

# Molecular characterization of *Encarsia inaron* (Walker, 1839) naturally associated with *Bemisia tabaci* (Gennadius, 1889) in Brazil

*Caracterización molecular de Encarsia inaron* (Walker, 1839) asociada naturalmente con *Bemisia tabaci* (Gennadius, 1889) en Brasil

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## ABSTRACT

This work is the first record of *Encarsia inaron* (Walker, 1839) (Hymenoptera: Aphelinidae) parasitizing *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) on soybeans in Brazil. Morphological and molecular characterizations are provided, in addition to verification of the presence of *E. inaron* in areas infested by whiteflies, indicating that the wasp can potentially be used in integrated management programs. Additionally to morphological verification, the molecular analysis allowed the identification of some degree of genetic variability in the genome of the parasitoid.

**Keywords:** biological control; natural enemy; parasitoid; whiteflies; integrated pest management.

## RESUMEN

Este es el primer registro de *Encarsia inaron* (Walker, 1839) (Hymenoptera: Aphelinidae) parasitando *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) en soja en Brasil. Se proporcionan caracterizaciones morfológicas y moleculares, además de la verificación de la presencia de *E. inaron* en áreas infestadas por mosca blanca, lo que indica que la avispa puede potencialmente ser utilizada en programas de manejo integrado. Además de la verificación morfológica, el análisis molecular permitió identificar algún grado de variabilidad genética en el genoma del parasitoide.

**Palabras clave:** control biológico; enemigo natural; parasitoide; moscas blancas; manejo integrado de plagas.

## Introduction

Problems with *Bemisia tabaci* (Gennadius 1889) (Hemiptera: Aleyrodidae), one of the main threats to agriculture worldwide, began increasing two decades ago. This sap-sucking insect can feed on more than 600 host plants (Polston *et al.*, 2014) and is characterized as a supervector involved in transmitting more than 300 species of virus (Gilbertson *et al.*, 2015). The species *Bemisia tabaci* was initially classified into two biotypes; however, it is now considered to be a complex of cryptic species that differ genetically, ecologically, and biologically but are morphologically identical (De Barro *et al.*, 2011, Kanakala and Ghanim, 2015, Geng and Li, 2017).

The consensus among the scientific community does not exist in identifying *B. tabaci* due to the lack of distinguishing characters among these cryptic species. However, molecular analyses so far have revealed 43 cryptic species based on the rule concerning 3.5% divergence in the mitochondrial cytochrome oxidase I (mtCOI) gene (Boykin and De Barro, 2014). Among these cryptic species, those species that cause worldwide concern are the Middle East-Asia Minor 1 (MEAM1, also known as “biotype B”) and Mediterranean (MED, known as “biotype Q”). These whiteflies are very invasive and widely disseminated, especially by international trade of plant material (Dalton, 2006). The ease of adaptation of these species of *B. tabaci* to various environmental conditions is key to their success

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in becoming established in several regions of the world (Hadjistylli *et al.*, 2016).

The first report of *B. tabaci* in Brazil dates from 1928 in Bahia. Up to the late 1980s, sporadic outbreaks of *Bean golden mosaic virus* (BGMV) were reported in beans, but these outbreaks were not concerning as the vector belonged to the native whitefly species of the New World group (biotype A), which presents low efficiency as a vector and as a colonizer of cultivated plants (Inoue-Nagata *et al.*, 2016). However, issues with whitefly occurrence began to intensify in the early 1990s, when biotype B was recorded in São Paulo. After the introduction of biotype B, begomovirus outbreaks became increasingly frequent, especially in tomato crops, from which 14 new species of virus were identified (Inoue-Nagata *et al.*, 2016).

In Brazil, MEAM1 is the species associated with high infestation rates and significant losses in agricultural regions. Control of this whitefly is difficult and costly, and the species is also an efficient virus vector. MED was recently found in Brazil in the state of Rio Grande de Sul (Barbosa *et al.*, 2015) and subsequently found in São Paulo and Paraná (Moraes *et al.*, 2017). MED seems to present some specificity for colonizing bell pepper crops and is less susceptible to neonicotinoid and pyriproxyfen insecticides compared to MEAM1. In Israel, in the absence of insecticides, MEAM1 tends to outcompete MED in cotton, but with the continuous use of insecticides, MED is favored and replaces MEAM1 in the fields (Horowitz and Ishaaya, 2014). This response suggests that MED might become a severe pest in Brazil since chemical control applications are continuous in several crops. Integrated pest management (IPM) practices are essential to control economic losses to suppress whiteflies.

One of the IPM approaches that help keep pest populations below the economic damage level is the biological control, which consists of regulating living organisms through mortality caused by natural enemies. Biological control is an important strategy for the management of *B. tabaci*, and parasitoids of the genus *Encarsia* (Förster 1878) (Hymenoptera: Aphelinidae) are notable because species such as *Encarsia formosa* (Gahan 1924) and *Encarsia inaron* (Walker) are considered natural parasitoids of whiteflies (Liu *et al.*, 2015). Most *Encarsia* are autoparasitoids; that is, females develop from eggs laid in a primary host, but males

develop as parasitoids of conspecific females or other endoparasitoids (Hunter and Woolley, 2001). However, *E. inaron* (Walker) females and males are primary parasitoids of whitefly nymphs (Hunter and Woolley 2001).

