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First record of *Telenomus fariai* Costa Lima, 1927 (Hymenoptera, Scelionidae, Telenominae) as a parasitoid of *Triatoma dimidiata* (Latreille, 1811) (Hemiptera, Reduviidae, Triatominae) eggs in Mexico

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Abstract

The egg parasitoid *Telenomus fariai* Costa Lima (Hymenoptera, Scelionidae), is reported for the first time in Veracruz, Mexico. *Telenomus fariai* was discovered in 2019 during a field collection of *Triatoma dimidiata* L. (Hemiptera, Reduviidae), representing the first report of its association with *Tr. dimidiata* in Mexico. This species is here redescribed and sequencing of a portion of the cytochrome oxidase 1 gene (COI) was performed to facilitate future identifications and to examine host associations between species of *Telenomus* Haliday and Reduviidae in a broader context.

Keywords

Biological control, COI, triatomines

Introduction

Wasps in the family Scelionidae (Hymenoptera, Platygastroidea) are endoparasitoids of insect and arachnid eggs. They therefore play a fundamental role as natural enemies of Coleoptera, Diptera, Embiidina, Hemiptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera, and Araneae (Masner and Hanson 2006). The subfamily Telenominae contains the most important species for biological control of insect pests of agricultural, forestry and medical importance, primarily from the orders Hemiptera and Lepidoptera (Johnson 1984, 1987). This lineage has a strong association with Hemiptera, and several species are known to attack the eggs of reduviids (Taekul et al. 2014). Masner (1975) described two species reared from the eggs of *Triatoma* Laporte: *Hadronotus triatomae* (Masner) and *H. linshcostei* (Masner). These were originally treated as species of *Gryon* Haliday but have since been placed in *Hadronotus* Förster (Talamas et al. 2021). Johnson (1984) described two species reared from the eggs of *Zelus* Fab., *Te. sulculus* Johnson and *Te. zeli* Johnson. *Telenomus fariai* was reported to parasitize the eggs of *Triatoma infestans* Klug, and *Panstrongylus megistus* (Burmeister) from Brazil (Pellegrino 1950). Zeledon (1957) demonstrated in experimental tests with *Te. fariai* from San Salvador, El Salvador, that the eggs of triatomine colonies maintained at the University of Costa Rica, including *Tr. dimidiata* from Costa Rica, *Tr. phyllosoma* (Burmeister) from Mexico, *Panstrongylus chinai* (Del Ponte) from Ecuador, *P. megistus* from Brazil, were preferred over eggs of *Tr. infestans* from Chile, *Rhodnius prolixus* Stål, 1859 from El Salvador and *R. palescens* Barber from Panama.

Triatoma dimidiata is considered one of the most important vectors of Chagas disease in southern Mexico, Central America and even in northern South America, second only to *Tr. infestans* (Dorn et al. 2017). This species has been reported in the state of Veracruz, Mexico by Sandoval-Ruiz et al. (2014) who reported a colonization index (percentage of infested houses where nymphs were found) of 88% in the location of Estacion Chavarrillo in the municipality of Emiliano Zapata.

Despite the medical relevance of these bugs and the potential for *Te. fariai* to control them, the only taxonomic treatment of *Te. fariai* is the original description by Costa Lima (1927). De Santis et al. (1980) stated that the characters provided by Costa Lima (1927) were not very detailed, making it difficult to identify the species, and their identifications were based on an examination of his specimens. However, they did not present any further information by which future workers might more reliably identify the species. We also found the original description of *Te. fariai* to be insufficient for identification and our identification was made by comparison to specimens identified by Costa Lima. Because reliable identification of parasitoids is essential for biological control efforts, including those that manage insects of medical importance, we here provide a new description of *Te. fariai*, images of males and females, and the COI barcoding sequences for both sexes. Our treatment of this species will facilitate future examination of intraspecific variation and the possibility of cryptic species, which may be relevant concepts for *Te. fariai*, given that De Santis et al. (1980) separated it into

subspecies. It should be noted, that De Santis et al. (1980) mentioned that *Te. fariai* is distributed from Mexico to Argentina, but these records are uncertain because data on the hosts and locations were not provided.

