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Occurrence and distribution of *Diaphorina citri* (Hemiptera: Liviidae) and *Tamarixia radiata* (Hymenoptera: Eulophidae) in Pará state, Brazil

Ocorrência e distribuição de **Diaphorina citri** *(Hemiptera: Liviidae) e* **Tamarixia radiata** *(Hymenoptera: Eulophidae) no estado do Pará*

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ABSTRACT: This study presents and discusses the results of a survey for the presence of *Diaphorina citri* Kuwayama and its natural enemies in *Citrus* spp. citrus and *Murraya* sp. myrtle plants in Pará State, Brazil. From 2013 to 2017, observations were made in five mesoregions of the State, and where *D. citri* was present, branches were examined to quantify eggs, nymphs and mummies with parasitoid emergence holes and to obtain parasitoids. *D. citri* adults were used to detected the bacterium through nested polymerase chain reaction (NESTED-PCR). Psyllids were found in citrus and myrtle plants. A total of 583 parasitoid specimens of *Tamarixia radiata* (Waterston) were obtained. The bacterial detection test was negative for *Candidatus* Liberibacter spp. Psyllids and parasitoids were present in the Lower Amazon, Metropolitan area, Northeastern Pará, Southeastern Pará and Southwestern Pará mesoregions. The presence of *D. citri* in citrus, with was previously absent in this host, alters the risk situation for Huanglongbing (HLB) in the State. These results will aid in developing preventive or containment measures through phytosanitary protection.

KEYWORDS: Huanglongbing; citrus; *Murraya* sp.; biological control.

RESUMO: No presente estudo, são apresentados e discutidos os resultados de levantamento para a presença de *Diaphorina citri* Kuwayama e inimigos naturais em plantas de citros *Citrus* spp. e murta *Murraya* sp. no estado do Pará. No período de 2013 a 2017 foram realizadas observações em cinco mesorregiões do estado. Na presença de *D. citri*, ramos foram coletados com a quantificação de ovos, ninfas, múmias com orifício de emergência de parasitoide e obtenção de parasitoides. Adultos de *D. citri* foram analisados para a detecção da bactéria por meio de *nested polymerase chain reaction* (NESTED-PCR). O psilídeo foi constatado em citros e murta. Foram obtidos 583 exemplares do parasitoide *Tamarixia radiata* (Waterston). O teste de detecção da bactéria foi negativo, indicando ausência de *Candidatus* Liberibacter spp. O psilídeo e o parasitoide estão presentes nas mesorregiões do Baixo Amazonas, Metropolitana, Nordeste Paraense, Sudeste Paraense e Sudoeste Paraense. A presença de *D. citri* em citros, até então ausente nesse hospedeiro, altera a situação de risco do Huanglongbing (HLB) para o Estado. Esses resultados auxiliarão na formulação de medidas preventivas ou de contenção pela defesa fitossanitária.

PALAVRAS-CHAVE: Huanglongbing; citros; *Murraya* sp.; controle biológico.

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Huanglongbing (HLB) is considered one of the most serious diseases in citrus fruit (*Citrus* spp.) and is the main phytosanitary problem in countries where the disease occurs (BOVÉ, 2006). The first report of HLB in Brazil occurred in 2004 in the Araraquara municipality, São Paulo state, and the disease is currently present in Paraná and Minas Gerais states (COLETTA-FILHO et al., 2004; CASTRO et al., 2010). HLB is associated with the *Candidatus* Liberibacter spp. bacteria, which is restricted to plant phloem vessels (MACHADO et al., 2010). In the Americas, the *Ca.* L. asiaticus bacterium is the most widespread species and is present in all countries where the disease has been reported (YAMAMOTO et al., 2014).

The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is important because it is a vector of the bacterium associated with HLB (MACHADO et al., 2010). *D. citri* is present in nearly all citrus-producing regions of Brazil, and the ornamental myrtle plant, *Murraya paniculata* (Rutaceae), is its preferred host for development (NAVA et al., 2007). Female *D. citri* place egg clusters at the shoot tips; thus, shoot availability is a limiting factor in oviposition (PARRA et al., 2010). To control insect vectors, synthetic insecticides are used to eliminate infective individuals. Another alternative is to use biological control (parasitoids, predators and pathogens). Among the parasitoids, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) has been successfully used in countries where HLB occurs (PARRA et al., 2010).

