

Rapid Communication**First report of *Greenidea ficicola* Takahashi and *G. psidii* van der Goot (Hemiptera: Aphididae) in Chile**

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OPEN ACCESS**Abstract**

Two species of the genus *Greenidea* Shouteden, 1905, associated with *Psidium guajava* L. and *Ficus benjamina* (L.), are described morphologically and molecularly, expanding their distribution. The latest reports of the plague are in South America and probably follow trade routes from the east. This is the first record in Atacama Desert, northern Chile.

Key words: *Ficus benjamina*, *Psidium guajava*, Atacama Desert, invasive species

Introduction

In the order Hemiptera, the superfamily Aphidoidea consists of three families: Adelgidae (2 genera with 49 species), Phylloxeridae (8 genera with 75 species) and Aphididae (483 genera with 4,277 species). The latter includes the genus *Greenidea*, of Asian origin, described by Schouteden (1905) (Blackman and Eastop 1994). In 1917, van der Goot described *G. psidii* on guava (*Psidium guajava*); and in 1921, Takahashi reported *G. ficicola* on *Ficus* sp. (Blackman and Eastop 1994). In the last 5 years, the records have extended to the Neotropics, frequently present in urban spaces, constituting a plague on ornamental and economically important trees (Table 1). Due to diet, this pest causes the leaves of branches and twigs of infested trees to curl, distort and become covered with honeydew. Eventually the trees weaken and lose their ornamental and productive value. Given its ornamental value, *Ficus benjamina*, along with other species of this genus, occupies both urban and rural spaces. These trees are commonly found along streets and in parks, which facilitates the colonization of aphids and other insects. The host registry for this pest includes species of Fagaceae, Moraceae, Betulaceae, Juglandaceae and Myrtaceae. Less frequently they are associated with Rosaceae and Rubiaceae (La Rossa et al. 2011).

The objective of this work is to complement the information on the distribution of *Greenidea ficicola* Takahashi and *G. psidii* Van der Goot (Hemiptera: Aphididae) in Chile by reporting their occurrence.

Table 1. Report of colonization of new areas by *Greenidea ficicola* Takahashi and *G. psidii* van der Goot (Hemiptera: Aphididae) in the world.

Greenidea	Continent, Country, approach (Reference)
<i>G. ficicola</i> and <i>G. psidii</i>	South America, Chile, DNA Barr Code, (This record 2022)
<i>G. ficicola</i>	Complete mitochondrial genome (Liu et al. 2020)
<i>G. ficicola</i>	Europe, Madeira Archipelago (Portugal), (Bella and Aguiar 2020)
<i>G. ficicola</i>	Asia, India, Aromatic Plants (Rohini et al. 2018)
<i>G. ficicola</i>	Western Asia, Syria (Ali 2015)
<i>G. ficicola</i>	Africa, Tunisia, (Ben Halima-Kamel 2009)
<i>G. ficicola</i>	South America, Venezuela, (Cermeli et al. 2012)
<i>G. ficicola</i>	North America, Hawaii, (Nagamine and Garcia 2012)
<i>G. ficicola</i> and <i>G. psidii</i>	North America, Central México, Natural control (Salas-Araiza et al. 2011)
<i>G. ficicola</i>	Ecology (Lazzarotto et al. 2011)
<i>G. ficicola</i>	South America, Argentina, (La Rossa et al. 2011)
<i>G. psidii</i>	Central America, Costa Rica, (Pérez Hidalgo et al. 2009)
<i>G. ficicola</i>	Africa, Tunisia, (Ben Halima 2012)
<i>G. ficicola</i>	Origen and (Bella et al. 2009)
<i>G. ficicola</i>	Europe, Spain (Pérez Hidalgo et al. 2009)
<i>G. ficicola</i>	South America, Antioquia, Colombia (David et al. 2009)
<i>G. ficicola</i>	Southern Europe, Malta Island (Mifsud 2008)
<i>G. ficicola</i>	South America, Brazil (Lazzari et al. 2006; Soussa-Silva et al. 2005)
<i>G. ficicola</i>	Europe, Meridional Italy (Barbagallo et al. 2005)
<i>G. ficicola</i> and <i>G. psidii</i>	North America, United States, key of two species (Halbert 2004)

