First Report of a Proconiine Sharpshooter, *Anacuerna centrolinea* (Hemiptera: Cicadellidae), in Chile, with Notes on Its Biology, Host Plants, and Egg Parasitoids

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ABSTRACT The first representative of the leafhopper tribe Proconiini (subfamily Cicadellinae), *Anacuerna centrolinea* (Melichar) is reported herein from the Tarapacá region in northern Chile. This species was discovered at high elevation (≈4,000 m) in the course of a survey conducted in South America by the USDA–ARS for the neoclassical biological control program against the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) in California. New data are given on the biology and host plants of *A. centrolinea*. Information also is provided on its egg parasitoid, *Gonatocerus tuberculifemur* (Ogloblin) (Hymenoptera: Mymaridae) which also was a first record from Chile. This discovery encourages further exploration for leafhopper egg parasitoids in northern and central regions of Chile to identify new perspective biological control agents that are more adapted to Mediterranean climate (winters and wet summers), which are similar to California climate. In addition, it is possible that *G. tuberculifemur* may be a good candidate for the biological control of the recently discovered *H. coagulata* in Easter Island, Chile.

KEY WORDS Pierce's disease, *Homalodisca coagulata*, *Gonatocerus tuberculifemur*, biological control

The self-introduction of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae: Cicadellinae: Proconiini), native from the southeastern United States, into southern California in the 1990s has resulted in the outbreak of Pierce's disease in grapes in that state. *H. coagulata* is an effective vector of the bacterium *Xylella fastidiosa* (Wells) that causes Pierce's disease. Estimated losses of $37.9 million were caused by this disease in California in 1998 and 1999 (Siebert 2001). About $7 million on pesticide applications were spent by growers to mitigate the effect of *H. coagulata* (Pilkington et al. 2005). *H. coagulata* invaded many islands in the Pacific Ocean: Hawaii, Tahiti, and in 2005 Easter Island in Chile. If *H. coagulata* moves to continental Chile, the wine and table grape industries there also may be threatened, provided *X. fastidiosa* is also present.

Since November 2000, personnel of the USDA–ARS South American Biological Control Laboratory in Buenos Aires, Argentina, have performed surveys of egg parasitoids in specific areas of South America (in Argentina and Peru) for a possible neoclassical biological control program against *H. coagulata* in California (Jones 2001; Logarzo et al. 2003, 2004; Virla et al. 2005). During 2004 and 2005, exploration surveys for proconiine sharpshooters and their egg parasitoids were conducted by G.A.L. in Chile.

There are >360 species described in the cicadelline leafhopper tribe Proconiini, all of which are restricted to the New World, with Chile being one of the few South American countries that had no prior records of proconiine sharpshooters (Young 1968). The absence of data on proconiine sharpshooters in Chile implies absence of data on their egg parasitoids, particularly mymarid wasps belonging to the *ater* species group of the genus *Gonatocerus* Nees (Hymenoptera: Mymaridae). Considering that Chile has areas that correspond more closely to the subclimate types of the grape-growing regions of California (Jones 2001), the discovery of proconiini sharpshooters and their associated parasitoids there may have important implications for the *H. coagulata* biological control program in California and elsewhere.

Materials and Methods

Two trips were performed in January of 2004 and 2005 to collect adult leafhoppers on wild and culti-

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vated plants in central and northern Chile. Most collections were carried out either from plants growing along roadways or in crops. Collection methods differed with the kind of plants sampled. Herbs and small shrubs were sampled by sweeping with a standard sweep net, by hand beating over a white plastic tray (30 by 27 by 17 cm), or by classical beating over a sheet (100 by 100 cm). Medium and large shrubs were sampled by hand beating over a tray and by classical beating. Thirty seven sites were sampled between Rancagua (34° 06′ 25″ S, 70° 43′ 18″ W) and Colchane (19° 13′ 58″ S, 68° 43′ 34″ W). Also, plants sampled were examined for egg masses. When a proconiine sharpshooter was found, host plants, egg masses, and immature stages were collected and identified.

