

***Gryon aetherium* Talamas (Hymenoptera, Scelionidae): Parasitoid of *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae) Adventive in Chile**

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Abstract

A parasitoid wasp, *Gryon aetherium* Talamas (Hymenoptera, Scelionidae), was reared from eggs of the invasive stink bug *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae) in Chile. The identification of *G. aetherium*, which is under study as a biological control agent, was made with morphological and molecular data in the context of a recent taxonomic treatment of this species. The presence of an adventive population of *G. aetherium* in South America has implications for biological control of *B. hilaris* in Chile, and other countries on the continent, where this stink bug may become a pest.

Keywords

biological control, egg parasitoid, invasive species, DNA barcoding, stink bug

Introduction

Bagrada hilaris (Burmeister) (Hemiptera, Pentatomidae), also known as bagrada bug, is now a significant pest in the Western hemisphere, having invaded the western United States (Palumbo et al. 2016), Mexico (Sánchez-Peña, 2014) and Chile (Faúndez et al. 2016). In Chile, *B. hilaris* has spread rapidly to the north and south of the Metropolitan region where it was initially detected (Faúndez et al. 2018) and is associated with both brassicaceous crops and natural areas (Alaniz et al. 2021). Current control measures in Chile consist of reiterative applications of conventional insecticides which appear to be ineffective (SAG 2017a, b). Currently, there are no feasible options to control populations in urban or suburban settings, or in natural habitats that serve as refugia for *B. hilaris*.

At present, Centro Ceres, a research institute in Valparaíso, Chile, is investigating alternative solutions to this pest through diversification of the vegetative components of the agroecosystem. By increasing the functional biodiversity and employing push-pull strategies, the aims are to decrease the density of *B. hilaris* and damage on crops, and to favor the presence of natural enemies. However, knowledge regarding indigenous candidate agents for biocontrol against stink bugs in general, and *B. hilaris* in particular, is poor in Chile. Due to a need for rearing facilities and COVID restrictions, exposure of sentinel eggs of *B. hilaris* has occurred only opportunistically, but our efforts to study *B. hilaris* have serendipitously provided a substantive result that we present here.

Materials and methods

Bagrada rearing

Adults and nymphs of *B. hilaris* were collected in the field in October, 2020, in the region of Valparaíso (Region V) and used to establish a colony in the Centro Ceres laboratory. The colonies were maintained in 1.5 L glass containers covered by a fine mesh at approximately 25 °C during the day with heating at night, when necessary, to prevent the temperature from dropping below 12 °C. The containers were provided with cabbage and fresh water. The relative humidity was $60 \pm 10\%$ with a L:D cycle of 16:8 h. Nymphs and adults were kept separately. Eggs were collected regularly and transferred to an independent container until the emergence of nymphs or use for exposure in the field to survey for parasitoids.

Parasitoid rearing

Two parasitoid wasps were found in one of the colony containers of *B. hilaris*, which we isolated and exposed to 20 freshly-laid *B. hilaris* eggs. The exposed eggs were kept separately in a Petri dish in an incubator at 25 ± 3 °C and $60 \pm 10\%$ RH. The con-

tainer where the parasitoids were initially detected was monitored continuously for the appearance of additional specimens. The specimens retrieved were placed in 96% alcohol for further study.

DNA barcoding

DNA extraction was performed with 30 µL of buffer using the DNA extraction kit LUCIGEN (MA150E, QuickExtract DNA Extraction Solution, Middleton, WI, USA), following company specifications. This method allows a non-destructive extraction of the DNA, so that the exoskeleton (voucher) remains intact for morphological identification.

PCR amplifications were performed on a portion of the Cytochrome C Oxidase, subunit I (COI) locus using the LCO-HCO primers: HCO2198 (5'-TAAA CTT CAG GGT GAC CAA AAA ATC A-3'), LCO1490(5'-GGTC AAC AAA TCA TAA AGA TAT TGG-3') (Folmer et al. 1994), which amplifies a 600–700 bp portion of the COI locus. Amplicons were sent to Beckman Coulter Genomics Genewiz (Leipzig, Germany; Essex, UK) for bidirectional sequencing (Sanger method) using both primers. All residual DNA is archived at the Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Sophia-Antipolis, France. Correction, annotation, and alignment were performed manually using BioEdit Geneious R10 software.