Materials and methods

Arica is a city located in the western region of Atacama Desert, northern Chile, where guava trees are frequently found. In December 2021, specimens of a locally unknown species of winged or apterous viviparous aphid on guava leaves, *Psidium guajava* (Myrtaceae), were collected. Subsequent surveys allowed for a further detection of this aphid in *Ficus benjamina* (Moraceae). Material from shoots and new leaves of both hosts was collected. By means of a fine bristle brush, apterous and alate adults were extracted and placed separately in small glass containers with 70% alcohol. For assembly, the adults were treated with a solution of KOH (10%) in a water bath during 8 to 10 min, rinsed in 70% and 96% ethanol respectively to remove the excess potassium hydroxide, and afterwards mounted in glycerin. Subsequently, the taxonomic determination of the species was carried out with the help of the keys of Halbert (2004). The specimens collected in guava and ficus were deposited in glass tubes containing 70% alcohol to later be identified. They are in the Entomological Collection room of the Faculty of Agronomic Sciences, University of Tarapacá.

DNA extraction and sequencing

Genomic DNA was extracted from a pool of 3 adult aphids of each plant species following the procedures described in Huanca-Mamani et al. (2015). A fragment of the cytochrome *c* oxidase I gene (COI) was amplified by polymerase chain reaction (PCR) with the primers LepF and LepR (Martínez-Ortega et al. 2016). PCR reactions were performed in a final volume of 20 µl. Each reaction contained 1 µl of DNA extract, 10 pmoles of each primer, 2.5 mM of each dNTP, 2 mM MgCl₂, 1x PCR buffer (KCl),

Table 2. Main morphological characters of *Greenidea ficicola* and *G. psidii*, according to various authors.

Character	<i>Greenidea ficicola</i>	<i>Greenidea psidii</i>
Host	<i>Ficus</i> spp., Fagaceae, Moraceae, Betulaceae, Rosaceae and Myrtaceae	<i>Psidium guajava</i> , Fagaceae, Moraceae, Betulaceae, Rosaceae and Myrtaceae
Shape of body	Pear	Pear
N° segments cornicules antenna	6	6
Seta	Long	Long
Alate female	Body and the cornicules less elongated	Body and the cornicules more elongated
Siphons covered with robust setae	++	++
Current Distribution	Japan, from the east of Australia and India and the Philippines	Japan, from the east of Australia and India and the Philippines
Cross-links present in the siphuncle in apterous form	Cover a large part of them	Cover only at the base
Number of sensoria on the segment III of the winged form antennas	Between 17–21 sensoria in a single line and not touching each other	Between 20–31 sensoria in a circular or elliptical forms, not in line and some touching each other

1 unit of Taq DNA polymerase (Thermo Scientific) and sterile distilled water. The amplification program was 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 47 °C, 1 min at 72 °C and a final elongation step of 10 min at 72 °C. Three µl of each PCR product were visualized on 1.5% agarose gel stained with gel-red (Biotium). PCR products were purified and directly sequenced by a commercial facility (Macrogen, South Korea). The nucleotide sequences were visualized with 4Peaks software (<http://nucleobytes.com/4peaks/>), assembled from forward and reverse and edited with MEGA software (Kumar et al. 2016). The sequences obtained were compared with COI data sequences from strains available in GenBank (www.ncbi.nlm.nih.gov) by using BLASTn.

Results

According to Sugimoto (2008), the material studied helped determine that these species belong to the genus *Greenidea* Shouteden. They can be recognized by their morphological characteristics: pear-like shape, antenna with long 6-segmented cornicules with many long setae and a prominent middle cauda in both species. The winged females are similar, although showing a more elongated body and cornicules (Pérez Hidalgo et al. 2009). These characters were observed in all the specimens studied (Table 2).

This genus has about 50 species, as established by Shouteden (1905), characterized by the shape of their body and siphons covered by robust setae (Blackman and Eastop 1994). They are distributed in Japan, in the east of Australia, India and the Philippines (Bella et al. 2009). In America, two species of the genus *Greenidea* have been detected: *G. psidii* van der Goot, 1916, and *G. ficicola* Takahashi, 1921, which—to date—have not been considered as plagues of consequence in infected hosts (Cermeli et al. 2012).