To confirm host association of the emerged parasitoids, identified as *Gonatocerus tuberculifemur* (Ogloblin), with the discovered proconiine sharpshooter *Anacuerna centrolinea* (Melichar), its eggs were exposed to this parasitoid in the laboratory as described in Logarzo et al. (2003) and Virla et al. (2005), as follows. Females of *A. centrolinea* were caged on a *Vicia faba* L. potted plant (pot of 6.3 dm^3), which was checked daily for eggs. When egg masses were detected, the sharpshooters were removed from the plant; each leaf was introduced into a petri dish with one to three mated *G. tuberculifemur* females (24–48 h old) for 2–3 d. The petri dish was covered with clear plastic food wrap to prevent desiccation and to keep wasps from escaping. Parasitized egg masses were checked daily to ensure leaf quality until the emergence of the adult wasps or leafhopper nymphs.

The sharpshooter *Oncometopia orbona* (F.) (previously misidentified as *Oncometopia nigricans*), were collected in Raleigh, NC, in a vineyard with visible symptoms of Pierce's disease or scorching by John Meyer of North Carolina State University (de León et al. 2004). *O. orbona* was used as a positive control for detection of *X. fastidiosa* as described below.

Genomic DNA Isolation. Total genomic DNA was previously extracted from *O. orbona* as described in de León et al. (2004). A rapid crude DNA extraction procedure was used for *A. centrolinea* nymphs (Black et al. 1992, de León et al. 2006). The detergent Nonidet P-40, used in the original procedure, was replaced by IGEPAL CA-630 (Sigma-Aldrich, St. Louis, MO). Individual sharpshooter nymphs were homogenized in 60 μl of lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0, 1% IGEPAL CA-630, and 100 μg/ml proteinase K) with one 30-s burst on ice. The samples were incubated at 95°C for 5 min, followed by 1 min on ice. The samples were centrifuged for 10 min at 16,110 × *g* at 4°C. The supernatant was transferred to fresh microfuge tubes and stored at −20°C. To confirm for the presence of genomic DNA, amplification reactions were performed with 1 μl of stock DNA and 28S primers at an annealing temperature of 65°C (forward: 5′-CCCTGTTGAGCTTGACTCTAGTCTGGC-3′ and reverse: 5′-AAGAGCCGACATCGAAGGATC-3′) (Werren et al. 1995, de León et al. 2006) for 35 cycles and the amplification conditions described below.

Amplification of the 16S rDNA Gene of *X. fastidiosa*. To test for the presence of *X. fastidiosa* in *A. centrolinea*, the standard primers of Minsavage et al. (1994) were used (RST31, GCGTTAATTTTC-GAAGTGATTCGA; RST33, CACCATTCGTATC-CCGGTG) as described in de León and Jones (2004). Positive control reactions were performed with *X. fastidiosa* genomic DNA from two strains (ATCC 700964 and 35881). In addition, to confirm that *X. fastidiosa* could be detected in whole sharpshooters by using standard polymerase chain reaction (PCR) and the current primers, sharpshooters (*O. orbona*) collected from a vineyard with visible Pierce's disease or scorching symptoms were included as an additional positive control. PCR reactions were performed in a final volume of 20 μl with the following components: 1× PCR buffer [50 mM KCl, 20 mM Tris-HCl, pH 8.4, 1.5 mM $MgCl_2$, and 0.01% gelatin), 0.25 mM deoxynucleotide triphosphates, 0.25 μM primer, 3 μl of DNA template, and 2 U of *Taq*DNA Polymerase (New England Biolabs, Beverly, MA). The cycling parameters were as follows: 1 cycle at 94°C for 3 min followed by 45 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 40 s. Negative control reactions were performed in the absence of genomic DNA. Amplification products were loaded onto 2% agarose gels and submitted to electrophoresis in 1× TBE buffer (90 mM Tris-borate and 2 mM EDTA) in the presence of 0.2 μg/ml ethidium bromide. Photographs of the gels were taken with the Chemi Doc system (Bio-Rad, Hercules, CA). Positive control reactions were performed with two strains of *X. fastidiosa* (ATCC 700964 and 35881; Wells et al. 1987) genomic DNA (American Type Culture Collection, Manassas, VA). Amplification reactions were performed with the above-mentioned *X. fastidiosa* primer set with 5.0 ng each of genomic DNA. The reactions were amplified for 40 cycles.

