



Short Communication

First records of parasitoids attacking the Asian citrus psyllid in Ecuador



Diego E. Portalanza^a, Luis Sanchez^a, Marjorie Plúas^a, Imelda Felix^a, Valmir A. Costa^b, Nivia da Silva Dias-Pini^c, Sheryl Ferreira-Stafanous^d, Mariuxi L. Gómez-Torres^{e,*}

^a Agencia Ecuatoriana de Aseguramiento de calidad del agro, Guayaquil, Ecuador

^b Instituto Biológico, Campinas, SP, Brazil

^c Embrapa Agroindustria Tropical, Fortaleza, CE, Brazil

^d New York City Department of Education, New York, United States

^e Ministerio de Agricultura, Ganadería, Acuacultura y Pesca – MAGAP, Quito, Ecuador

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ABSTRACT

First records of parasitoids attacking the Asian citrus psyllid in Ecuador. The objective of the current study was to investigate the presence of natural enemies of *Diaphorina citri* (Hemiptera: Liviidae) (the Asian citrus psyllid) in Ecuador. Incidence of parasitoid *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) was assessed between November 2015 and March 2016, in Letamendi, Febres-Cordero and Tarqui, urban districts of Guayaquil. Highest incidence of parasitism occurred in those regions and seasons of the year with the highest temperatures commensurate with increase of citrus plant shoots. Similar to their host, these parasitoids appear to have established in Ecuador by accident, and were not the result of purposeful introduction. This fortuitous introduction is a potentially helpful tool in controlling the Asian citrus psyllid, and potentially Huanglongbing.

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Diaphorina citri Kuwayama (Hemiptera: Liviidae) is distributed throughout Southeast Asia, India, Saudi Arabia, Mauritius and Reunion islands, Brazil, Uruguay (EPPO, 2014), Colombia (Moncayo-Donoso et al., 2014), Ecuador (Cornejo and Chica, 2014), U.S. (Sétamou et al., 2008), Argentina, Venezuela, Mexico and in some countries of the Caribbean and Central America (EPPO, 2014; SENASICA, 2012).

Among *D. citri* cataloged hosts there are 25 genera in the family Rutaceae, primarily *Citropsis*, *Citrus* and *Murraya* (Ikeda and Ashihara, 2008). Due to their distribution and biological characteristics it is the most important vector of huanglongbing (HLB) or greening, the main citrus disease worldwide (Bove, 2006; Wang and Trivedi, 2013).

Transmission of pathogens by arthropods is a serious problem for agriculture. In order to address these agricultural problems and to maintain the quality of agro-ecosystems, biological control tactics are being used to reduce the pest. The idiobiont ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and the arrhenotokous endoparasitoid *Diaphorencyrtus aligarhensis*

(Shaffee, Alam and Agarwal) (Hymenoptera: Encyrtidae) have been used in programs of classical biological control to the population of *D. citri* in different regions of the world (Gómez-Torres et al., 2006; Parra et al., 2007).

Tamarixia radiata is the main agent of biological control of *D. citri*. This parasitoid has high efficiency of parasitism and a great capacity for dispersal, establishment, and field adaptation. Thus, the success of *T. radiata* releases for biological control of *D. citri*, and consequently the HLB, was reported in the Reunion, Mauritius and Guadeloupe islands (Étienne et al., 2001), Taiwan (Chien et al., 1991) and in USA (Hall et al., 2008).

Diaphorencyrtus aligarhensis preferentially parasitizes second and third instar *D. citri* (Sule et al., 2014). In addition to parasitization, females can kill *D. citri* nymphs via host feeding (Rohrig et al., 2011). *D. aligarhensis* has been used against *D. citri* in various citrus-growing regions including Taiwan, Reunion Island, Saudi Arabia, and Florida (Rohrig et al., 2012). Florida populations of *D. aligarhensis*, sourced from Taiwan and China, have failed to establish, despite repeated release efforts (Rohrig et al., 2012; Bistline-East et al., 2015).

Despite the presence of *D. citri* being reported in Ecuador since 2014 (Cornejo and Chica, 2014), so far no parasitoid populations have been detected which might help to keep balance of this pest. Within this context, the aim of this study was to survey

* Corresponding author.

E-mails: mgomezt@magap.gob.ec, gomez.torres.ml@gmail.com (M.L. Gómez-Torres).

