**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**

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**Reporting Period:** The results reported here are from work conducted July 2006 to September 2006.

**ABSTRACT**
At least sixteen species of *Gonatocerus* were reared in Argentina and Neotropical Mexico from eggs of the proconiine sharpshooters in the course of classical and neoclassical biological control projects against the glassy-winged sharpshooter (GWSS) *Homalodisca vitripennis* (Germar) in California. The objectives of this project are to identify them taxonomically and to prepare a pictorial, easy-to-use, annotated key to more than 60 Neotropical species of the *ater* group of *Gonatocerus* – mymarid egg parasitoids of the proconiine sharpshooters including *Homalodisca*. Results obtained during the first three months of this new project are being reported.

**INTRODUCTION**
In North America, eggs of proconiine sharpshooters, which are known vectors of *Xylella fastidiosa* (*Xf*), are parasitized by various Mymaridae and Trichogrammatidae. An illustrated, annotated key to the genera and species of proconiine-parasitizing Trichogrammatidae is available (Triapitsyn 2003), and a key to such North American Mymaridae was published recently (Triapitsyn 2006).

Recommendation 3.12 (NRC 2004) calls for support for the classical biological control (over augmentative approach) against the glassy-winged sharpshooter (GWSS). Recently (during 2000-2006), major efforts have been undertaken to survey for egg parasitoids of GWSS and the related proconiine sharpshooters in Mexico (Hoddle & Triapitsyn, 2004, 2005; Morgan et al., 2000; Pilkington et al., 2005; Triapitsyn et al. 2002; Triapitsyn & Hoddle 2001, 2002; Triapitsyn et al. 2006) as well as in Argentina (Jones 2001; Jones et al. 2005; Logarzo et al. 2004; Pilkington et al. 2005; Virla et al. 2005). As the result, 12 species of *Gonatocerus* (Mymaridae) were reared in Argentina from eggs of the proconiine sharpshooter genera related to *Homalodisca* (G. Logarzo, unpublished USDA-ARS South American Biocontrol Lab. reports for 2004 and 2005). During 2002-2004, some of these species were imported under permits into the Universiey of California, Riverside (UCR) and USDA-APHIS, Mission, Texas quarantine facilities and their colonies were established on GWSS eggs. Several species are still being maintained and evaluated (Jones, Logarzo, Triapitsyn et al. 2005; Jones, Logarzo, Virla et al. 2005; Hoddle & Triapitsyn 2005). However, importation and quarantine evaluation of other available species from Argentina have not been initiated because their identification is not possible without a careful comparison with more than 60 already described Neotropical species of *Gonatocerus*. Several other species of *Gonatocerus* were also reared from eggs of proconiine sharpshooters in Mexico (Triapitsyn et al. 2002; Hoddle & Triapitsyn 2005), Chile (Logarzo et al. 2006), and Peru (Logarzo et al. 2004). The major problem, however, is taxonomic identification of these species, which has been impossible, except for a few of them. Thus, further introductions of these unnamed species (including applications for their release) are hampered because no positive identifications could be made before this project was initiated.

The *ater* species group of the genus *Gonatocerus* is mostly associated with Proconiini in the New World (Triapitsyn 2002; Triapitsyn et al. 2002). It is extremely speciose in the Neotropical region, with at least 60 described species, mainly from Argentina by A. A. Ogloblin, and probably with at least 100-150 undescribed species. Some of them have wide distributions from Mexico to Argentina (Triapitsyn et al. 2006). Unfortunately, almost 50 species described from Argentina and Ecuador by A. A. Ogloblin cannot be positively identified at present because there are no taxonomic keys for their separation, no adequate illustrations that accompany their descriptions in Spanish, and because the type specimens of almost half of these species were not available. Some of these were not marked by A. A. Ogloblin as types; they were located among the miscellaneous slides of *Gonatocerus* in his collection deposited in La Plata Museum in La Plata, Argentina, and needed to be identified. Therefore, to make identification of any specimen of *Gonatocerus* reared from eggs of proconiine sharpshooters anywhere from Mexico to Argentina, it needs to be compared with about 60 already described Neotropical species from the

