006 Pierce's Disease Research Symposium, November 27-29, 2006, The Westin Horton Plaza Hotel, San Diego, California, organized by California Department of Food and Agriculture* (Esser, T., Chief Ed., compiled by Tariq, M.A., Medeiros, R., Mochel, M. & Veling, S.). Copeland Printing, Sacramento, California, pp. 111-113.

Triapitsyn, S. V. 2007. Identifying the species of Mymaridae reared in Argentina and Mexico for potential introduction to California against the glassy-winged sharpshooter and prepare and submitting for publication a pictorial, annotated key to the *ater*-group species of *Gonatocerus* – egg parasitoids of the proconiine sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the Neotropical region. *In*: *Proceedings of the 2007 Pierce's Disease Research Symposium, December 12–14, 2007, The Westin Horton Plaza Hotel, San Diego, California, organized by California Department of Food and Agriculture* (Esser, T., Chief Ed., compiled by Blincoe, P., West, D., Mochel, M. & Veling, S.). PIP Printing and Document Services, Sacramento, California, pp. 58–61. Available online at http://www.cdfa.ca.gov/pdcp/Research_Symposium_Index.html.

Triapitsyn, S. V. & J. S. Bernal. 2008. Egg parasitoids of Proconiini (Hemiptera: Cicadellidae) in northwestern Mexico, with description of a new species of *Gonatocerus* (Hymenoptera: Mymaridae). Journal of Insect Science, in press.

Triapitsyn, S. V., G. A. Logarzo, J. H. De León & E. G. Virla. A new *Gonatocerus* (Hymenoptera: Mymaridae) from Argentina, with taxonomic notes and molecular data on the *G. tuberculifemur* species complex. Ms almost ready, to be submitted to Zootaxa in April 2008 (40 ms pages, 29 figures, 3 tables).

Virla, E. G., Logarzo, G. A., Paradell, S. L. & Triapitsyn, S. V. 2008. Bionomics of *Oncometopia tucumana* (Hemiptera: Cicadellidae), a sharpshooter from Argentina, with notes on its distribution, host plants, and egg parasitoids. Florida Entomologist, in press.

VI. Presentations on Research.

2006 Pierce's Disease Research Symposium, November 27-29, 2006, The Westin Horton Plaza, San Diego, California. The PI made a poster presentation: S. V. Triapitsyn, G. A. Logarzo, and J. H. de León, "Identify the Species of Mymaridae Reared in Argentina and Mexico for Potential Introduction to California against Glassy-winged Sharpshooter and Prepare and Submit for Publication a Pictorial, Annotated Key to the *ater*-group species of *Gonatocerus* – Egg Parasitoids of the Proconiine Sharpshooters (Hemiptera: Cicadellidae: Proconiini) in the

Neotropical Region". Also had 5 other poster presentations as a third co-author (with J. H. de León and G. A. Logarzo; the titles are indicated above in Section V).

II Reunión Argentina de Parasitoidólogos, 22-23 November 2007, Córdoba, Argentina. Presentation: E. Luft Albarracin, E. Virla & S. Triapitsyn, "Complejo de parasitoids oófilos de *Hortensia similis* (Hemiptera: Cicadellidae) sobre cultivo de maíz, en Tucumán, Argentina".

2007 Pierce's Disease Research Symposium, December 12–14, 2007, The Westin Horton Plaza Hotel, San Diego, California, organized by California Department of Food and Agriculture. Presented 3 posters (1 as first author [for this project], and 2 as co-author and/or collaborator).

VII. Research Relevance Statement. This project identifies more than 20 species of *Gonatocerus* (Mymaridae), which were reared in South America and Neotropical Mexico from eggs of the proconiine sharpshooters related to *Homalodisca*. Some of them are considered to be promising neoclassical biological control agents for introduction to California against the glassy-winged sharpshooter. Many of them need new scientific names, so that permits could be applied for their release and evaluation. Several species, colonies of which are being maintained at University of California, Riverside and USDA-APHIS Mission, Texas quarantine laboratories, were identified. The project will result in a published illustrated key to more than 70 described species of *Gonatocerus*, and that would help identify potential biocontrol agents in the future. This project provides necessary identification support to the classical and neoclassical biological control programs in California, which currently are being conducted by the CDFA and USDA.

VIII. Lay Summary of Current Year's Results. We are pleased to report some major accomplishments that have been achieved during the second year of research (partly Objective 1, partly Objective 2). We were able to locate all but one types of the South American species of *Gonatocerus*. More than 2,500 non-type specimens of *Gonatocerus* from South America and Mexico (reared from eggs of the proconiine sharpshooters or sorted from bulk samples) were sorted to morphospecies and compared with the types, and identified, including at least 30 undescribed species. 10 new species are being described. High quality digital photographs were taken from most of the types and also from both females and males of the species of *Gonatocerus*, reared by G. Logarzo in Argentina from eggs of the proconiine sharpshooters (Objective 2). Taxonomic keys and redescriptions were prepared for two species groups, and a portion of the third (the largest, *ater*) species group. The project has already resulted in 13 scientific papers and reports that either were published, submitted, or are ready to be submitted. Results of this project will be of significant benefit to biological control (especially to the CDFA/PD Biological Control Program) specialists, ecologists, and other researchers that manage the Pierce's disease threat posed by the glassy-winged sharpshooter. When completed, the key will make possible identifications of the mymarid egg parasitoids of proconiine sharpshooters in America south of the USA, differentiation of native vs. introduced species of *Gonatocerus*, and also will provide information on the candidate species of Mymaridae for introduction as part of biological control programs, facilitate surveys for assessing levels of egg parasitism of the proconiine sharpshooters, and indicate all known host associations of the mymarid species important for classical and neoclassical biological control of GWSS and other Proconiini.

IX. Status of Funds. It is expected that the fund allocation for Year 2 of the project will not be exhausted by the end of the fiscal year and the balance by 6/30/2008 will be approximately $19,000. Funds were used to hire a highly skilled Principal Museum Preparator (Vladimir Berezovskiy), who sorted and dried the material from alcohol, and also point- and slide-mounted specimens. However, because of other obligations, he was hired to less time than originally planned, and that resulted in the positive balance, which the PI is asking to be carried over to Fiscal Year 2008-2009 as a no-cost extension of the project. The remaining funds thus will be used to hire the Principal Museum Preparator to finish the work he could not complete during the current year (there are approximately 1,200 specimens that need to be sorted, curated, and many of them slide-mounted); also, collections were made during the current year in Argentina and Mexico that need to be processed during next year. Jennifer Walker (an undergraduate art major student) was hired over the summer to make high quality illustrations.

X. Summary and Status of Intellectual Property Produced during this Research Project. Not applicable.