In Brazil, the highest population density of *D. citri* in commercial citrus orchards and myrtle was recorded in Bahia. The insect was not found in citrus orchards in the states of Amazonas and Pará, which are nonendemic for HLB (NASCIMENTO et al., 2016).

Pará state is the largest citrus producer in the north region, with two citrus production centers in the microregions of Guamá (orange) and Santarém (Tahiti); in 2017, it accounted for 1.97% of national production, representing 81% of regional production (IBGE, 2017). In regions where HLB remains undetected, exclusion and eradication measures are essential to avoid its introduction and to enable its early eradication (LARANJEIRA et al., 2011).

This study presents and discusses the results of a survey conducted to determine the presence of *D. citri* and its natural enemies (parasitoids) on citrus and myrtle plants to obtain information on the HLB vector's distribution range and on parasitoids in Pará state.

Nonconsecutive observations were made from April 2013 to September 2017 in five mesoregions of the state: The Lower Amazon, the Metropolitan area, Northeast Pará, Southeast Pará and Southwestern Pará. *D. citri* presence was evaluated in new plant shoots on farms and in public places (squares, sidewalks), gardens and roadsides. When adult insects were present, eggs and/or nymphs and branches (up to 15 cm) were collected and packed in labelled plastic bags for transport to the laboratory. The number of branches per plant varied depending on the plant's vegetative development (height, flowering, fruiting, new shoots). Adult specimens, when detected, were collected directly in plastic tubes containing 70% alcohol, with a sample representing each sampling/observation point. The number of observation points per mesoregion varied (8 to 97), and all were georeferenced, with each point corresponding to one or more citrus and/or myrtle plants (sampling up to 30 plants/point).

In the laboratory, the numbers of eggs, yellow or darkened nymphs with parasitoid development (NYD), and *D. citri* mummies with characteristic parasitoid emergence holes (NH) were quantified. To obtain the parasitoids, branches with nymphs were individually placed in glass tubes sealed with polyvinylchloride film and observed daily for up to 15 days. The *D. citri* adults and emerged parasitoids were collected and preserved in 70% alcohol. The parasitism rate was obtained using the ratio of the number of parasitized nymphs to total nymphs (parasitized and nonparasitized). Adult *D. citri* specimens collected at 27 observation points and stored in 96°C alcohol were analyzed to screen for the bacterium using nested polymerase chain reaction (NESTED-PCR).

Observations were made in 16 microregions in the state's 5 mesoregions (Fig. 1). Regarding *D. citri* presence, 48 points with citrus plants, 146 with myrtle plants and 2 with both citrus and myrtle were observed, totaling 196 evaluation sites (sampling points) in 46 municipalities. *D. citri* was found in myrtle plants in 32 municipalities at 95 sampling points. *D. citri* was present in only one of the two sites containing both citrus and myrtle plants, a fruit (including citrus) and ornamental seedling nursery, but the insect was only present in myrtle seedlings. Some points were visited more than once, and the insect's presence was not observed on all visits. Of the 146 observation points containing myrtle, 36 were visited two or three times, and *D. citri* was absent in nine of them.

In the myrtle, 881 adult *D. citri* specimens were collected, considering both the specimens collected in the field and those obtained in the laboratory from nymphs present on the branches. Of the 1,415 myrtle branches collected, 7,072 eggs and 5,635 nymphs were counted, averaging 5.0 eggs and 3.98 nymphs per branch (Table 1). In the citrus plants, *D. citri* was detected in 2017 in an urban area at two sampling points in the Castanhal (orange orchard) and Peixe-Boi (lemon plant) municipalities (Table 1). Although darkened and apparently parasitized nymphs were found on the branches from Castanhal, no parasitoids were found.