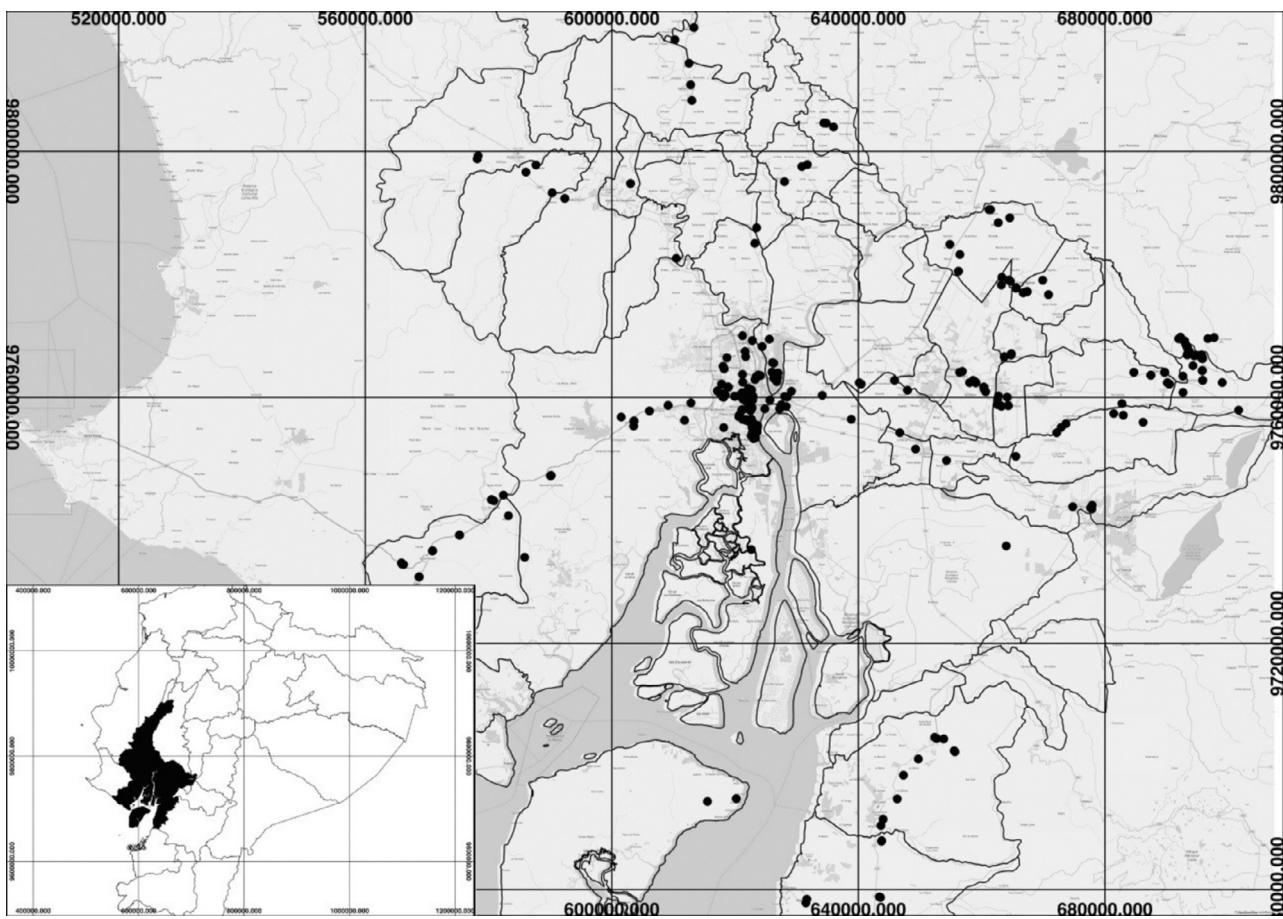


Fig. 1. *Diaphorina citri* surveys between April 2013 and 2016. Points represent monitored areas in Guayas province, Ecuador. The lower box represents where the province is located.

the population of *D. citri* in Ecuadorian regions where it has been reported, and determine variations among pest populations in different seasons and consequently identify the parasitoids associated with this psyllid.