Material and methods

Rearing

The parasitoids were reared from eggs of *Tr. dimidiata* that were collected outdoors in the area of Chavarrillo, Emiliano Zapata in the state of Veracruz, south of Mexico (19°25'30.378"N, 96°47'56.767"W). Fifty-six *Tr. dimidiata* eggs were taken to the laboratory (HR 70%, T 25 ± 2 °C) and placed into Petri dishes. Observation were made until *Tr. dimidiata* nymphs and parasitoids emerged. Parasitoids were placed in 96% ethanol for morphological and molecular analyses. The number of eggs and sex ratio of the parasitoids was recorded.

Identification

We used the descriptions of Costa Lima (1927) and De Santis et al. (1980) in combination with the host information to make an initial identification of *Te. fariai*. This was followed by comparison to images of a female specimen of *Te. fariai* that was identified by Costa Lima (USNMENT01795654). We were unable to contact curatorial staff at Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, where the primary types are housed, and thus were not able to compare our specimens to primary type material. For comparison purpose, we used the specimens of *Te. sulculus* and *Te. zeli* reared from eggs of *Zelus renardii* Kolenati 1857 and *Zelus* sp., respectively, collected in corn crops in Sinaloa, Mexico, and were identified using the key in Johnson (1984). Specimens of *Tr. dimidiata* were identified using the keys of Lent and Wigodzinsky (1979).

Morphology

Slides of male genitalia were prepared following the protocol of Polaszek and Kimani (1990), which consists of permanent preparations in Canada balsam (Sigma-Aldrich, St. Louis, MO). Morphological terminology follows that of Johnson (1984) and Talamas et al. (2017).

Collections

Voucher specimens of *Te. fariai* are deposited in the "Coleccion de Insectos Benefi-cos Entomofagos" (Facultad de Ciencias Biologicas-Universidad Autonoma de Nuevo Leon, Mexico) and the Florida State Collection of Arthropods, Gainesville, Florida (FSCA), all of which have identical collection data. Specimens deposited

in FSCA, including the molecular vouchers of *Te. fariai*, *Te. sulculus* and *Te. zeli*, have been assigned collecting unit identifiers and the associated data is available at mbd-db.osu.edu.

Imaging

Photographs were produced with a Macropod imaging system using 10X and 20X objective lenses, with images rendered with Helicon Focus. Dissections for scanning electron microscopy were performed with a minuten probe and forceps. Body parts were mounted to a 12 mm slotted aluminum mounting stub using a carbon adhesive tab and sputter coated with approximately 70 nm of gold/palladium using a Denton IV sputter coater. Micrographs were captured using a Phenom XL Desktop SEM.

DNA barcoding

Genomic DNA was nondestructively isolated from whole specimens of *Te. fariai*, *Te. zeli*, and *Te. suculus* using the Qiagen DNeasy kit (Hilden, Germany) as described by Giantsis et al. (2016). PCRs were carried out to amplify the DNA barcode region of the cytochrome oxidase subunit I (COI) using the LCO/HCO primers of Folmer et al. (1994). The PCRs were performed in a 25 µl reaction volume using the KAPA HiFi Hotstart Ready Mix (Roche) per the manufacturer's standard protocol. PCR conditions were as follows: 95 °C for 2 min, followed by 32 cycles of 95 °C for 30 s, 50 °C for 40 seconds, 72 °C for 1 min with a final extension at 72 °C for 7 min. The fragments to be amplified by PCR were separated by electrophoresis on 1.5% agarose gels. After verification, the samples were sequenced at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida. Newly generated barcodes submitted to GenBank are listed in Table 1.

Molecular analysis

As there is no previous record of the sequence in GenBank of the CO1 region of *Te. fariai* we undertook the task of determining this through phylogenetic analysis comparing with other nearby species. All available COI-5P barcodes from *Telenomus* were downloaded from BOLD along with the two BOLD BINS nearest to *Te. fariai* (Ratnasingham and Hebert 2007). This database query returned over 14,000 *Telenomus* barcodes. *Baeoneurella* Dodd was selected as the outgroup based on its position sister to *Telenomus* in Chen et al. (2021). These sequences were aligned using MAFFT (Katoh and Standley 2013) with FFT-NS-1 settings. The resulting alignment was then trimmed of short sequences that confounded neighbor-joining (NJ) analysis. The final alignment consisted of 14,580 terminals (640 bp) which were used for a NJ analysis (Suppl. material 1). The NJ analysis was conducted in MEGAX (Kumar et al. 2018) with the following settings: 1) K2P model (Kimura 1980) including transitions and transversions, 2) uniform rates among sites, and

Table 1. Specimens for which new DNA barcodes data were generated.