Genidae can be distinguished by the cross-links in the siphuncle that cover a large part of them, (Figure 1A, B). These sensoria are not presented in a line and some are touching each other (Figure 2A, B). Whereas in *G. ficicola* 17–21 sensoria are in a single line and not touching each other (Figure 2C, D). Both species can be easily confused because of their morphological resemblance

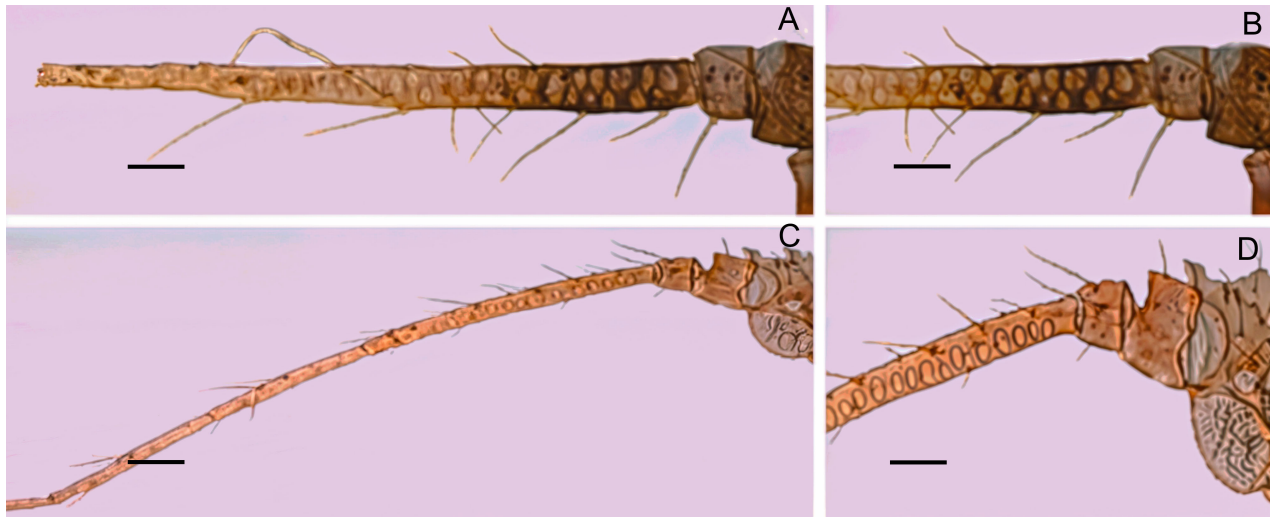


Figure 1. Reticulations on siphunculi of the apterous form. A–B *Greenidea ficicola*. Reticulations on a significant part of the siphunculus. Scale bar: 0.1 and 0.04 mm, respectively. C–D *Greenidea psidii*. Reticulations are restricted to the basal part of the siphunculus. Scale bar: 0.1 and 0.04 mm, respectively. Photographs by D. Bobadilla.