***Diaphorina citri* population's assessment in urban and rural areas of Guayas province:**

To identify *D. citri*'s associated parasitoids, samples were taken in urban districts in the city of Guayaquil: Ximena ($2^{\circ} 13' 35'' S$ - $79^{\circ} 53' 45'' W$), Febres-Cordero ($2^{\circ} 11' 45'' S$ - $79^{\circ} 54' 54'' W$), Urdaneta ($2^{\circ} 12' 08'' S$ - $79^{\circ} 54' 20'' W$), Rocafuerte ($2^{\circ} 11' 16'' S$ - $79^{\circ} 52' 44'' W$), Tarqui ($2^{\circ} 09' 52'' S$ - $79^{\circ} 56' 14'' W$), Chongon ($2^{\circ} 16' 56'' S$ - $80^{\circ} 11' 08'' W$), Letamendi ($2^{\circ} 12' 15'' S$ - $79^{\circ} 54' 12'' W$), and other cities of Guayas province: El Empalme ($0^{\circ} 56' 32'' S$ - $79^{\circ} 37' 49'' W$), Balzar ($1^{\circ} 17' 36'' S$ - $79^{\circ} 52' 49'' W$), Colimes ($1^{\circ} 32' 22'' S$ - $79^{\circ} 58' 25'' W$), Santa Lucía ($1^{\circ} 41' 30'' S$ - $79^{\circ} 59' 14'' W$), Pedro Carbo ($1^{\circ} 48' 58'' S$ - $80^{\circ} 18' 22'' W$), Daule ($1^{\circ} 51' 26'' S$ - $79^{\circ} 52' 08'' W$), Alfredo Baquerizo Moreno ($1^{\circ} 55' 24'' S$ - $79^{\circ} 32' 31'' W$), Milagro ($2^{\circ} 09' 01'' S$ - $79^{\circ} 35' 48'' W$), Naranjal ($2^{\circ} 40' 41'' S$ - $79^{\circ} 38' 24'' W$), Balao ($2^{\circ} 53' 38'' S$ - $79^{\circ} 41' 12'' W$) between April 2013 and April 2016 at every 15 days (Fig. 1).

In each case, a collection was taken from ten branches with buds from thirty plants showing nymphs of *D. citri*. The collection was made by following a protocol previously defined and with reference established by Gómez-Torres et al. (2006), samples were taken very carefully using secateurs being careful not to disturb the insects present in each stratum until packaging for transport.

The collected twigs were placed in paper bags (30 cm × 15 cm), properly labeled, and conditioned at $22 \pm 3^{\circ}\text{C}$ in a Waeco® electric

Cooler box model CarFridge (17.5 cm × 31 cm × 31 cm) where they were transported to the laboratory and immediately transferred to acrylic cages (35 cm × 45 cm × 35 cm). Then, they were deposited on properly sterilized plastic trays containing a moistened filter paper sheet. For proper conservation of the collected material, the laboratory was maintained at $25 \pm 1^{\circ}\text{C}$, relative humidity of $80 \pm 10\%$ and a 12 h photo phase.

Every 24 h, the acrylic cages containing the nymphs were observed for the extraction of emerged parasitoids, which were dried in a critical point dryer (Leica CPD 030) and double-mounted on points. Parasitoid identification was done according to Noyes (1980) and Hayat (1981) for genus and to Shafee et al. (1975) for species. The percentage of emergence was 78.05. The specimens were deposited at Coleção de Insetos Entomófagos Oscar Monte (Instituto Biológico, Campinas, São Paulo, Brazil). Parasitoids collected emerged on nymphs of *D. citri* were identified as *D. aligarhensis*. Female and male specimens of *Tamarixia radiata* were also recorded.

Images of the identified material taken under the stereoscopic microscope and scanning electron microscope (SEM) were examined by Dr. John S. Noyes (Natural History Museum, London, UK), who confirmed the identification.

With these initial studies in Ecuador, the various collections demonstrated the natural presence of parasitoids *D. aligarhensis* and *T. radiata* in Letamendi, Febres-Cordero and Tarqui, urban districts of Guayaquil (Fig. 2). Thus, population variations of the pest and the parasitoid may occur at different times of the year, depending on the region and the management. The absence of



Fig. 2. Letamendi, Febres Cordero and Tarqui (triangles), Guayaquil city urban districts where *Diaphorencyrtus aligarhensis* was detected. The lower box represents where the city is located.

natural enemies, in some localities, could be directly related to the indiscriminate application of chemicals used to control insects and mites, as well as *D. citri*.

Establishment of this parasitoid in other countries of South America has not been reported. Similar to *D. citri*, the endoparasitoid *D. aligarhensis* appears to have established in Ecuador accidentally, and were not the result of purposeful introduction. It is not unusual for this parasitoid to be discovered in non-release locations in Hawaii (Matsunaga, 2014).

This work fits within the objectives of the national plan of well living of Ecuador (*Sumak Kawsay*) 2012–2017 Article 7 "To guarantee the rights of nature and to promote environmental sustainability". With this record the possibility of adopting a new tactic for sustainable management of *D. citri* and thus maintain an ecological balance that allows sustainable and economically fair agriculture opens.

Conflicts of interest

The authors declare no conflicts of interest.

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