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Infección de huevos de *Triatoma dimidiata* (Hemiptera; Reduviidae) con *Telenomus fariai* (Hymenoptera: Scelionidae) en Alajuela, Costa Rica

Infection of eggs of *Triatoma dimidiata* (Hemiptera; Reduviidae) with *Telenomus fariai* (Hymenoptera: Scelionidae) in Alajuela, Costa Rica

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Resumen: Los microhimenópteros de la especie *Telenomus fariai* (Hymenoptera: Scelionidae) son reconocidos parasitoides de huevos de triatominos. Estas avispas constituyen un potencial modulador poblacional de *Triatoma dimidiata* (Hemiptera: Reduviidae), principal vector de la enfermedad de Chagas en Centroamérica. El presente estudio describe la infección con *T. fariai* en huevos de *T. dimidata* colectados en el campo. Los huevos fueron obtenidos en el peridomicilio de una vivienda ubicada en Tuetal Sur (provincia de Alajuela, Costa Rica). La progenie de avispas, observada 24 horas después de su recepción el laboratorio, consistió principalmente de hembras (94,0%). La confirmación de especie se efectuó mediante análisis de la genitalia masculina y por secuenciación de ADN de un fragmento del gen correspondiente a la subunidad 1 del citocromo oxidasa *c* mitocondrial (COI). La identidad de los insectos, basada en análisis de secuencias, sitúa a la cepa local en proximidad con ejemplares colectados en Veracruz, México. La caracterización de cepas de *T. fariai* podría ser de utilidad en la identificación de los mejores candidatos para ser empleados en el control biológico de *T. dimidiata*.
Palabras clave: avispas parasitoides, biología de microhimenópteros, control biológico, control vectorial de la enfermedad de Chagas.

Abstract: Microhymenoptera of the species *Telenomus fariai* (Hymenoptera: Scelionidae) are recognized parasitoids of eggs of kissing bugs. These wasps constitute a potential modulator of populations of *Triatoma dimidiata* (Hemiptera: Reduviidae), the main vector of Chagas disease in Central America. This study describes the infection by *T. fariai* in field-collected eggs of *T. dimidiata* (Hemiptera: Reduviidae). The eggs were obtained in the peridomicile of a house in Tuetal Sur (Alajuela Province, Costa Rica). The wasp progeny, observed 24 h after receiving the eggs, consisted mainly of females (94.0%). The species confirmation was carried out by analysis of the male genitalia and DNA sequences corresponding to a fragment of the cytochrome *c* oxidase subunit 1 mitochondrial gene (COI). The identity of the wasp, based on analysis of sequences, places the local strain in proximity to specimens collected in Veracruz, Mexico. The characterization of strains of *T. fariai* could be useful for the identification of the best candidates to be employed in the biological control of *T. dimidiata*.
Keywords: biological control, Chagas disease vector control, microhymenopteran biology, parasitoid wasp.

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Introduction

Chagas disease is considered a neglected parasitic disease that affects the seven countries of Central America; it is estimated that around 12% of the population lives in risk areas, where *Triatoma dimidiata* (Hemiptera: Reduviidae) is the main vector (Peterson *et al.*, 2019). Despite the elimination of *Rhodnius prolixus* (Hemiptera: Reduviidae), as a result of the Initiative for Chagas Disease Control in Central America, "IPCA" (Hashimoto y Schofield, 2012; Ponce, 2007), the domiciliary infestation by *T. dimidiata* and its presence in sylvatic ecotopes continues to be highly significant (Dorn *et al.*, 2022). For this reason, the search for effective control tools for *T. dimidiata*, as alternatives to traditional insecticide spraying, represents a priority in Central American countries (Dorn *et al.*, 2022).

Microhymenopterans in the family Scelionidae are oophagous parasitoids of insects and arachnids, and, in the case of the subfamily Telenominae, specifically of triatomine bug eggs (Reduviidae: Triatominae) (Austin *et al.*, 2005). *Telenomus farai* Costa-Lima 1927 has been reported as a parasite of eggs in various species of the genera *Triatoma* and *Panstrongylus* (Bosque y Rabinovich, 1979;Costa-Lima, 1928; Peláez, 1944; Pellegrino, 1950a, 1950b; Zeledón, 1957).