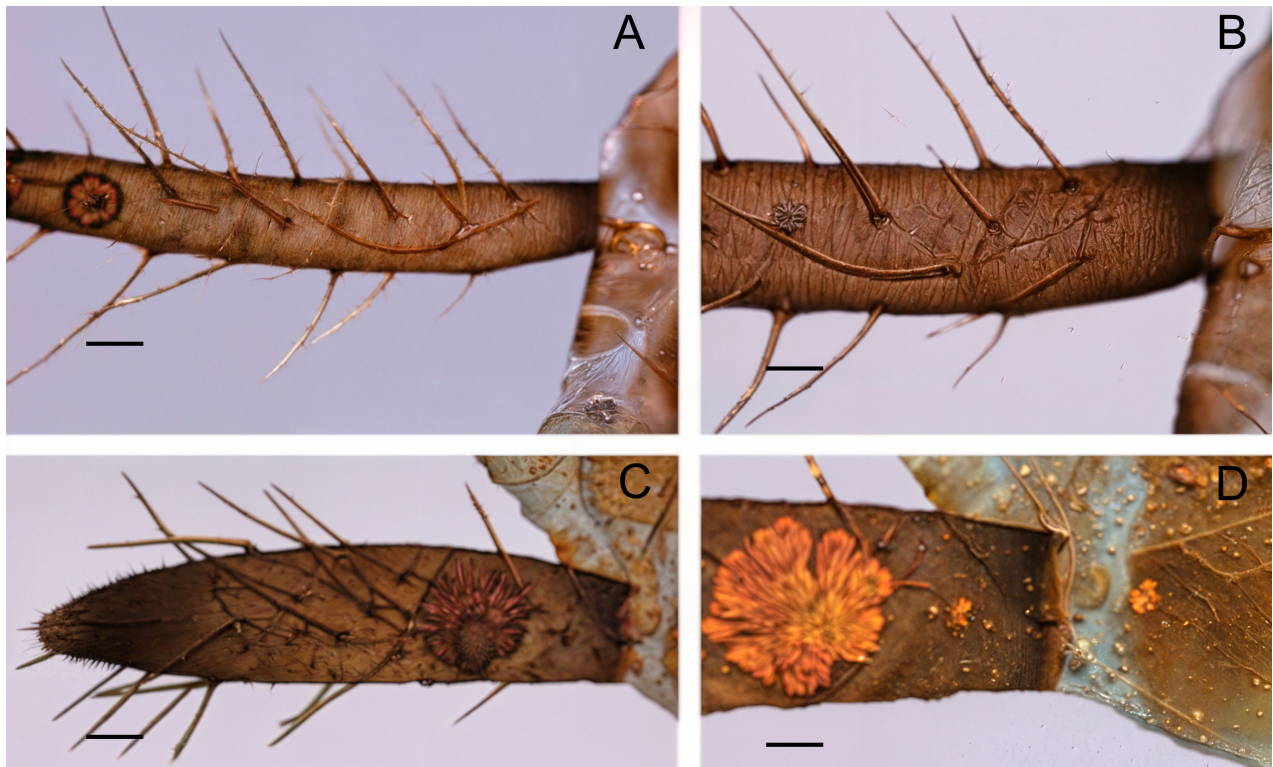


Figure 2. Rhinaria on antennal segment III of the apterous form. A–B *Greenidea psidii*. Scale bar 0.09 mm. C–D *Greenidea ficicola*. Scale bar: 0.2 and 0.1 mm, respectively. Photographs by D. Bobadilla.

and because they have common host plants in the Myrtaceae and Moraceae families. *Greenidea ficicola* prefers *Ficus* (Moraceae) and *Psidium guajava* (Myrtaceae) (Halbert 2004; La Rossa et al. 2011); and *G. psidii* prefers *P. guajava* and other Myrtaceae species (*Callistemus*, *Eucalyptus*, *Ficus*), *Engelhardtia* (Juglandaceae), *Scurrula* (Loranthaceae), *Lagers troemia* (Lythraceae) and *Nesua ferrea* (Clusiaceae) (Halbert 2004; ONPP 2018).

Discussion

Each aphid COI PCR fragment was successfully amplified and sequenced to confirm the morphological identification of *G. ficicola* and *G. psidii*, yielding 658 bp fragments for each specimen, which were deposited in GenBank (accession numbers OP562908 and OP562907, respectively). The Blastn analysis performed with COI sequence of aphid collected from *F. benjamina* showed a 100% similarity to *G. ficicola* from China (NC_048525, OL619512, OL619470), Korea (GU978831) and India (KJ702465). The aphid collected from *P. guajava* showed a 100% similarity to *G. psidii* from China (NC_041198, MH844624, OL619497, OL619495, OL619478) and USA, Hawaii (EU701673).

These species are native to Asia; however, there has been a report of both in the New World in recent decades. *Greenidea ficicola* was recorded in the United States in 2003 (ONPP 2018); it was subsequently cited in Brazil (Soussa-Silva et al. 2005), Peru (Rubin de Celis et al. 2006), Colombia (David et al. 2009) and Argentina (La Rossa et al. 2011). *Greenidea psidii* was reported in California in 1998 and later in Florida (Halbert 2004). It was reported by Lazzari et al. (2006) in Brazil and, in Costa Rica, by Pérez Hidalgo et al. (2009) and Cermeli et al. (2012) in Venezuela and Panama. Collectively, with these aphids, the presence of larvae of *Chrysoperla externa* (Hager) (Neuroptera: Chrysopidae) was observed preying on the specimens.

Due to the heavy traffic of plant material to and from Arica and Parinacota Region in the north of Chile, these insects will probably spread to the rest of the country, even though guava is restricted only to Arica and Tarapacá Regions (ODEPA 2021). In parallel, at the same time, the high ornamental value of *Ficus benjamina* allows it to occupy a larger surface area. This situation maximizes the possibilities for *G. ficicola* to expand. These aphids have not been previously recorded in Chile (Blackman and Eastop 1994; Nieto Nafría et al. 2016), and as far as our knowledge extends, this note represents the first documented occurrence of *Greenidea ficicola* and *Greenidea psidii* in the country.

Conclusion

Two invasive aphid species, *Greenidea psidii* and *G. ficicola*, were identified in association with *Pisum guajava* and *Ficus* sp. These findings were confirmed through morphological and molecular data, marking the first documented occurrences of these species in Chile.

Authors' contribution

All authors contributed to the study conception and design. Material preparation, sampling and experimental procedures were performed by G.F.S.Ch., W.H.M., M.A.M. and D.B.G. Data analyses were executed by G.F.S.Ch., W.H.M., M.A.M. and D.B.G. The first draft of the manuscript was written by D.B.G. and G.F.S.Ch. All authors have read and agreed to the published version of the manuscript.

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