Received: 15 May 2018 | Revised: 5 November 2018 | Accepted: 3 January 2019

DOI: 10.1111/aab.12490

WILEY Annals of Applied Biology

RESEARCH ARTICLE

Genetic diversity and geographic distribution of *Bemisia tabaci* and *Trialeurodes vaporariorum* in Costa Rica

Natalia M. Barboza[1] | Paul Esker[2] | Alice K. Inoue-Nagata[3] | Enrique Moriones[4]

[1]Centro de Investigación en Biología Celular y Molecular (CIBCM), Escuela de Tecnología de Alimentos, Centro Nacional en Ciencia y Tecnología de Alimentos (CITA), Universidad de Costa Rica, San José, Costa Rica

[2]Centro de Investigación en Protección de Cultivos (CIPROC), Escuela de Agronomía, Universidad de Costa Rica, San José, Costa Rica

[3]Embrapa Vegetables, Brasília, Brazil

[4]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora"-Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM UMA-CSIC), Estación Experimental "La Mayora", Málaga, Spain

Correspondence
N. Barboza, Centro de Investigación en Biología Celular y Molecular, San José 2060, Costa Rica.
Email: natalia.barboza@ucr.ac.cr

Funding information
National Council for Scientific and Technological Development (CNPq); Consejo Nacional de Rectores-Fondo Especial para la Educación Superior (FEES), Grant/Award Number: VI-801-B1-650; Ministerio de Economía y Competitividad and Ministerio de Economía; Industria y Competitividad; European Regional Development Fund (ERDF) and the European Social Fund (ESF), Grant/Award Number: AGL2016-75819-C2-2

The tobacco whitefly *Bemisia tabaci* (Gennadius) cryptic species complex and of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) are extensively reported as destructive pests in vegetable crops worldwide. A survey was conducted in 2011 and 2012 to determine the occurrence and genetic diversity present in the populations of these whiteflies in the major vegetable production areas of Costa Rica. Insect samples were collected from sweet pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), common bean (*Phaseolus vulgaris* L.) and weeds present in commercial crops either in open field or greenhouse conditions. PCR-RFLP analysis of mitochondrial cytochrome *c* oxidase subunit 1 gene (mtCOI) sequences of 621 whitefly individuals confirmed the presence of the Mediterranean (MED) type of the *B. tabaci* and of *T. vaporariorum* in most sampled regions. Also, individuals of the Middle East-Asia Minor 1 (MEAM1) type of the *B. tabaci* were observed in low numbers. Contingency analyses based on type of crop, geographical region, whitefly species, year of collection and production system confirmed that *T. vaporariorum* was the most frequent species in vegetable production areas in Costa Rica, both in greenhouses and in open fields. *B. tabaci* MED is likely spreading to new areas of the country, whereas *B. tabaci* MEAM1 was mostly absent or rarely found. Comparisons of mtCOI sequences from *B. tabaci* individuals revealed the presence of four *B. tabaci* sequence haplotypes (named MED-i, MED-ii, MEAM1-i, MEAM1-xviii) in Costa Rica, three of them identical to *B. tabaci* haplotypes previously reported in the Western Hemisphere and other parts of the world. Analysis of sequences of *T. vaporariorum* individuals revealed a more complex population with the presence of 11 haplotypes, two of which were identical to *T. vaporariorum* sequences reported from other countries.

KEYWORDS

Bemisia tabaci, genetic diversity, *Trialeurodes vaporariorum*, whitefly

1 | INTRODUCTION

The whiteflies (Hemiptera: Aleyrodidae) *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) are consistently associated with the emergence and global spread of plant viruses (Gilbertson, Batuman, Webster, & Adkins, 2015; Navas-Castillo, López-Moya, & Aranda, 2014; Wainaina et al., 2018). These whiteflies are devastating pests to agriculture, both in protected cultures or in outdoor crops (De Barro, Liu, Boykin, & Dinsdale, 2011; Dinsdale, Cook, Riginos, Buckley, & De Barro, 2010; Hu et al., 2011; Liu, Colvin, & De Barro, 2012; Wang et al., 2011).