Of the 6,438 nymphs observed in myrtle, 12.47% had characteristic parasitoid emergence holes. A total of 583 parasitoids were identified as *T. radiata*. Confirmed parasitism

or signs of parasitism (contained a hole or were darkened in color without parasitoid emergence) by *D. citri* nymphs were observed in 23 municipalities in all five mesoregions.

To detect the bacteria, adult *D. citri* specimens collected from myrtle in Belém, Castanhal, Capitão Poço, Igarapé-Açu, Irituia, Tomé-Açu, Breu Branco, Tucuruí and Santarém municipalities were analyzed. The analyzed samples were negative for the bacteria, indicating the absence of *Candidatus* Liberibacter spp.

D. citri was found in 94% of the microregions visited, although some microregions, such as Conceição do Araguaia, Marabá and Redenção (IBGE, 2017), were not listed as

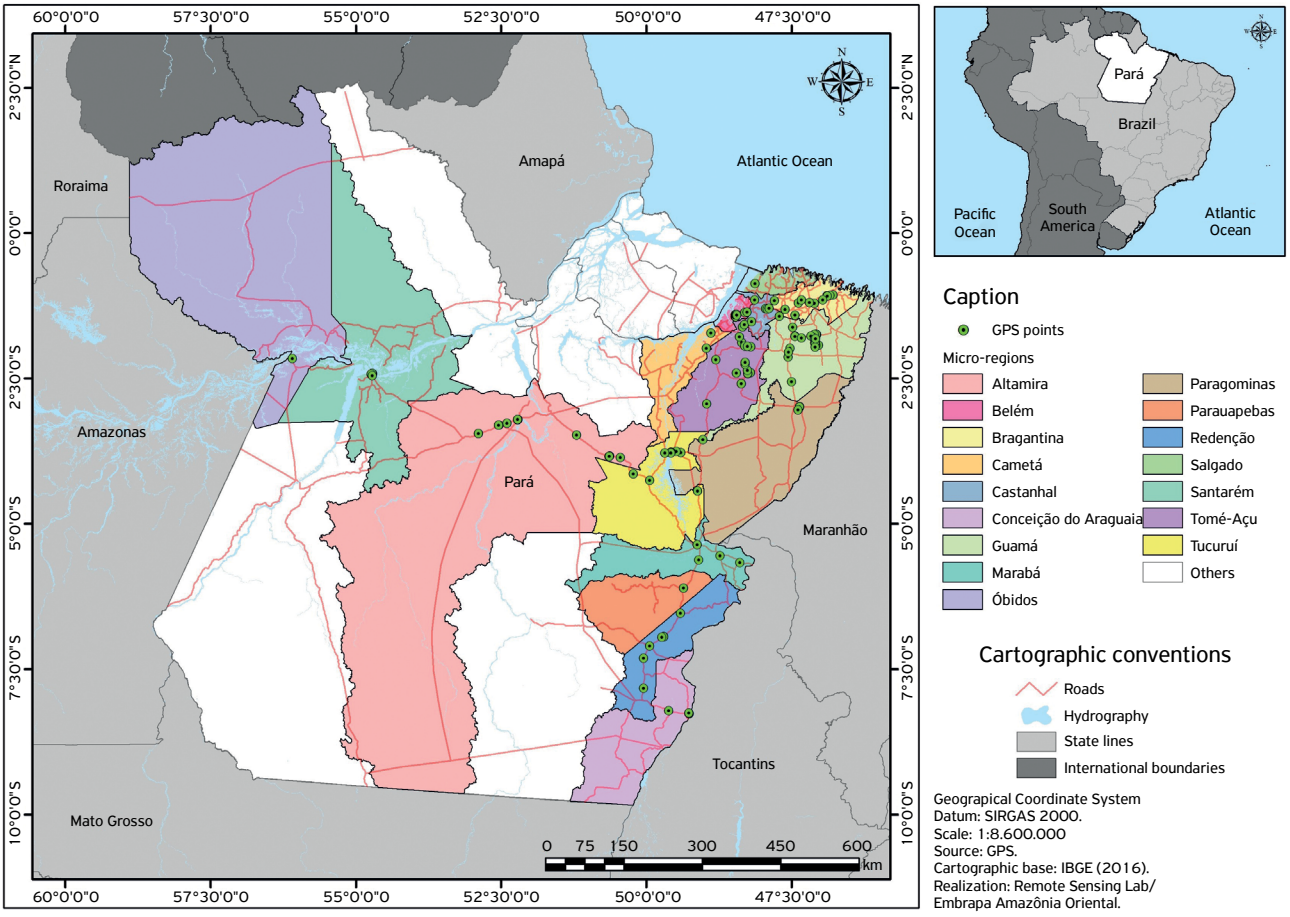

Figure 1. *Diaphorina citri* observation and/or sampling points in citrus (*Citrus* spp.) and myrtle (*Murraya* sp.) plants in microregions in Pará state.

Table 1. Number of *Diaphorina citri* eggs and nymphs in myrtle (*Murraya* sp.) and citrus (*Citrus* spp.) branches and of the parasitoid *Tamarixia radiata*, female (♀) and male (♂), obtained in the laboratory from samples collected in Pará state from 2013 to 2017.

Year	Host	Number						
		Branches	Eggs	Nymphs*		*T. radiata*		
				NYD	NH	♀	♂	Total
2013	Myrtle	94	551	226	122	27	33	60
2014	Myrtle	309	1,758	1,070	39	101	68	169
2015	Myrtle	598	2,886	2,270	466	163	120	283
2016	Myrtle	382	1,906	1,831	112	23	17	40
2017	Myrtle	32	0	238	64	18	13	31
	Citrus	31	80	116	0	0	0	0
Total		1,446	7,152	5,751	803	332	251	583

*NYD: yellowish or darkened color; NH: *D. citri* mummy with parasitoid emergence hole.

citrus-producing regions. Therefore, psyllids were present in five mesoregions of the state. *D. citri* was detected in citrus in an urban area; however, it was unobserved during two years of biweekly monitoring in rural citrus-producing areas in Capitão Poço and Castanhal (NORONHA et al., 2014). In the Recôncavo region of Bahia, population density of *D. citri* in myrtle was approximately 1.4 times higher than that in the citrus host (NASCIMENTO et al., 2016).