The presence of *T. fariai* in Central America has been documented. Previous studies performed in Costa Rica with insects collected in El Salvador revealed phenotypic characteristics, levels of infestation, progeny structure, and the ability of these microhymenopterans to parasitize eggs of *T. dimidiata* of the region (Zeledón, 1957). Considering that *T. dimidiata* is an important vector of Chagas disease in Latin America, the density-dependent population regulation between these triatomines and *T. fariai* represents a potential tool for the biological control of these vectors (Costa Costa-Lima, 1927; Zeledón, 1957). In this context, studies by Zeledón (1957) described that the temperature-dependent development time of *T. fariai* inside *T. dimidiata* eggs was approximately 35 days, and that the average number of microhymenopterans per egg was 6.66 females and 1.15 males, with a relative frequency of adult emergence of 15% for males and 85% for females (Zeledón, 1957). Reports of *T. fariai* parasitizing field-collected eggs of *T. dimidiata* have been recorded in Mexico (Arisqueta-Chablé *et al.*, 2022; Ramírez-Ahuja *et al.*, 2021) and Costa Rica (Zeledón *et al.*, 1970). In addition, recent studies based on molecular analyses have confirmed that populations of *T. fariai* associated with *T. dimidiata* in Central America and Mexico correspond to at least three different haplotypes (Ramírez-Ahuja *et al.*, 2021), and multiple genetic sequences of *T. fariai* from the Pailas Frías - Rincón de la Vieja Volcano (Guanacaste province, Costa Rica) appear deposited in systems such as BOLD BINS, but not associated with any publication. This paper reports the infection of field-collected *T. dimidata* eggs with *T. fariai* in Alajuela, Costa Rica, with combined morphological and molecular analyses to confirm the species. The information presented could support future biological control strategies and contributes to the limited knowledge of this species in the region.

Materials and methods

Collection Site and Entomological Data

As part of a project that receives arthropod samples of medical importance, on June 28, 2022, the Vector Research Laboratory (LIVE) received a group of *T. dimidiata* eggs collected manually from the peridomicile of a house, with a previous positive history of infestation by *T. dimidiata,* located in the town of Tuetal Sur, province of Alajuela, 10,0265 N and -84,2318 W (Figure 1).

The eggs collected were placed in a borosilicate glass vial and observed until *T. dimidiata* nymphs and/or parasitoids emerged. Most of these eggs showed a dark coloration when they were received in the laboratory, and the presence of some microhymenopterans was documented in the container after a couple of hours. After 24 h, the microhymenopterans that emerged were removed, sexed according to the morphology of the antennae, and freeze-inactivated. A small fraction of the females was transferred to Eppendorf tubes for further analysis by polymerase chain reaction (PCR) and sequencing of the cytochrome *c* oxidase subunit 1 mitochondrial gene (COI). The remaining eggs of *T. dimidiata* were cleared in lactophenol and used for the estimation of the intensity of parasitism. The measurement of the length of 34 emerged females was recorded; each specimen was placed on a microscope slide with a drop of Hoyer's medium and measured with a calibrated slide micrometer. The morphological corroboration of the species was done by dissection and observation of the male genitalia, according to criteria previously established (Polaszek y Kimani, 1990), as it is accepted that the male genitalia unquestionably provides the best means of species-level identification (Polaszek y Kimani, 1990). The dissection and mounting of two male genitalia were performed as follows: males were cleared in lactophenol for two weeks and placed in a drop of Hoyer's medium for entomological manipulation. The pleural borders of the metasoma of each male were cut sagittally with minuten pins and then, the tergal and sternal sclerites were

FIGURE 1. Map of the central portion of Costa Rica showing the collection site (Tuetal Sur) of eggs of *Triatoma dimidiata* (Hemiptera: Reduviidae) infected with *Telenomus fariai* (Hymenoptera: Scelionidae).