Voucher Specimens. Voucher specimens of *A. centrolinea* were deposited in the entomological collections of Miguel Lillo Foundation and Institute (San Miguel de Tucumán, Argentina), La Plata Museum (La Plata, Argentina), and the USDA–ARS South American Biological Control Laboratory. *G. tuberculifemur* specimens were deposited in the Entomology Research Museum, University of California at Riverside (Riverside, CA) and the USDA–ARS South American Biological Control Laboratory.

Results and Discussion

Only one proconiine sharpshooter species, *Anacuerna centrolinea* (Melichar), was collected in January 2005 in the area surveyed of Chile (Colchane, Tarapacá region (region I), ≈300 km northwest from Iquique City). The collection site is ≈4,000 m above sea level. It belongs to the Central Andean dry Puna ecoregion; the vegetation type there is tropical andine herbs with small shrubs. The annual rainfall in this region is between 200 and 350 mm (Davis et al. 1997). *A. centrolinea* was previously reported only from Perú and Bolivia (Young 1968), and it is also the first report of a proconiine sharpshooter from Chile.

Table 1. Plants examined for adults, nymphs, and eggs of *A. centrolinea* in two sites at Colchane, Tarapacá region, Chile

Plant species[a]	Family	No. plants examined	No. of plants (%) with *A. centrolineal* (nymphs and adults)
Cultivated			
V. fava	Fabaceae	100	100 (100)
M. sativa	Fabaceae	20	20 (100)
A. sativa	Poaceae	30	30 (100)
C. quinoa	Chenopodaceae	200	23 (11.5)
Wild			
A. pusillus	Fabaceae	65	51 (78.5)
L. medicinalis	Verbenaceae	30	16 (53.3)
B. incarum	Asteraceae	30	13 (43.3)
P. lepidophylla	Asteraceae	25	7 (28.0)
Festuca sp.	Poaceae	8	7 (87.5)

[a] *V. fava* and *C. quinoa* were the only two plant species where eggs of *A. centrolinea* were found.

The entomological collection of the Facultad de Agronomía, Universidad de Chile, has 23 specimens of *A. centrolinea:* CHILE: Tarapacá Province: 13 specimens, Altiplano de Isluga, 4,600 m, 13-I-79; six specimens, Cotasaya, 10-I-79; three specimens, Sitani, 3,600 m, 22-I-69, C. Klein; two specimens, Cusallapo (maybe is Cusayapu), 6-X-69. All specimens were collected on *C. quinoa* and formerly labeled as *Tapajosa* nr. *doeringi.*

Host Plants. The pattern of plant use was influenced by the host plant species. Some plants supported only adults and nymphs, whereas others supported adults, nymphs, and eggs. Adult and nymphs were collected on cultivated and wild plants (Table 1). The crops were broad bean, *V. fava* and alfalfa, *Medicago sativa* L. (Fabaceae); oat, *Avena sativa* L. (Poaceae); and quinoa, *Chenopodium quinoa* Willd. (Chenopodiaceae).

Quinoa, a fruit of the *Chenopodium* family, is like a cereal grain. This grain comes from the Andes Mountains of South America. Quinoa was one of the three staple foods, along with corn and potatoes, of the Inca civilization. Whole grain of quinoa can be used like rice or can be grounded and used as flour.

The wild host plants were *Astragalus pusillus* Vogel (Fabaceae), *Baccharis incarum* Wedd. and *Parastrephia lepidophylla* (Wedd). (Asteraceae), *Lampaya medicinalis* Phil (Verbenaceae), and *Festuca* sp. (Poaceae). We found a hot spot of *A. centrolinea* where thousands of adults and nymphs were on almost all plants in the area. Interestingly, many adults and nymphs (≈30–40% of the observed individuals) were collected on the soil surface and rocks. The vegetation cover in the Puna region is very scarce (≈50%). The adults and nymphs preferred cultivated over wild plants. Among crops, broad bean was the most preferred by the adults and nymphs, with 100% of the 100 checked plants held adults and nymphs of *A. centrolinea.* Egg masses were found only on two crops: Broad bean (113 egg masses) and quinoa (1), although all the plants were checked for eggs.