same group. Thus, a key is needed, which would include all the previously described species of *Gonatocerus* (*ater* group) and also all the new species reared in Argentina and Mexico in the course of the recent surveys. Such a key will also be a useful tool to distinguish the species already present in California from other species of the same genus with similar host associations following possible release and establishment of the exotic egg parasitoids from Argentina and Mexico. Moreover, because of the easy availability of proconiine sharpshooter eggs in California due to the establishment and outbreak of the GWSS, non-intentional introductions of exotic egg parasitoids from countries in Central and South America are also quite possible.

**OBJECTIVES**

1. 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 by UCR researchers from eggs of *Homalodisca* and other proconiine sharpshooters (Year 1).
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).

**RESULTS**

**Progress on Objective 1.**

***Types***

To locate the unmarked or previously unavailable types of the *Gonatocerus* species, described by A. A. Ogloblin from Argentina and Ecuador, S. Triapitsyn and G. Logarzo visited the entomological collection of La Plata Museum in La Plata, Argentina, in August 2006 and studied the entire Ogloblin collection of *Gonatocerus*. Sorted and examined were numerous specimens stored in more than 20 boxes, each containing 100 microscopic slides. We found all but one primary types of Ogloblin's *Gonatocerus* species, which were borrowed for further study; digital photographs have already been taken from some of those (unfortunately, all the holotypes of the species described from Ecuador will need to be remounted as the mounting medium is so dark that the specimens are not visible). The holotype of *G. dorsiniger* (Ogloblin), described from Ecuador, is still missing but that would not affect our work as it belongs to a different (*membraciphagus*) species group of *Gonatocerus*. We were also able to curate a significant portion of the Ogloblin collection of *Gonatocerus*, labeling unmarked syntypes, paratypes, etc.

***Identification***

Morphologically, we recognized three more unidentified species among altogether at least 15 species of *Gonatocerus* reared in Argentina by G. Logarzo from eggs of the proconiine sharpshooters. So far we were able to positively identify the following species (including the three species identified during the reporting period, i.e., spp. #2, 8, and 12): *G. nigrithorax* (Ogloblin) [sp. #2], *G. annulicornis* (Ogloblin) [sp. #4], *G. metanotalis* (Ogloblin) [sp. #5], *G. tuberculifemur* (Ogloblin) [sp. #7]; *G. abbreviatus* (Ogloblin) [sp. #8], *G. uat* S. Triapitsyn [sp. #9], *G. atriclavus* Girault [sp. 10], and possibly *G. nigriflagellum* (Girault) [sp. #12]. Sp. # 1 from Argentina and a similar, yet clearly different species, reared from eggs of *Homalodisca* or *Oncometopia* in Veracruz, Mexico, and also sp. #6 from Argentina (a colony of which is being maintained in UCR quarantine) seem to be new, undescribed species, which will be described taxonomically later in the course of this project. The identities of other species remain to be figured out.

***Specimen preparation***

Due to the late availability of funding (in mid-September), work on slide-mounting of the hundreds of specimens will be conducted mostly during October 2006 – October 2007.

***Preparation of the illustrations (mostly Objective 2)***

High quality digital photographs (later arranged in plates) were taken, using the Automontage system, of the first 12 species reared in Argentina by G. Logarzo from eggs of the proconiine sharpshooters. Additionally, scanning electron micrographs were taken from some of them to facilitate their recognition and to illustrate some key morphological features.

**CONCLUSIONS**

Although work on this project has just begun, we are pleased to report some major accomplishments. We were able to locate all but one missing type of the South American species of *Gonatocerus*, described by A. A. Ogloblin from Argentina and Ecuador. High quality digital photographs were taken from both females and males of the first 12 species of *Gonatocerus*, reared by G. Logarzo in Argentina from eggs of the proconiine sharpshooters. Positive identifications of the additional three species from Argentina will make possible submission of several publications of the available biological and molecular data on these species (unpublished data by G. Logarzo, J. de León, and E. Virla). 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 GWSS. When completed, this 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.