FIGURA 1. Mapa de la porción central de Costa Rica mostrando el sitio de colecta (Tuetal Sur) de huevos de *Triatoma dimidiata* (Hemiptera: Reduviidae) infectados con *Telenomus fariai* (Hymenoptera: Scelionidae).

separated to expose the genitalia. Each genitalia was transferred to a new drop of Hoyer's medium and covered with a cover glass to complete the mounting process. Voucher specimens of *T. fariai* were deposited in the Medical Entomological Collection of the University of Costa Rica (Facultad de Microbiología, Sección de Entomología Médica).

MOLECULAR IDENTIFICATION

To support morphological identification, genomic DNA from one microhymenopteran specimen was extracted using a NucleoSpin® tissue kit (Macherey-Nagel), according to the manufacturer's instructions. A one-step endpoint PCR protocol was applied to amplify a fragment of the COI gene with primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR conditions were 95 °C for 1 minute for primary denaturation, followed by 35 cycles at 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1.5 minutes, and a final extension at 72 °C for 7 minutes. The product of amplification was visualized on 1.0% agarose gel electrophoresis. The amplicon was purified with Exonuclease I (EXO I) and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific Inc.) and sequenced in both directions at Macrogen, Inc. (Seoul, South Korea). The software BioEdit Sequence Alignment Editor version 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used to assemble the sequences, and BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search the closest sequences with the consensus sequence. First, the protein sequences of the COI gene were aligned using MAFFT (Katoh y Standley, 2013). The corresponding nucleotide sequences downloaded from GenBank (MZ810544 and MZ810543; https://www.ncbi.nlm.nih.gov/genbank/) and BOLD BINS (BOLD:ADW5671, BOLD:ADB0583; https://v3.boldsystems.org/) of the aligned protein sequences, subsequently were aligned codon by codon, using the PAL2NAL (Suyama *et al.*, 2006). *Trissolcus brochymenae* was included as an outgroup based on its position as sister to *Telenomus* sp. Phylogenetic analyses were performed on maximum likelihood (ML) to determine the relationship between *Telenomus* specimens. The TIM2+F was determined to be the most suitable model in maximum likelihood (ML) analyses using IQ-TREE web server, version 1.6.12; (Nguyen *et al.*, 2015). Branch support was estimated with 1000 ultrafast bootstrap replicates.

RESULTS

MORPHOLOGICAL IDENTIFICATION

The sample of triatomine eggs received in the laboratory consisted of 29 eggs. After 24 hours, 36 wasps emerged from 6 eggs (Fig. 2), 16 were still parasitized, and 7 were uninfected. The total egg parasitism rate was 76%, with an average of 5.5 wasps per egg.

The average number of microhymenopterans per egg was corroborated by direct visualization of the individuals in the parasitized eggs (Fig. 3).

Of the emerging wasp progeny, 34 were females (94.0%), and only 2 were males (6.0%). The average length of the females was 1.12 ± 0.093 mm. The morphology of the male genitalia presented the following characteristics, compatible with descriptions of *T. fariai*: basal ring (Br) measuring nearly a third of the aedeagus length (Ae) and less the half of the length of the aedeagus volsellar shaft