Eggs. Of 114 egg masses examined, the average number of eggs per mass was 3.9 ± 2.5 (range 1–12).

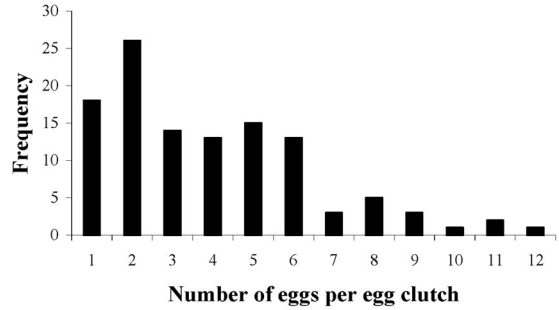

Fig. 1. Number of eggs per egg mass in *A. centrolinea.*

Approximately 87% of the egg masses contained one to six eggs, the mode was two eggs (Fig. 1). Egg masses of *A. centrolinea* presented the following characteristics: 1) Eggs were laid in both sides of the leaves. This is very unique because most Proconiini are known to lay endophytic eggs in clusters in the abaxial surface of the leaves (Rakitov 2004); 2) On 50 leaves with egg masses collected, 66% had >1 egg mass (between two and five egg masses per leaf) (Fig. 2). Few leaves in a single plant were selected for oviposition; 3) The eggs were not powdered with brochosomes (at least we were not able to detected brochosomes). The study of powdering of egg nests of the Proconiini by Rakitov (2004) does not show any correlation between the presence of powdering and the phylogeny of this tribe. Indeed, some genera, such as *Cuerna* Melichar, have species whose females do or do not powder their egg nests.

Egg Parasitoids. One egg parasitoid species, the mymarid *G. tuberculifemur* emerged from the field-collected eggs and also from eggs of *A. centrolinea* in the laboratory. This parasitoid emerged from 67% (n = 114) of the wild egg masses collected. The host association between *A. centrolinea* and *G. tuberculifemur* was confirmed: Seven adults of *G. tuberculifemur* emerged from the 12 exposed eggs of this leafhopper. *G. tuberculifemur* is also a known egg parasitoid of the proconiine sharpshooter *Tapajosa rubromarginata* (Signoret) in Argentina (Virla et al. 2005).

Amplification of the 16S rDNA Gene of *X. fastidiosa.* Fig. 3 shows the results of the control *X. fastidiosa* amplification reactions. Both strains of *X. fastidiosa*

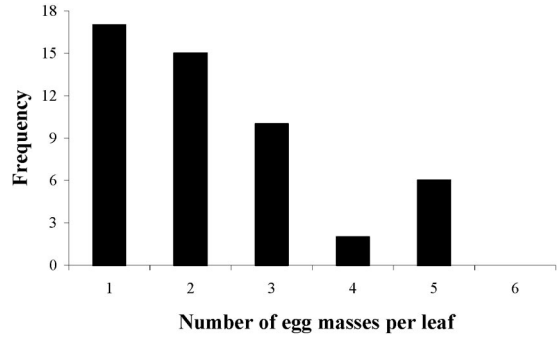

Fig. 2. Number of egg masses of *A. centrolinea* per leaf.