The tobacco whitefly, also commonly referred to as the sweetpotato or cotton whitefly, *B. tabaci*, constitutes a cryptic species complex (De Barro et al., 2011). Currently, at least 47 *B. tabaci* sister clades (species) have been proposed based on the differences observed in the sequence of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) fragment (Alemandri et al., 2012; Boykin, Savill, & De Barro, 2017; Chowda-Reddy et al., 2012; Firdaus et al., 2013; Hu et al., 2011; Hu et al., 2017; Mugerwa et al., 2018; Parrella, Scassillo, & Giorgini, 2012). Divergence of at least 3.5% in mtCOI sequences is accepted as criterion for separation of *B. tabaci* species (Boykin & De Barro, 2014; De Barro, 2012), although some authors consider that this delimitation rule should be revised (Boykin & De Barro, 2014; Lee, Park, Lee, Lee, & Akimoto, 2013; Qin, Pan, & Liu, 2016). Two invasive *B. tabaci* species, Middle East-Asia Minor 1 (MEAM1;

formerly known as B biotype) and Mediterranean (MED; formerly known as Q biotype), have spread to a significant number of countries in the world in recent decades. They are found on a broad range of hosts, from cotton to ornamental plants, and particularly on vegetable crops (De Barro, 2012; Hu et al., 2011). The origin of *B. tabaci* MEAM1 and MED seems to be the Mediterranean/Asia Minor/North Africa region (Boykin et al., 2007; Dinsdale et al., 2010; Simón, Cenis, & De la Rúa, 2007). *B. tabaci* MEAM1 has spread worldwide since the late 1980s (Bellows, Perring, Gill, & Headrick, 1994). Recently, *B. tabaci* MED has emerged in many parts of the world, displacing or co-existing with native species of *Bemisia* (Barboza et al., 2015; Bethke, Byrne, Hodges, McKenzie, & Shatters, 2009; Dalton, 2006; Grille, Gauthier, Buenahora, Basso, & Bonato, 2011; Guevara-Coto, Barboza-Vargas, Hernández-Jiménez, Hammond, & Ramírez-Fonseca, 2011; Martínez-Carrillo & Brown, 2007; McKenzie, Hodges, Osborne, Byrne, & Shatters, 2009). *B. tabaci* MEAM1 and MED cause considerable damage to a large number of crops through direct feeding and because of the transmission of plant viruses (Chu et al., 2006; De Barro et al., 2011; Dinsdale et al., 2010; Gilbertson et al., 2015; Hu et al., 2011; Navas-Castillo et al., 2014). Damage caused by members of the *B. tabaci* complex has been reported in all continents, though in cold climates low temperatures prevent high infestations, except in protected cultivated crops (Dinsdale et al., 2010).

Trialeurodes vaporariorum, known as the greenhouse whitefly, is another important whitefly pest. It has been the focus of many researchers because of its ability to develop resistance to commonly used insecticides such as neonicotinoids (Gorman et al., 2007; Karatolos et al., 2011). This whitefly, unlike *B. tabaci*, is better adapted to cold climates, and is common at high elevations. *T. vaporariorum* may co-exist with *B. tabaci*, especially in greenhouses, although under warm conditions the latter whitefly tends to compete more successfully (Zhang, Li, Liu, Wan, & Wang, 2011). A recent study about phylogeographical structuring and genetic diversity of *T. vaporariorum* indicates that a single large group is present, in which 16 different genetic haplotypes can be distinguished based on mtCOI sequence, with extensive overlap across countries (Wainaina et al., 2018).