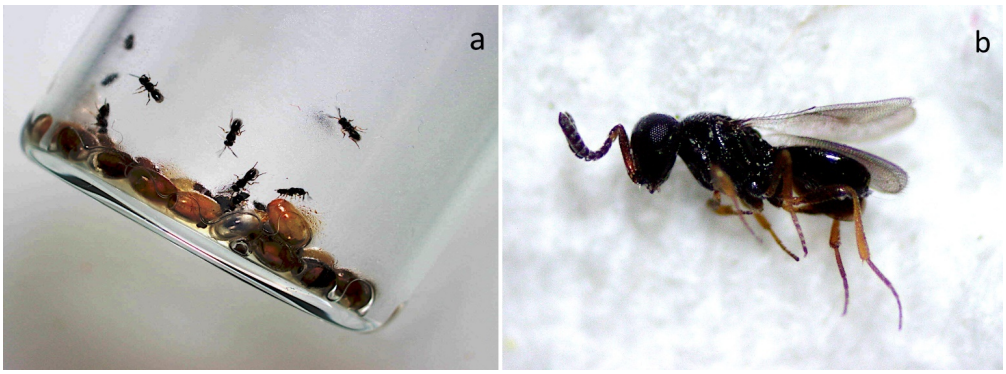

FIGURE 2. *Telenomus fariai* (Hymenoptera: Scelionidae) adults hatched from *Triatoma dimidiata* (Hemiptera: Reduviidae) eggs. a: emerging parasitoids, b: female in lateral view.

FIGURA 2. Adultos de *Telenomus fariai* (Hymenoptera: Scelionidae) eclosionados de huevos de *Triatoma dimidiata* (Hemiptera: Reduviidae). a: parasitoides emergentes, b: vista lateral de la hembra.

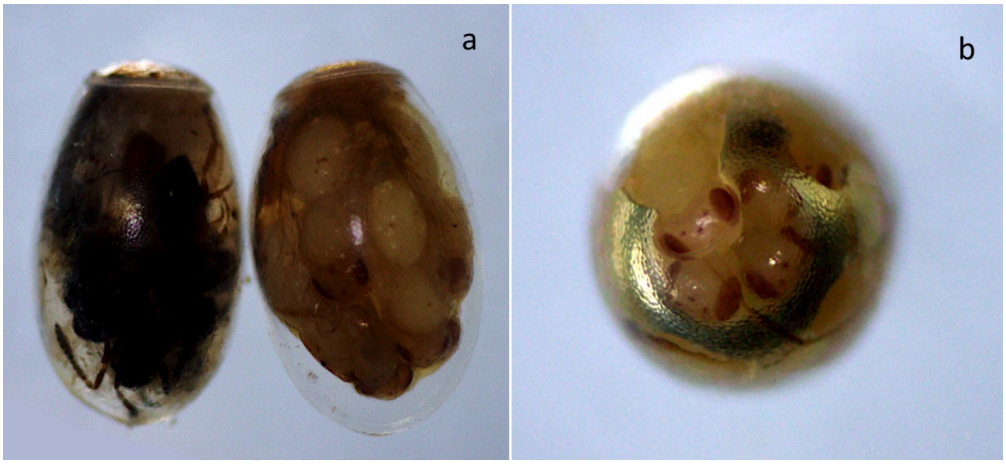

FIGURE 3. Direct visualization of the microhymenopterans in the parasitized eggs. **a:** lateral view, **b:** ventral view.

FIGURA 3. Visualización directa de las microhimenópteros en los huevos parasitados. **a:** vista lateral, **b:** vista ventral.

(Vs); two large digits (Di), each one with 3 small teeth at the latero-distal border; laminae volsellares (Lv) with elongated plates sclerosed in the medial margin; and aedeagal lobe (Al) short and truncated at the tip (Fig. 4).

MOLECULAR IDENTIFICATION

A COI partial sequence of 554 bp was recovered from one specimen submitted for molecular identification. BLAST analyses showed high similarity (97.6% - 98.0%) with a sequence identified as *T. fariai* from Mexico (GenBank accession numbers: MZ810544 and MZ810543). The consensus sequence of *T. fariai* generated in this study was deposited in GenBank as *T. fariai* isolate Tf-CR2022 with the accession number OQ448504.

The phylogenetic tree (Fig. 5), using the sequences obtained and additional *Telenomus* species available in GeneBank and BOLDSystems showed that all the species belonging to *Telenomus* were clustered together, and *T. fariai* clustered with members of the same species in the genus *Telenomus,* forming a well-supported group. Our specimens of *T. fariai* clustered with a sister group of homologs from Guanacaste, Costa Rica (BOLD: ADB0583 and BOLD: ADW5671).