Species	Sex	CUID	GenBank Accession
Te. fariai	female	FSCA 00091164	MZ810543
	male	FSCA 00091165	MZ810544
Te. sulculus	female	FSCA 00091160	MZ905522
	male	FSCA 00091161	MZ905523
Te. zeli	female	FSCA 00091158	MZ905520
	male	FSCA 00091159	MZ905521

3) partial deletion of missing data with a site coverage cutoff at 95%. The resulting Newick tree file (Suppl. material 2) was manipulated in the Interactive Tree of Life portal [https://itol.embl.de/] (Letunic and Bork 2021) to collapse terminal clusters and highlight taxa with heteropteran hosts. Host data was taken from Taekul et al. (2014) and Johnson (1984).

Results

Rearing

Sixty-six specimens of *Te. fariai* (48 females, 18 males) emerged from 48 of the 56 *Tr. dimidiata* eggs. This represented an egg parasitism rate of 86% (48/56) with an average of 1.4 parasitoids per egg and a female:male sex ratio of 2.67:1. These results are consistent with previous studies that documented that over 70% of the eggs were parasitized with a sex ratio of 2.7:1 (Fernandes et al. 1990) and that superparasitism occurred with an average of 1.36 parasitoids per egg (Stehr 1990).

Telenomus fariai Costa Lima

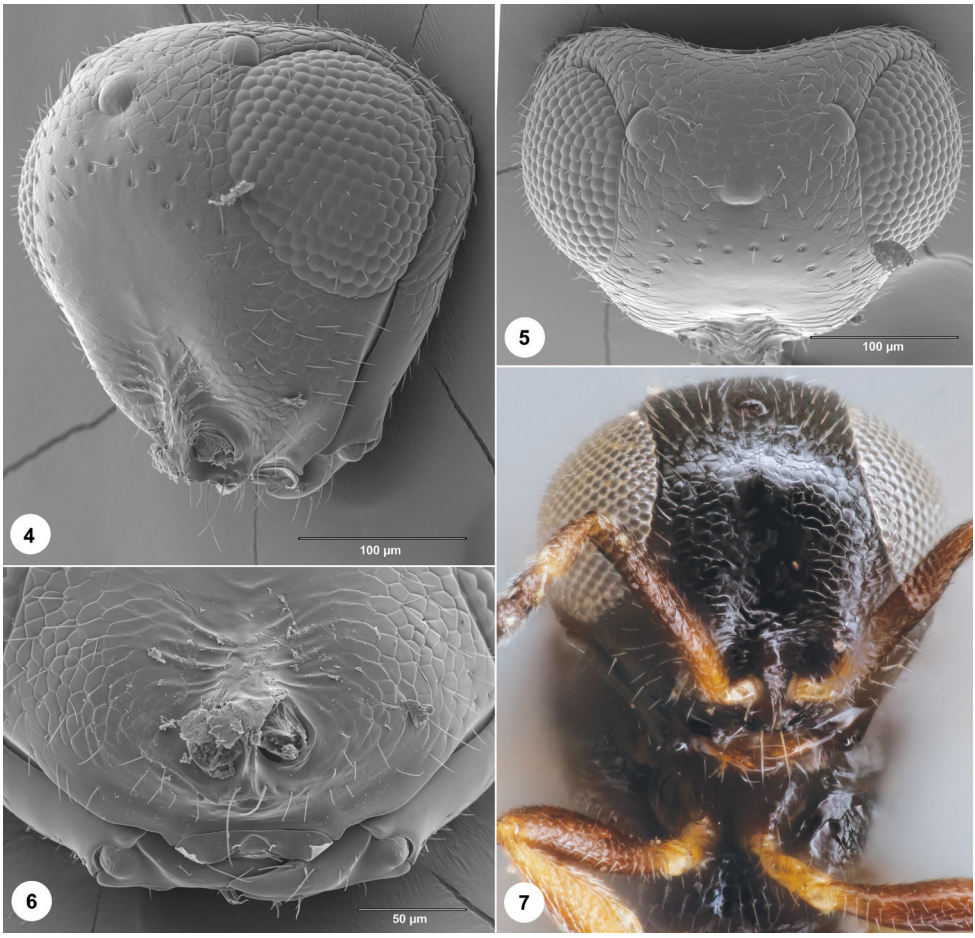
Figures 1–15

Telenomus fariai Costa Lima, 1927: 451 (original description)
Telenomus fariai Costa Lima: De Santis, de Regalia, de Silva & de Larramendy, 1980: 197 (key to subespecies), Johnson, 1992: 587 (cataloged, type information)
Telenomus fariai Rabinovichi De Santis & Vidal Sarmiento, 1980: 198 (original description).
Telenomus fariai fariai Costa Lima: De Santis, de Regalia, de Silva & de Larramendy, 1980: 198 (description).
Telenomus fariai rabinovichi De Santis & Vidal Sarmiento: Loiácono & Díaz, 1996: 10 (type information).