In Costa Rica, whiteflies and whitefly-borne viruses cause serious damage to many crops (Hilje, Cubillo, & Segura, 1993; Morales et al., 2005; Vargas-Asencio et al., 2013). Previous studies showed that the predominant whitefly was *B. inconspicua*. This species, known as the New World 2 (NW2, biotype A) species of the *B. tabaci* complex (Boyking, 2014) was the first whitefly reported in Costa Rica (Morales et al., 2005). In 2011, the *B. tabaci* MED species was reported for the first time in the agricultural highlands of the Alfaro Ruiz region of Costa Rica (Guevara-Coto et al., 2011). *T. vaporariorum* has been present in Costa Rica since 1993 (Hilje et al., 1993). Recently, a high abundance of *T. vaporariorum* was reported in the Cartago region of Costa Rica (Vargas-Asencio et al., 2013), while the presence of mixed populations of *B. tabaci* MED and *T. vaporariorum* has been shown in the Alfaro Ruiz region (Guevara-Coto et al., 2011).

Because of the importance of whiteflies as agricultural pests, the aim of this study was to determine the current geographic distribution and genetic diversity of whiteflies of the *B. tabaci* complex and *T. vaporariorum* species in the two most important vegetable crops of Costa Rica, sweet pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.). The study was conducted in the Central Valley, where the major vegetable production area of Costa Rica is concentrated. Factors that could be driving whitefly species abundance were analysed.

2 | MATERIALS AND METHODS

2.1 | Sampling sites and sample collection

Whitefly samples were collected from commercial tomato and sweet pepper fields (Table S1, Supporting information). Whiteflies were also collected from common bean (*Phaseolus vulgaris* L.) crops grown close to the visited tomato and sweet pepper fields and from weeds present in the sampling sites (Table S1). Surveys were conducted during the dry (January–April) and rainy (July–September) seasons of 2011 and 2012 in the Central Valley of Costa Rica where most vegetable production of the country is concentrated. Three regions in the Central Valley were considered in the surveys: Zarcero, Grecia and Cartago (Figure 1). In Zarcero, sampling sites were located at about 1,700 m above sea level (m.a.s.l.), and have temperatures ranging from around 13°C to 24°C. In Grecia, sampling sites were located at altitudes ranging from 425 to 2,500 m.a.s.l., with an average of 1,000 m.a.s.l. Temperatures vary from 16°C to 27°C year-round. In Cartago, sampling sites were located at about 1,400 m.a.s.l., with temperatures ranging from 15°C to 26°C. The three regions have a bimodal rainfall regime, with average rainfall ranging from 950 to 2,000 mm annually (Hilje, Cubillo, & Segura, 1993). In each region, independent sites were visited including greenhouses and open field production areas. The same sites were visited during subsequent samplings whenever tomato and sweet pepper plants were cultivated. Regular insecticide applications were made to reduce the whitefly populations in the crops visited. Imidacloprid, which is effective against both adults and nymphs, was the active ingredient most commonly applied.

Adult whiteflies were collected at each site from randomly selected plants, using a mouth aspirator. Insects from each collection site and sampling date were deposited in 1.5 mL vials containing 750 μL of 70% ethanol and stored at −20°C until analysis. All insects collected at one site on a single date constituted a sample. A total of 66 samples were collected. About 10 whitefly individuals were analysed per sample, with some exceptions where fewer individuals were collected.

2.2 | Nucleic acid isolation and partial mitochondrial cytochrome *c* oxidase subunit 1 gene (mtCOI) analysis

Nucleic acids were extracted from individual insects in microcentrifuge tubes using Chelex® 100 sodium form (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's recommendations. Whitefly adults were crushed individually in 30 μL of H_2O, and then 30 μL of 50% Chelex solution were added and homogenised. The tube was incubated at 56°C for 15 min and at 99°C for 3 min. After centrifugation at 14,000 rpm for 5 min, 30 μL of the supernatant was collected, stored at −20°C and used as a template for PCR amplification.