**REFERENCES**


Hoddle, M.S. & S.V. Triapitsyn. 2004. Searching for and collecting egg parasitoids of the glassy-winged sharpshooter and other *Homalodisca* species in southeastern and southwestern Mexico. In: Proceedings of the 2004 Pierce's Disease Research Symposium, pp. 339-341.

Hoddle, M.S. & S.V. Triapitsyn. 2005. Maintaining and evaluating quarantine cultures of *Gonatocerus* spp., promising egg parasitoids from Argentina and Mexico, for the classical biological control of the glassy-winged sharpshooter in California. In: Proceedings of the 2005 Pierce's Disease Research Symposium, pp. 341-342.

Jones W.A. 2001. Classical biological control of the glassy-winged sharpshooter. In: Proceedings of the Pierce's Disease Research Symposium, pp. 50-51.

Jones, W.A., G.A. Logarzo, S.V. Triapitsyn, M. Casas, E.G.Virla & A.H. Purcell. 2005. Biology and host range of 2 South American egg parasitoids (Hymenoptera: Mymaridae), possible biocontrol agents for glassy-winged Sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae; Proconiini). Submitted Papers T7-T8. In: Submitted Papers T1-T21, 12th International Auchenorrhyncha Congress and 6th International Workshop on Leafhoppers and Planthoppers of Economic Significance, University of California, Berkeley, 8-12 August 2005. Available online at http://nature.berkeley.edu/hoppercongress/.

Jones, W.A., G. Logarzo, E.G. Virla, & E. Luft. 2005. Environmental risk assessment of egg parasitoids from South America: non-target field and laboratory host range in Argentina and the U.S. In: Proceedings of the 2005 Pierce's Disease Research Symposium, pp 343-344.

Logarzo, G.A. , J.H. de León, S.V. Triapitsyn, R. Rodriguez & 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.

Logarzo, G., Triapitsyn, S.V. & W.A. Jones. 2004 (2003). New host records for two species of *Gonatocerus* (Hymenoptera: Mymaridae), egg parasitoids of proconiine sharpshooters (Hemiptera: Clypeorrhyncha: Cicadellidae), in Peru. Florida Entomologist 86 (4): 486-487.

Morgan, D.J.W., S.V. Triapitsyn, R.A. Redak, L.G. Bezark, & M.S. Hoddle. 2000. Biological control of the glassy-winged sharpshooter: current status and future potential, pp.167-171. In: [Proceedings] California Conference on Biological Control, held July 11-12, 2000 at the historic Mission Inn, Riverside, California (M. S. Hoddle, Ed.), 205 pp.

NRC. 2004. California agricultural research priorities Pierce's disease. National Research Council of the National Academies. The National Academies Press, Washington, D.C., 161 pp.

Pilkington, L.J., N.A. Irvin, E.A. Boyd, M.S. Hoddle, S.V. Triapitsyn, B.G. Carey, W.A. Jones & D.J.W. Morgan. 2005. Introduced parasitic wasps could control glassy-winged sharpshooter. California Agriculture 59 (4): 223-228.

Triapitsyn, S.V. 2002. Species-level taxonomy of Mymaridae (Hymenoptera): current status and implications for biological control of leafhoppers of economic importance, pp. 89-94. In: Melika, G. & Thuróczy, C., editors. Parasitic wasps: evolution, systematics, biodiversity and biological control. Agroinform, Budapest, 480 pp.

Triapitsyn, S.V. 2003. Taxonomic notes on the genera and species of Trichogrammatidae (Hymenoptera) - egg parasitoids of the proconiine sharpshooters (Hemiptera: Clypeorrhyncha: Cicadellidae: Proconiini) in southeastern USA. Transactions of the American Entomological Society 129 (2): 245-265.

Triapitsyn S.V. 2006. A key to the Mymaridae (Hymenoptera) egg parasitoids of proconiine sharpshooters (Hemiptera: Cicadellidae) in the Nearctic region, with description of two new species of *Gonatocerus*. Zootaxa 1203: 1-38.