The species *Encarsia inaron* may reach up to 85% parasitism in populations of *Neomaskellia andropogonis* (Corbett, 1926) (Hemiptera: Aleyrodidae). They are the most active parasitoids between September and November, which indicates some adaptation to higher temperatures. *Encarsia inaron* was introduced in California in 1989 to control *Siphoninus phillyreae* (Haliday 1835) on *Fraxinus uhdei* (Wenzig.) Lingelsh., 1907. *Encarsia inaron* was recorded in Brazil from Brasilia, associated with *Nicotiana tabacum* L., 1763; *Gossypium hirsutum* L., 1763; *Lycopersicon esculentum* Mill., 1768; *Brassica oleracea* L., 1753; *Glycine max* (L.) Merr., 1917; *Phaseolus vulgaris* L., 1753; *Cucumis melo* L., 1753; *Solanum gilo* Raddi., 1820; *Emilia sonchifolia* (L.) DC., 1834; in Lavras (Minas Gerais), on *Brassica oleracea* L.; and in Campinas (São Paulo state), on *Gossypium hirsutum* L., attacking *B. tabaci* MEAM1 and *Trialeurodes vaporariorum* (Westwood 1856) (Lourenção *et al.*, 2014, Torres *et al.*, 2014). However, the identification, molecular characterization, genetic variability, importance, and efficiency in the control of *B. tabaci* have not yet been studied. Thus, the objective of this work was to conduct the identification and molecular characterization of the parasitoid attacking *B. tabaci* nymphs in soybean crops in open fields in the state of São Paulo.

## Materials and methods

Samples of *B. tabaci* were obtained from soybean (*Glycine max* (L.)) crops in the municipality of Casa Branca, São Paulo, Brazil (21°44'16.7"S; 47°09'40.1"W) in November 2016 and May 2017. The parasitoids were reared and kept in isolated cages with collard green (*Brassica oleracea* L.) plants infested with *B. tabaci*.

The samples were identified based on morphological and molecular analyses. Morphological identification followed Myartseva and Evans (2008). The 102 examined specimens (84 females and 18 males) were identified as *E. inaron*. The parasitoid vouchers were deposited at the "Oscar Monte" Entomophagous Insects collection at the Instituto Biológico in Campinas, SP, Brazil, under reference number IB-CBE-685.

The molecular analyses were conducted on DNA extracts from 10 samples (one insect/tube) obtained using Chelex 100 (Walsh, Metzger & Higuchi, 1991). PCR reactions were conducted using primers for 28S-D2 rDNA, as Campbell *et al.* (2000) described. The primers amplified part of the *Encarsia* genome, and sequencing allowed the comparison with other sequences in the database.

Three amplified samples were selected for sequencing. The PCR product was purified, and the resulting sequences were compared with data in the NCBI database using Blast-N (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). These comparisons led to matches with *E. inaron*. The nucleotide sequences were aligned to a data set composed of another 51 sequences of *Encarsia* samples available from GenBank, using MAFFT v7.222 (Kato *et al.*, 2002) through Geneious v9.1.3 (Kearse *et al.*, 2012).

## Results and discussion

The taxonomically diagnostic morphological features observed were tarsal formula 5-5-5; fore wings uniformly setaceous, antennal club two-segmented; head and mesosoma dark; gaster yellow; scutellar placoid sensilla approximately five times their diameter apart; mesofemur yellow, mesotibial spur 0.57× length of basitarsus, ovipositor short, shorter than or as long as mesotibia; linear sensilla

present in F1 through F6 (Myartseva and Evans, 2008).

The final molecular alignment consisted of 54 sequences with 481 bp. This data set was analyzed using Bayesian phylogenetics (Figure 1) in MrBayes 3.2.2 (Huelsenbeck *et al.*, 2011).

The obtained data showed that the primers and amplified sequences allowed efficient detection of *E. inaron*. Phylogenetic analysis of 28S-D2 region fragments of *E. inaron* indicated low variability in that region of the genome.

The confirmed identification of *E. inaron* attacking different cryptic species of *B. tabaci* demonstrates the potential of this parasitoid for the management of whitefly populations. The use of *E. inaron* for control of *B. tabaci* might initially be implemented through conservation methods such as the use of selective pesticides and indicates opportunities for biology and field efficiency experiments for prospective mass production and field release of the parasitoid.

The occurrence of the parasitoid in commercial soybean plantation areas indicates the species tolerance to varying environmental conditions, which reinforces the argument for the potential of this species as a biocontrol agent.

Although parasitism rates in Brazil have not been studied, the occurrence of *E. inaron* in populations of *B. tabaci* established in various plant



Figure 1. Phylogenetic tree of the partial region of the 28S-D2 gene of *Encarsia inaron* collected in Brazil (highlighted) plus the references of samples of the genus *Encarsia* available from GenBank.

hosts indicates the parasitoid can potentially be effective in various agricultural regions, especially in those where *B. tabaci* occurs as a primary pest in succession planting, e.g., cotton followed by beans, soybeans followed by beans or tomatoes, and others.

Importantly, *B. tabaci* MEAM1 has caused damage to agriculture in Brazil since its introduction in the 1990s. However, with the appearance of MED (Moraes *et al.*, 2017), the problems are likely to increase, as MED is less susceptible to pesticides used for whitefly control (Wang *et al.*, 2018), which might lead to it predominating over MEAM1 in the field (Horowitz and Ishaaya, 2014). In addition to the competition between these two cryptic species, virus transmission is also a concern. MED is more efficient as a virus vector than MEAM1 and species of the New World 2 group (De Marchi *et al.*, 2017). The importance of MED as a vector may increase with the emergence of new virus strains.

In conclusion, the use of natural enemies like *E. inaron* for biocontrol is increasingly important due to the impact of MEAM1 on economically important crops and the lower susceptibility of MED to insecticides. Thus, providing efficient control methods to farmers is essential. The information provided in this work will serve as a foundation for future research on this parasitoid, which needs to be evaluated for its potential application in the management of *B. tabaci* MEAM1 and MED in Brazil.

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