Tamarixia radiata and signs of parasitoid presence (nymphs with parasitoid emergence holes or darkened without parasitoid emergence) were found in 50% of the municipalities visited, corresponding to 72% of the municipalities where *D. citri* was present. The parasitism rate in myrtle plants was 11%, considering that only darkened nymphs collected from plants without insecticide application were quantified. PAIVA; PARRA (2012) found a mean parasitism rate of 12% by *T. radiata* in *D. citri* nymphs, primarily 4th and 5th instar, in citrus plants subjected to regular insecticide applications. *T. radiata* presence in citrus-producing and nonproducing regions is an alternative for biological control to manage *D. citri*.

Although a low *D. citri* population density was observed in myrtle in Pará, the insect's presence in citrus, which was previously absent in this host (NASCIMENTO et al., 2016), changes the risk situation for HLB in the state. Several quarantine and exotic pests have entered Brazil through the Amazon. MORAIS et al. (2016) cite several reasons for the region's vulnerability to quarantine pests: The large border, the intense flow of people, the transit of plant material and the poor performance of neighboring countries in phytosanitary protection. HLB is present in Colombia, Panama and Trinidad and Tobago (ICA, 2017). The risk for citrus farming in the Amazon is imminent, given the presence of *D. citri* in lemon trees and in myrtle in the Boa Vista municipality, in Roraima state (MARSARO JÚNIOR et al., 2014), which has terrestrial access routes to Guyana and Venezuela.

The present study's results regarding *D. citri* and *T. radiata* distribution in the state will allow the necessary phytosanitary protection measures to be defined (contingency plan), with preventive or containment actions before HLB is possibly disseminated to other regions of Brazil or before it enters through the Amazon region.

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