Description. Body length of male: 0.75–0.91 mm (n = 4). Body length of female: 0.87–1.05 mm (n = 10) color of body: dark brown to black: color of legs: coxae and femora brown; trochanters, tibiae and tarsi yellow to pale brown: color of antenna in female: brown.

Figures 1–3. *Telenomus fariai* **1** female (USNMENT01795654), identified by Costa Lima, reared from *Triatoma* eggs in Brazil, lateral habitus **2** female (FSCA 00091164), reared from *Tr. dimidiata* in Mexico **3** male (FSCA 00091165), reared from *Tr. dimidiata* in Mexico.

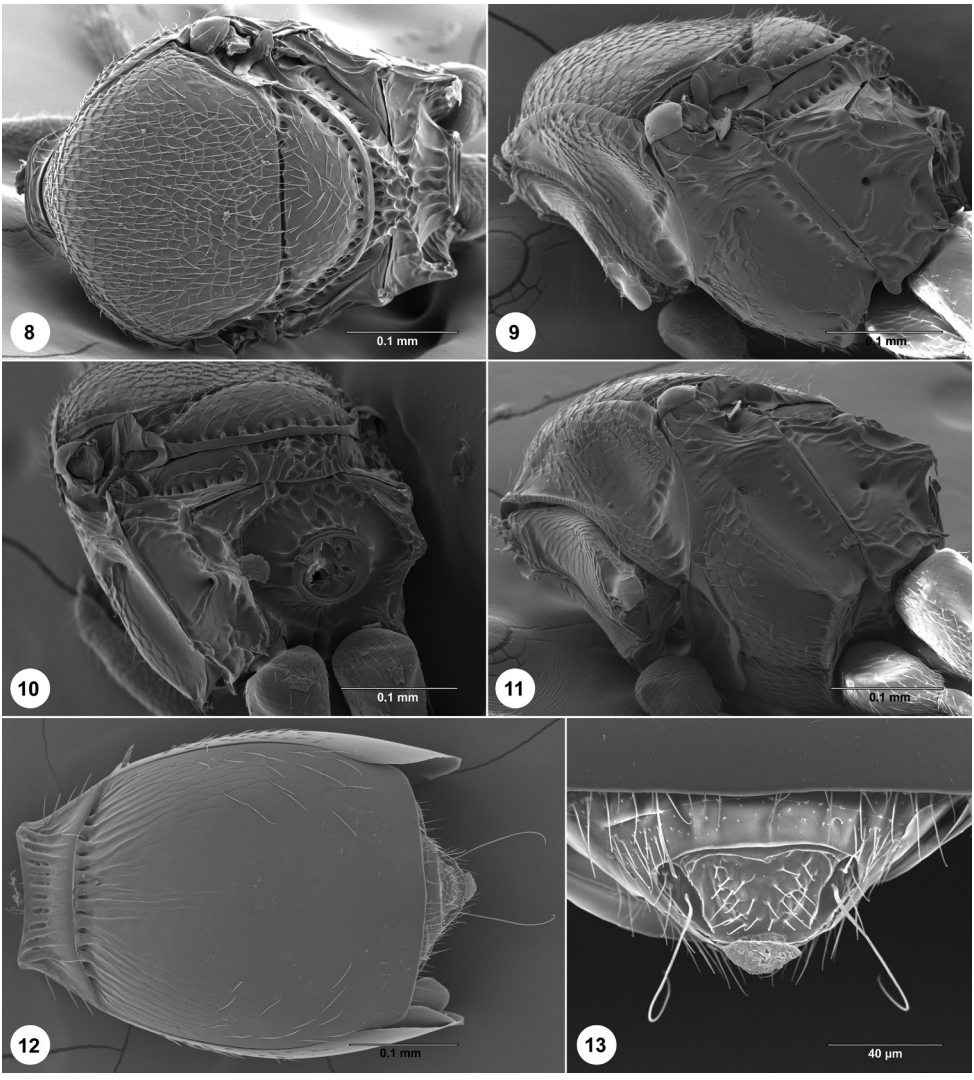
Head. Claval formula: 1-2-2-1. Number of mandibular teeth: 2, dorsal tooth the largest. Labium: transverse with median notch. Shape of clypeus: concave, apical margin straight, not dentate, not protruding anteriorly. Number of clypeal setae: 6, dorsal pair distinctly longer. Central keel: absent. Sculpture of frons: with arcuate rugae present around interantennal process, otherwise smooth or with coriaecious microsculpure, setal bases punctate. Frontal depression: weakly developed, frons not bulging between antennal insertions and inner orbits. Compound eyes: with short setation throughout, inner orbits rounded at the level of lateral ocelli. Lateral ocellus:



Figures 4–7. *Telenomus fariai* **4** female (FSCA 00091199), head, anterolateral view **5** female (FSCA 00091199), head, dorsal view **6** female (FSCA 00091199), ventral frons, anterior view **7** female (USNMENT01795654), head and mesosoma, anterior view.

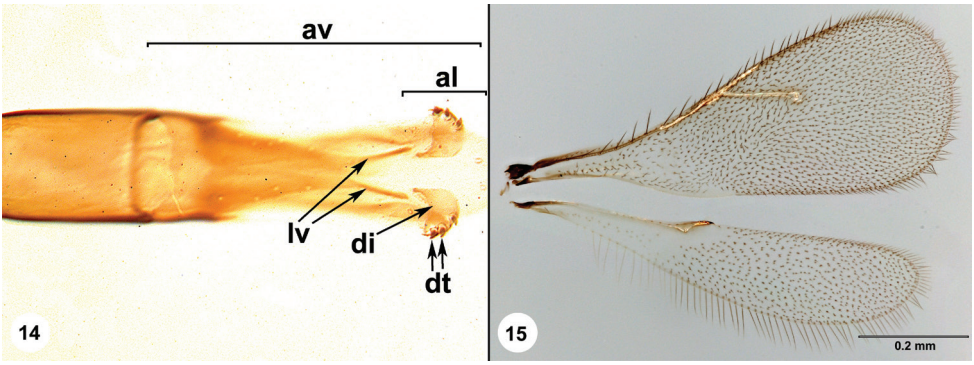
contiguous with compound eye. Ocellar setae: absent. Sculpture of vertex: shallowly and evenly coriaceous. Shape of vertex: rounded, without hyperoccipital carina. Occipital carina: complete, extending to base of mandibles ventrally, continuous dorsally. Anterior margin of occipital carina: weakly crenulate dorsally, smooth laterally and ventrally. Sculpture of gena: shallowly and evenly coriaceous.

Mesosoma. Epomial carina: absent. Sculpture of lateral pronotum: coriaceous microsculpture anterior to netrion. Netrion sulcus: present, weakly defined medially. Sculpture of netrion: smooth. Setation of mesoscutum: evenly covered with white setae throughout. Sculpture of mesoscutum: scaly reticulate microsculpture. Mesoscutal suprahumeral sulcus: absent. Mesoscutal humeral sulcus: present as a smooth furrow. Interior of axillar crescent: smooth. Setation of mesoscutellum: evenly covered with white setae throughout. Sculpture of mesoscutellar disc: smooth. Posterior mesoscutellar sul-



Figures 8–13. *Telenomus fariai*, female (FSCA 00091199) **8** mesosoma, dorsal view **9** mesosoma, lateral view **10** mesosoma, posterolateral view **11** mesosoma, ventrolateral view **12** metasoma, dorsal view **13** T6–T7, dorsal view.

cus: foveate. Sculpture of metascutellum: rugose throughout. Metanotal trough: foveate, foveae less than half the lenght of the metanotum. Number of setae in metanotal trough: 3–4. Acetabular carina: present, wide and flat dorsally and directly posterior to fore coxa. Intercoxal space: greater than the length of fore coxa. Setation of ventral mesopleuron: densely setose surrounding mesodiscrimen. Episternal foveae: absent. Sculpture of anteroventral mesopleuron: scaly reticulate microsculpture. Sculpture of femoral depression: smooth. Mesopleural carina: weakly indicated in posteroventral corner of mesopleuron. Prespecular sulcus: indicated by shallow foveae. Sculpture of speculum:



Figures 14–15. *Telenomus fariai* **14** male genitalia, ventral view **15** female (FSCA 00091164), wings, dorsal view. Annotations. al: aedeagal lob; av: aedeago-volsellar shaft; di: digitus; dt: digital teeth; lv: laminae volsellares.

transversely rugulose. Mesepimeral sulcus: comprised of circular foveae. Sculpture of posterior mesepimeral area: smooth. Paracoxal sulcus: absent. Metapleural epicoxal sulcus: indicated by a line of shallow foveae. Anteroventral extension of the metapleuron: blunt, triangular, extending to base of mesocoxa. Sculpture of metapleuron: variably rugose in posterior and ventral portions, anterior part of dorsal metapleuron mostly smooth. Lateral propodeal area: mostly smooth with some rugae. Metasoma depression: with sparse rugae radiating from surrounding carinae and from propodeal foramen.

Wings. Length of postmarginal vein in fore wing: twice as long as stigmal vein.

Metasoma. Sculpture of T1: striate throughout. Number of sublateral setae on T1 (on one side): 1. Number of lateral setae (on one side): 3 or 4. Sculpture of T2: faint striation extending from basal costae, striae in lateral portion and along midline extending half the length, otherwise smooth. Length of T2: about 4/5 the length of the metasoma. Setation of T2 (mediotergite): sparse, present in a broad patch located in lateral third, roughly one half the length of the tergite. Setation of laterotergite 2: present in a patch adjacent to setose area on mediotergite. Sculpture of T7: rugulose; setation of T7: short and dense. Setation of S2: sparse and evenly distributed in area between laterotergites. Sculpture of S6: densely punctulate. Setation of S6: dense.

Male genitalia. Number of digital teeth (on each side): 3. Size of digital teeth: small. Laminae volsellares: elongated plate with lateral indications of more intensely sclerotized rods. Aedeagal lobe: very short and rounded apically.

Material examined. Brazil: Rio de Janeiro, ex. Triatoma eggs (1♀, USNMENT01795654). **Mexico**: Estacion Chavarrillo, Municipality Emiliano Zapata, state Veracruz, 13-VII- 2019; Cristina Bobadilla-Utrera (CIBE 19-032), (48♀, 18♂, FSCA 00091145, 00091154, 00091164–00091165, 00095774–00095780, 00095785–00095786).

Species-group placement. *phymatae*-group.

Host(s). *Panstrongylus chinai*, *P. megistus*, *P. herreri*, *Tr. brasiliensis* Neiva, *Tr. dimidiata*, *Tr. infestans*, *Tr. pallidipennis* (Stal), *Tr. phyllosoma*, *Tr. maculata* (Erichson),

Tr. rubrovaria (Blanchard, in Blanchard & Bulle), *Tr. sordida* (Stal), *Tr. tibiomaculata*, *Tr. vitticeps* (Stal), *Rhodnius prolixus*, *R. palescens* (Zeledon 1957; Ravinovich 1971).

Comments. The specimens of *Te. fariai* from Mexico (Figures 2–6, 8–15) match the specimen from Brazil (Figures 1, 7) in every character that we could assess. The degree to which the frons is covered in microsculpture appears to vary within the species, as it may cover the frons (Figure 7) or be present only in the areas surrounding the antennal scrobe (Figures 4–5).

DNA barcoding

We generated two COI sequences for male and female pairs of *Te. fariai*, *Te. sulculus*, and *Te. zeli*, and for each species the male and female sequences were identical (Table 1). The *Te. fariai* sequences matched two BOLD BINS from Costa Rica in the Barcode of Life Database (BOLD:ADW5671, BOLD:ADB0583) at approximately 97.4% identity. The images associated with these records do not allow for a detailed morphological comparison, but to the extent that characters can be seen, they are congruent with *Te. fariai*, supporting the notion that this is a widespread Neotropical species.

Molecular analysis

The NJ analysis included a total of 498 nucleotide positions and returned an optimal tree with the sum of branch lengths equaling 21.82 (Figures 16A–B). *Telenomus* species associated with Reduviidae did not cluster together in our NJ tree.