Sequences were compared to those available in the NCBI database (GenBank) using BLASTN (Altschul et al. 1990) with standard settings and to COI data made available in Talamas et al. (2021). *Gryon prisma* Mineo (Hymenoptera, Scelionidae) was selected as the closest outgroup to our specimens based on the phylogenetic analysis in Talamas et al. (2021). Three *Hadronotus* species *i.e.* *H. drunoris*, *H. ancilla* and *H. charon* group sp. 2, were added to this analysis because they were originally described under the genus *Gryon*, until they were synonymized with *Hadronotus* by Talamas et al. (2021). *Trissolcus basalis* (Wollaston) (Hymenoptera, Scelionidae) [MN615650] was used as the outgroup to root the tree. These data were analyzed with MegaX software (Tamura et al. 2013) using the neighbour joining (NJ) method (Saitou and Nei 1987) with bootstrap values based on 500 replications. Nucleotide distances in NJ trees were estimated by the Kimura's two-parameters method (Kimura 1980).

Morphological analysis

Ethanol-stored and DNA-extracted specimens were dried and glued on card points for optical observation using a Zeiss Macroscope AxioZoom.v16. Morphological confirmation of *Gryon* specimens was performed using the description and diagnosis of *Gryon aetherium* in Talamas et al. (2021). Voucher specimens are deposited at INRAE UMR 1355 ISA in Sophia-Antipolis, France.

Results

Parasitoid rearing

Sixteen parasitoids emerged from the 20 eggs that were exposed to the initial two parasitoids detected in the *B. hiliaris* colony, yielding a parasitism rate of 80%. Monitoring of the *B. hiliaris* colony yielded additional specimens: 11 parasitoids were collected 26 days after the initial detection and eight were collected at day 29.

DNA barcoding

Sequences from two specimens were 607 bp in length and correspond to one haplotype. Sequences of each specimen were deposited in GenBank (Table 1).

The best matches (100% identity) in GenBank were with sequences of *G. aetherium* from Mexico (MK720832.1 and MK720831.1, reported as *G. myrmecophilum* in Felipe-Victoriano et al. (2019)). Furthermore, they are identical to sequences of *G. aetherium* from California reported by Talamas et al. (2021) (Figure 1).

Morphological analysis

According to the diagnosis in Talamas et al. (2021), the specimens clearly belong to the genus *Gryon* Haliday (Hymenoptera, Scelionidae) based on the glabrous metapleuron (Figure 2A), striate axillula (Figure 2B) and frons without transverse sculpture (Figure 2C). They also match the diagnosis for *G. aetherium* provided in Talamas et al. (2021), including the absence of a mesopleural carina (Figure 2A), the acetabular carina and ventral mesopleural carina intersecting ventrally (Figure 2A) and the posterior margin of mesoscutellum extending posteriorly over the metascutellum and metanotal trough (Figure 2B).