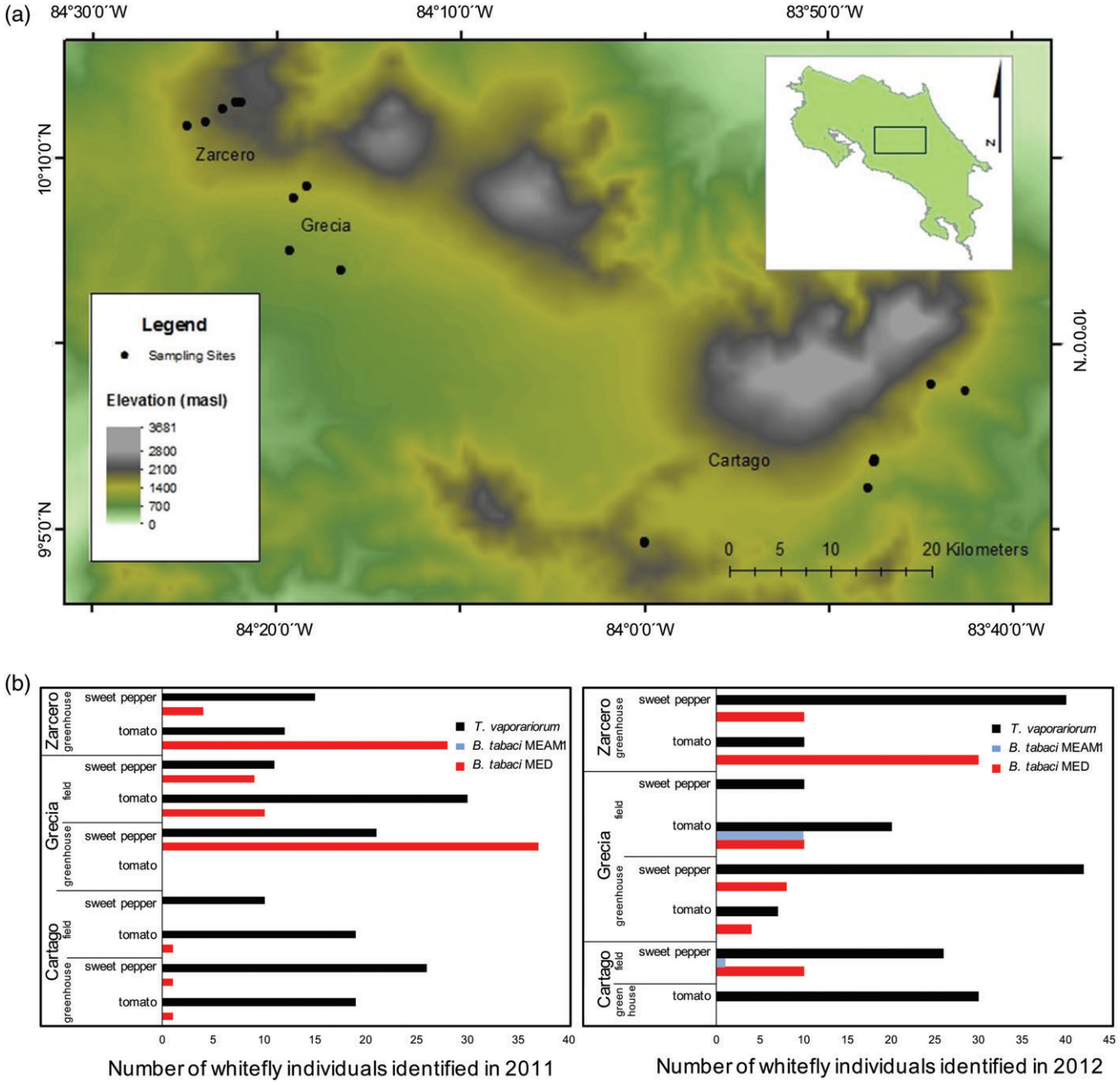

FIGURE 1 Map of Costa Rica with the surveyed sites (black solid circles) (a). Abundance (%) of whitefly species (*Bemisia tabaci* Mediterranean (MED), *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and *Trialeurodes vaporariorum*) in tomato and pepper detected in the survey conducted during 2011 and 2012, according to crop and type of production (greenhouse and open field) and region (Zarcero, Grecia and Cartago) (b). masl: metres above sea level