Triapitsyn, S.V., L.G. Bezark & D.J.W. Morgan. 2002. Redescription of Gonatocerus atriclavus Girault (Hymenoptera: Mymaridae), with notes on other egg parasitoids of sharpshooters (Homoptera: Cicadellidae: Proconiini) in northeastern Mexico. Pan-Pacific Entomologist 78 (1): 34-42.

Triapitsyn, S.V. & M.S. Hoddle. 2001. Search for and collect egg parasitoids of glassy-winged sharpshooter in southeastern USA and northeastern Mexico. In: Proceedings of the Pierce's Disease Research Symposium, pp. 133-134.

Triapitsyn, S.V. & M.S. Hoddle. 2002. Search for and collect egg parasitoids of glassy-winged sharpshooter in southeastern USA and northeastern Mexico. In: Proceedings of the Pierce's Disease Research Symposium, pp. 94-95.

Triapitsyn S.V., D.B. Vickerman, J.M. Heraty & G.A. Logarzo. 2006. A new species of *Gonatocerus* (Hymenoptera: Mymaridae) parasitic on proconiine sharpshooters (Hemiptera: Cicadellidae) in the New World. Zootaxa 1158: 55-67.

Virla, E.G., G.A. Logarzo, W.A. Jones & S. Triapitsyn. 2005. Biology of *Gonatocerus tuberculifemur* (Hymenoptera: Mymaridae), an egg parasitoid of the sharpshooter, Tapajosa rubromarginata (Hemiptera: Cicadellidae). Florida Entomologist 88 (1): 67-71.


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# Section 3: Pathogen Biology and Ecology

# BIOLOGY OF THE *XYLELLA FASTIDIOSA*-VECTOR INTERFACE

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## ABSTRACT
This project will build (and test) a framework to study the interactions between *Xylella fastidiosa* and insect vectors at the molecular, cellular and transmission biology levels. Understanding these interactions will lead to a better understanding of the transmission process and its biology, and potentially highlight promising strategies to disrupt pathogen retention by vectors and inoculation into plants. In addition, it will further elucidate how these genes function in infected grape and other hosts.

## INTRODUCTION
Current data suggest that *Xylella fastidiosa* (*Xf*) is transmitted to plants from the precibarium of vectors where it attaches, multiplies, forms a 'carpet' of cells and eventually detaches to be injected into plants (Almeida and Purcell 2006). The details about the mechanics of an inoculation event (from the insect's probing behavior perspective) are still to be determined. In addition, there is no information on the interactions between *Xf* and vectors. To our knowledge, there is no datum on any molecular aspect of the vector-*Xf* interface, with the exception of Newman et al. (2004) who demonstrated that a cell-cell signaling mutant was not transmissible to plants by insects (which showed that signaling controls transmission, but did not identify genes associated with attachment or retention per se). This project will start to fill an essential gap in *Xf* transmission and biology research with a study on the molecular determinants of the vector-pathogen interface.

## OBJECTIVES
1. Determine the effects of rpfC mutant on vector transmission.
2. Determine the transmission biology of *Xf* mutants hypothesized to be important in early and late stages of insect colonization.

## RESULTS
This project is being initiated. We will first focus our studies on *Xf* attachment mutants characterized for some biological attributes by Meng et al. (2005), in addition to cell-cell signaling (rpf) mutants currently being studied by Steve Lindow's group at UC Berkeley.

## REFERENCES
Almeida, R.P.P. and Purcell, A.H.. 2006. Patterns of *Xylella fastidiosa* colonization on the precibarium of leafhopper vectors relative to transmission to plants. Annals of the Entomological Society of America 99: 884-890

Meng, Y.Z., Li, Y.X., Galvani, C.D., Hao, G.X., Turner, J.N., Burr, T.J. and Hoch, H.C. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. Journal of Bacteriology 187: 5560-5567.

Newman, K.L., Almeida, R.P.P., Purcell, A.H. and Lindow, S.E. 2004. Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. PNAS 101: 1737-1742.

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