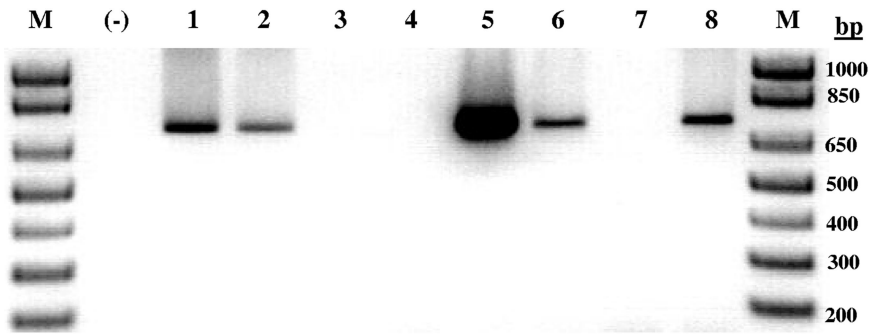

Fig. 3. Positive control: detection of *X. fastidiosa* in whole sharpshooters (*O. orbona*) collected in a vineyard with Pierce's disease or scorching symptoms. Amplification reactions were performed with 1 ng of genomic DNA for *O. orbona* and 5 ng for the *X. fastidiosa* strains. Lane −, negative control (no template DNA); lanes 1 and 2, positive controls, *X. fastidiosa* strains ATCC 35881D and 700964D, respectively; and lanes 3–8, individual sharpshooters. Expected size of the amplification product is 733 bp. M, 1.0-kb Plus DNA ladder.

produced amplification products of the correct size (733 bp); in addition, *X. fastidiosa* was detected in whole *O. orbona.* However, screening of 40 randomly chosen *A. centrolinea* nymphs resulted in negative amplification with the *X. fastidiosa* primers, even though amplification with the 28S internal control resulted in positive amplification (data not shown). These results indicate that negative amplification was not due to PCR failures, PCR inhibitors, or lack of DNA (Pooler et al. 1997, de León et al. 2006), but rather because *X. fastidiosa* was not present, at least within the sensitivity limits of the current amplification assay. It is possible that a more sensitive assay, such as real-time PCR (Bextine et al. 2005), may detect *X. fastidiosa* in *A. centrolinea* sharpshooters. It is also possible that the host plants on which *A. centrolinea* were collected were not infected with *X. fastidiosa* (de León and Jones 2004).

The discovery of *A. centrolinea* in Chile encourages the exploration for leafhopper egg parasitoids around and to the North of Santiago de Chile (around 33° S) to identify new biological control agents climatically more adapted to the California-like climates. In addition, it is possible that parasitoids of *A. centrolinea* may help in the control of *H. coagulata* in Easter Island, Chile, given that in August 2005, *H. coagulata* was discovered there (by Ide, Pilkington et al. 2005; Servicio Agrícola y Ganadero de Chile 2005).

Two approaches could be used to reduce *H. coagulata* populations in Easter Island: 1) the introduction of natural enemies of *H. coagulata* from its native range, i.e., the southeastern United States and northeastern Mexico (classical biological control approach). de León et al. (2003, 2004) were the first authors to determine that Texas was the origin of the *H. coagulata* that invaded California. Genetic testing or DNA fingerprinting needs to be performed with the *H. coagulata* population discovered in Easter Island to confirm its origin before a classical biological control program can be attempted; and 2) the "new-association" approach (Pimentel 1963, 1991; Hokkanen and Pimentel 1989). The new-association strategy involves the selection of natural enemies of species closely related to the target pest (*A. centrolinea* or *T. rubromarginata*) with which the natural enemies have not had a previous association. Given that *H. coagulata* does not occur in South America, *G. tuberculifemur* may be used. This approach has particular merit when native natural enemies are not present as reported by Ide (Pilkington et al. 2005). The closer the taxonomic proximity between the new host and the main target, the higher the probability of success (Van Driesche and Bellows 1996).

Possible utilization of the parasitoid native to continental Chile, such as *G. tuberculifemur*, against *H. coagulata* in Easter Island could reduce research efforts, costs, and potential nontarget effects compared with an imported parasitoid. *G. tuberculifemur* has been under appraisal as a perspective agent for the control of *H. coagulata* in the United States since 2000. Colonies of *G. tuberculifemur* have been successfully maintained at the USDA–APHIS Mission (Edinburg, TX, since March 2001) and the University of California (Riverside, CA, since September 2002) quarantine facilities on eggs of *H. coagulata* (Jones et al. 2005, Virla et al. 2005). Nontarget tests were conducted in Argentina and the United States by using *G. tuberculifemur* on >25 potential host species and the parasitoid successfully attacked only the four proconiine sharpshooter species tested (Jones et al. 2005).

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