DISCUSSION

This constitutes the report of a case of field-collected *T. dimidiata* eggs infected with *T. fariai* oophage microhymenopterans in Costa Rica. The identity of the analyzed *T. fariai* matched, both morphologically and genetically, with the same species of microhymenopterans described in Mexico (Arisqueta-Chablé *et al.*, 2022; Ramírez-Ahuja *et al.*, 2021), as well as COI gene sequences reported in other locations of Costa Rica (Guanacaste province), suggesting that they are all conspecific haplotypes. Previous experiments, using *T. fariai* from El Salvador in Costa Rica or laboratory

populations from Costa Rica, reveal the presence of this hymenopteran in the country under controlled conditions (Bosque y Rabinovich, 1979; Zeledón, 1957). Furthermore, Zeledón (1981) mentions unpublished experiments where *T. fariai* wasps were released in San Rafael de Ojo de Agua (approximately 10 km from Tuetal Sur), Alajuela, to study different biological traits and their ability to control populations of *T. dimiata* during a three-year period (Zeledón, 1981). It is unknown if the specimens evaluated in the present study could be remnants of the population that was intentionally released at that time, or if they constitute populations that are naturally present in the area even before the reported releases.

Observed biology traits of the collected *T. fariai* were in accordance with the ones reported by other authors, such as egg parasitism over 70% and proportions higher than 85% of females emerging from the parasitized eggs (Monroy-Escobar *et al.*, 1998; Ramírez-Ahuja *et al.*, 2021; Zeledón, 1957). This level of parasitism allows us to intuit the high effectiveness of *T. fariai* in recognizing *T. dimidiata* eggs as a target for oviposition, although it is not clear if this effectiveness can significantly impact the wild populations of the bug. Previous experiments done by Zeledón (1981) in Costa Rica, concluded that after a long-term release of the microhymenopteran, the parasitism was constant but low, denoting a balance in the system that favors the persistence of both the microhymenopteran and *T. dimidiata* (Zeledón,

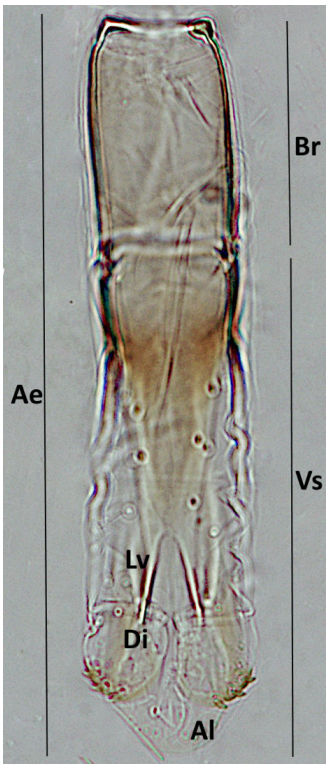

FIGURE 4. Morphology of the male genitalia of *Telenomus fariai* (Hymenoptera: Scelionidae). **Ae**: aedeagus, **Br**: basal ring; **Vs**: aedeagus volsellar shaft; **Lv**: laminae vollselares; **Di**: digit, **Al**: aedeal lobe.

FIGURA 4. Morfología de la genitalia masculina de *Telenomus fariai,* **Ae**: edeago, **Br**: anillo basal; **Vs**: eje volselar del edeago; **Lv**: láminas volselares; **Di**: dígito, **Al**: lóbulo del edeago.