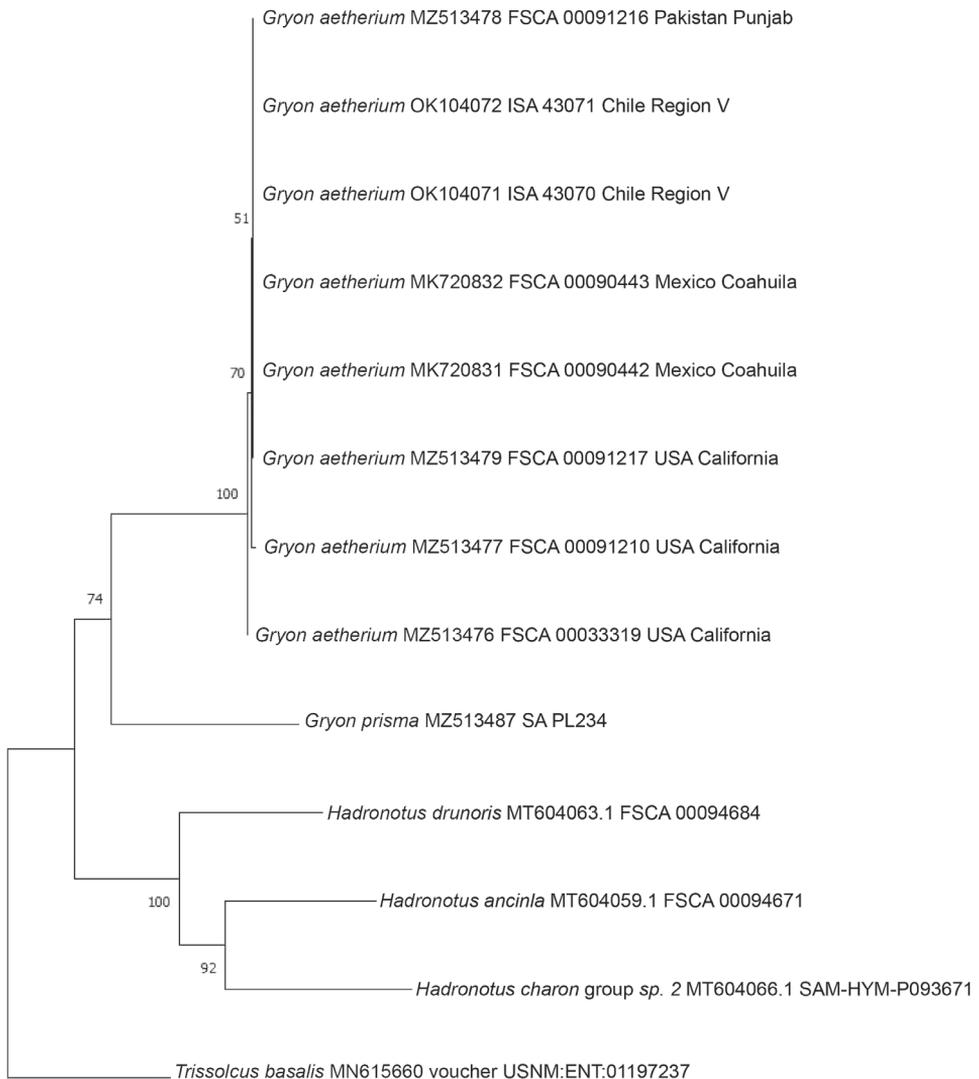
Discussion

The phenomenon of adventive biological control agents seems to be increasing in frequency. For scelionid parasitoids of stink bug eggs, surveys designed to determine their presence and efficacy are undoubtedly accelerating the rate of their detection. In the past decade, these include *Trissolcus japonicus* (Ashmead) from North America (Talamas et al. 2015; Milnes et al. 2016; Abram et al. 2019) and Europe (Stahl et al. 2019; Sabbatini-Peverieri et al. 2018; Dieckhoff et al. 2021), *T. mitsukurii* (Ashmead) in Europe (Sabbatini-Peverieri et al. 2018; Rot et al. 2021; Bout et al. 2021) and *T. hyalinipennis* Rajmohana & Narendran from the USA (Ganjisaffar et al. 2018).

Our analysis is facilitated by recent studies of the genus *Gryon* that were conducted to support biological control of *B. hiliaris* in North America. In North America, adventive populations of *G. aetherium*, a species under study as a biological control agent, have been reared from sentinel or naturally laid eggs of *B. hiliaris* (Felipe-Victoriano

Table I. GenBank accession numbers and sample information for COI sequences of *Gryon aetherium* presented in this study.

Species	Collection code	Region, country	Year of collection	GPS Coordinates (DMS)	Host species	GenBank Accession Number
<i>Gryon aetherium</i>	43070_HCO	Valparaiso, Chile	2021	32°52'58.19"S, 71°12'22.99"W	<i>Bagrada hilaris</i>	OK104071
<i>Gryon aetherium</i>	43071_HCO	Valparaiso, Chile	2021	32°52'58.19"S, 71°12'22.99"W	<i>Bagrada hilaris</i>	OK104072

**Figure 1.** Molecular clustering of 13 sequences including the 2 sequences of *Gryon aetherium* from Chile. Sequences of *G. aetherium* from California, Mexico and Pakistan are from the Talamas et al. (2021) study. *Gryon prisma* Mineo, three *Hadronotus* species and *Trissolcus basalis* were selected as outgroups. *Trissolcus basalis* was used to root the tree based on the topology of Talamas et al. (2021). Numbers at nodes indicate bootstrap support values derived from 500 replicates.