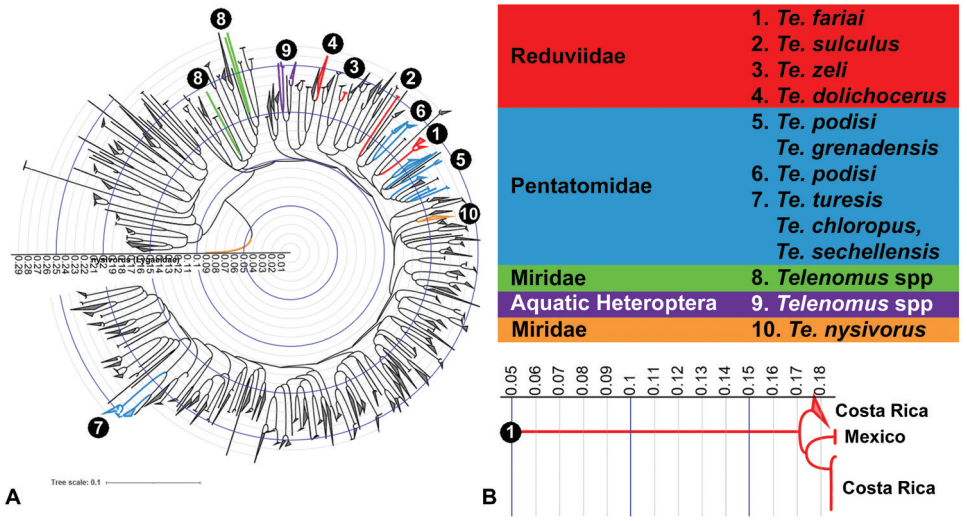


Figure 16. A neighbor joining tree of all *Telenomus* COI sequences in BOLD with heteropteran host associations annotated by color **B** pruned branch of *Te. fariai*.

However, they were all retrieved near species that parasitize the eggs of Heteroptera. This not surprising, given that Heteroptera was considered by Taekul et al. (2014) to be the ancestral host for *Telenomus* and the clades that contain most of these species are found near the base of the tree. Many branches without host data are present between the species that attack reduviid eggs (Figure 16A, in red). These data are needed to further interpret how parasitism of Reduviidae has evolved in *Telenomus*.

The COI data placed Mexican specimens of *Te. fariai* within a clade of specimens from Costa Rica (Figure 16B). Based on sequence similarity, we consider the three haplotypes in Figure 16B to be conspecific.

Discussion

Telenomus fariai has been reported as the most important biological control agent of *Triatoma* species in Central and South America (Zeledon 1957). Monroy (1998) proposed the use of *Te. fariai* as an alternative to the application of insecticides for the control of *Tr. dimidiata*, given that he observed a parasitism rate 5.7 eggs per female in the laboratory. Other authors report for *Te. fariai* parasitism rates of 14–15% in field conditions (Pellegrino 1950; Barrett 1976; Schofield 1979) and some studies have reported the capacity of *Te. fariai* to parasitize up to 60% of *Tr. infestan*s in short periods (Gorla 1994; Noya 2019). Other studies have addressed aspects of its biology (Pellegrino 1950; Fernandes et al. 1990) despite there being no detailed description or diagnosis for *Te. fariai*. The association of *Te. fariai* with triatomines was used to help identify this parasitoid wasp in previous studies, as well as in this work. However, multiple species of *Telenomus* have been reported from triatomine eggs (De Santis et al. 1980) and determining the degree to which species are host specific will require integration of species-level taxonomy with studies of parasitoid biology. To that end, we have here provided a step forward for the systematics of these parasitoids that will facilitate future studies. We hope that future efforts will be able to examine type specimens. This is essential to confirm identification of *Te. fariai* and to characterize the other species listed in De Santis et al. (1980), such as *Te. costalimai* Ortiz & Alvarez, and *Te. capito* De Santis & Loiácono.

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Supplementary material 1

Sequences of COI barcodes from *Telenomus* and *Baeoneurella* outgroup

Authors: Elijah J. Talamas, Matthew R. Moore

Data type: molecular data

Explanation note: Alignment of over 14,000 *Telenomus* barcodes.

Link: https://doi.org/10.3897/jhr.87.73546.suppl1

Supplementary material 2

Newick tree file

Authors: Elijah J. Talamas, Matthew R. Moore

Data type: phylogenetic data

Explanation note: Molecular phylogenetic analysis of *Telenomus COI* sequences.

Link: https://doi.org/10.3897/jhr.87.73546.suppl2