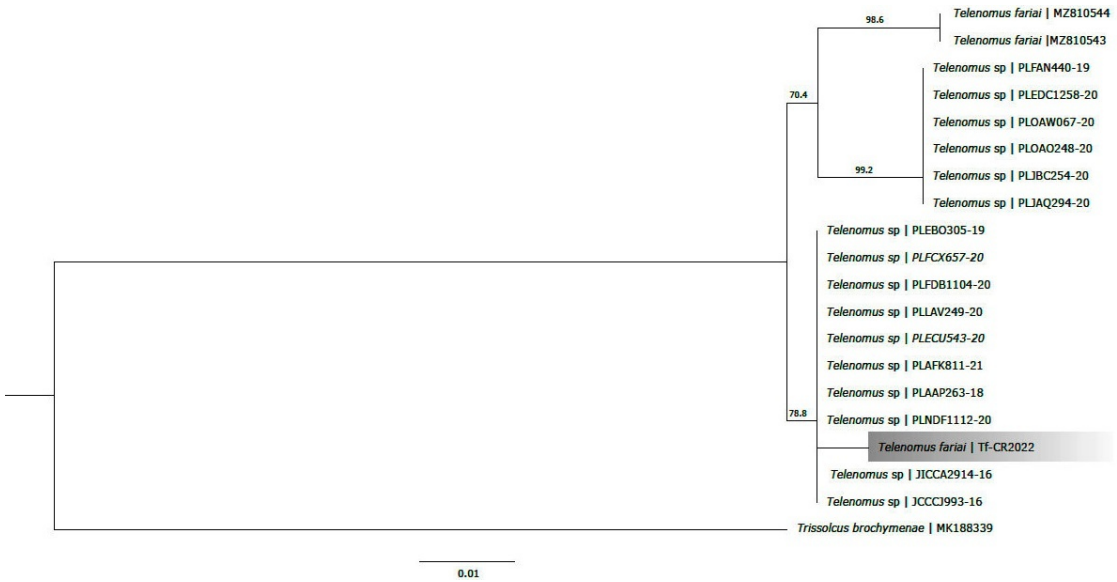

FIGURE 5. Maximum-likelihood phylogenetic analysis of *Telenemos fariai* (Hymenoptera: Scelionidae) and related species based on TIM2+F model using a partial fragment of the COI gene.

FIGURA 5. Análisis filogenético de *Telenemos fariai* y especies relacionadas basado en el modelo TIM2+F y el método de máxima verosimilitud utilizando un fragmento parcial del gen COI.

1981). From a practical perspective, it is accepted that the effectivity of the wasp parasitism requires the synchrony of the female-adult stage of *Telenomus* and the oviposition of *Triatoma* (Menu *et al.*, 2010). This coincidence of requirements represents a challenge for biological control based on the liberation of parasitoids.

Another important aspect to consider is that the interaction between both species may vary between tropical and temperate zones. In temperate zones, like Argentina, the main mortality of *Triatoma infestans* eggs is attributable to climatic effects associated with the low temperatures in the austral winter and this aspect was more important than the action of egg parasitoids such as *T. fariari* (Gorla y Schofield, 1985). According to Gorla (2020), previous experiments, also conducted in the 1980s, showed that *T. fariai* was ineffective at regulating *Triatoma infestans* population abundance under field conditions (Gorla, 2020). Although the conclusions of these studies suggest that the use of this parasitoid is not effective for the control of triatomine bugs, its synergistic use along with other control measures has been suggested (Monroy-Escobar *et al.*, 1998). A study of biological characteristics of *Telenomus fariai rabinovichi* performed in Guatemala, recommends the use of this parasitoid wasp in the control of *T. dimidita* six months after the application of insecticide treatment (Monroy-Escobar *et al.*, 1998). This represents a complementary action of vector control that would prevent the development of insecticide resistance in *T. dimidiata* populations, but the actual impact on wild populations of different regions and the logistics of its implementation must be assessed.

Genetic evidence supports the existence of at least three different haplotypes of *T. fariai* in Central America and Mexico (Ramírez-Ahuja *et al.*, 2021), but further studies are required to validate these haplotypes and characterize them in terms of parasite efficiency.

In conclusion, Costa Rica and the rest of Central America show favorable conditions for the biology of *T. fariai*. The dynamics of its reproductive cycles are linked to the populations of triatomine bugs, mainly *T. dimidiata*. The future selection and release of more infective strains of *T. fariai* could complement residual insecticide spraying in the control of this Chagas disease vector, which may also aid in preventing insecticide resistance.

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