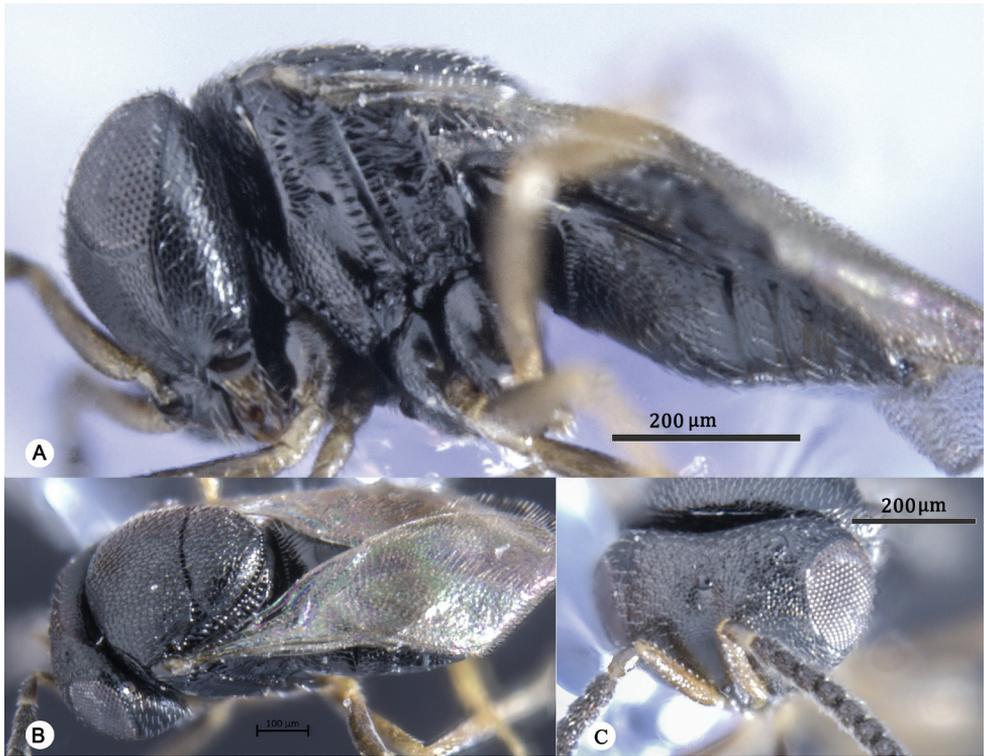


Figure 2. *Gryon aetherium*, male, ISA30411 **A** head, mesosoma, metasoma, lateral view **B** head, mesosoma, metasoma, dorsolateral view **C** head, anterodorsal view.

et al. 2019; Hogg et al. 2021). The taxonomy of *G. aetherium* has been challenging, but a recent treatment by Talamas et al. (2021) corrected prior misidentifications (as *G. myrmecophilum* (Ashmead) and *G. gonikopalense* Sharma) and provided morphological and molecular means to identify it. Our study used these data to identify parasitoids reared from the eggs of *B. hilaris* in the V region, Region of Valparaíso, Chile.

Detection of an adventive population of *G. aetherium*, a promising biological control agent of the invasive *B. hilaris* in Chile, is a great opportunity for the country to develop classical biological control programs against this pest. Indeed, *B. hilaris* displays a specific oviposition behavior among stink bugs by laying part of its eggs in isolation and underground. Nonetheless, *G. aetherium* can detect buried eggs and overcome the physical barrier constituted by the soil (Martel and Sforza 2021). Other parasitoids of *B. hilaris* eggs, such as *T. hyalinipennis* (Hymenoptera, Scelionidae), may not be capable of this (Tofangsazi et al. 2020).

Gryon aetherium was not recovered from sentinel eggs deployed near Centro Ceres. This may be the result of the low number of exposures in the 2020–2021 period (<100) and the normal low rate of parasitism typically observed with this methodology. We have no information yet on *G. aetherium* in Chile regarding its broader distribution, host range or host preferences. No stink bugs from the tribe Strachiini are recorded

from Chile (Prado 2008; Faúndez and Carvajal 2011) except for the invasive *B. hilaris*. So, there are no species close to *B. hilaris* or *Eurydema* species in Chile which could appear as a logical alternative host. It remains unclear if *G. aetherium* arrived simultaneously with *B. hilaris* and spread with the stink bug or if it is the result of a separate introduction. The 100% match of COI with adventive populations in Mexico and the United States is noteworthy, and may indicate that the parasitoid arrived in these three places from the same place, perhaps via similar pathways. Additional studies on populations of *G. aetherium* throughout the world may yield insight into this matter and are ongoing. Future monitoring and study of *G. aetherium* in Chile is needed to guide efforts for inoculative or augmentation releases that have the potential to regulate *B. hilaris* both inside and outside of agricultural ecosystems.

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