Part of the mtCOI gene was amplified from whitefly DNA extracts using the primer pair C1-J-2195 (Simon et al., 1994) and 801c (Frohlich, Torres-Jerez, Bedford, Markham, & Brown, 1999) (~800 base pairs product). The reaction was performed using Dream *Taq* polymerase (Thermo Fisher Scientific, Massachusetts) with the following cycling conditions: one cycle at 94°C for 5 min, 50°C for 2 min and 72°C for 3 min; 39 cycles at 94°C for 1 min, 50°C for 1.5 min, 72°C for 2 min; and a final 10 min extension at 72°C. A restriction fragment length polymorphism study of the obtained PCR products (PCR-RFLP) was done after digestion with TaqI (Thermo Fisher Scientific, Massachusetts) and Tru9I (New England BioLabs, Massachusetts), enzymes used to differentiate *B. tabaci* MEAM1, *B. tabaci* MED, *B. tabaci* Asia I, *B. tabaci* Sub-Saharan Africa 2, *B. tabaci* Italy and *T. vaporariorum* (Bosco, Loria, Sartor, & Cenis, 2006). DNA extracts previously identified to correspond to *B. tabaci* MEAM1, *B. tabaci* MED and *T. vaporariorum* were used as controls. The digested DNAs were visualised by electrophoresis in a 2% agarose gel (high resolution) stained with GelRed (10,000×) (Biotium, California). When needed, the amplified mtCOI gene fragment was directly sequenced in both orientations by Macrogen® (Seoul, South Korea).

2.3 | Sequences analysis

The obtained partial mtCOI gene sequences were assembled using the Staden program (Bonfield, Smith, & Staden, 1995). Sequence

similarity searches were performed using BlastN (Altschul, Gish, Miller, Myers, & Lipman, 1990) in order to compare the obtained sequences with sequences available in the databases (Firdaus et al., 2013) including new dataset published for *B. tabaci* (https://f1000research.com/articles/6-1835/v1) (Boykin et al., 2017) and more recent sequences available until February 23, 2018 in GenBank. Population genetics analysis was done with 283 mtCOI sequences of *B. tabaci*, which included 110 sequences representative of the *B. tabaci* complex proposed groups and 173 sequences of *B. tabaci* MED and MEAM1 (107 sequences of *B. tabaci* MED and 66 sequences of *B. tabaci* MEAM1). Information from Costa Rica included: 21 *B. tabaci* MED sequences and two *B. tabaci* MEAM1 sequences obtained from this research, and one previously reported *B. tabaci* MED sequence (Guevara-Coto et al., 2011) (Tables S2 and S3). A common sequence length of 657 nucleotides (nt one to 657 of mtCOI gene, accession KJ606633) was considered in all cases. For the analysis of *T. vaporariorum*, 76 mtCOI sequences were considered of a common length of 532 nucleotides (nt 77 to 608 of mtCOI gene, KF991608) including 33 sequences obtained from this research (Tables S2 and S4), 10 sequences previously reported from Costa Rica (Guevara-Coto et al., 2011; Vargas-Asencio et al., 2013), and a collection of sequences representative of the different sequence haplotypes detected among sequences available in databases.

For *B. tabaci* and *T. vaporariorum*, representatives of identical sequence haplotypes from other parts of the world were also included (Tables S3 and S4). All sequences were aligned using MUSCLE (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The DnaSP package (Librado & Rozas, 2009) was used to estimate the number of haplotypes. The nonsynonymous (nsSNPs) and synonymous substitutions found in haplotypes for MED, MEAM1 and *T. vaporariorum* were also calculated.

For phylogenetic comparison, 63 and 26 representatives of the sequence haplotypes detected in the Western Hemisphere were included for *B. tabaci* and *T. vaporariorum*, respectively. Also, 87 and 20 representatives of sequences haplotypes shared with *B. tabaci* and *T. vaporariorum* from other parts of the world were included, respectively. Complete information about partial mtCOI sequences of *B. tabaci* and *T. vaporariorum* obtained in this research and from other sources used in this study is detailed in Tables S2, S3 and S4. We initially used the jModelTest 2.1.7 (Darriba, Taboada, Doallo, & Posada, 2012) and AIC model selection to define the optimum substitution model for all sequences. The General Time Reversible (GTR) model considering gamma distribution with invariant sites was chosen for sequence comparisons. Bayesian phylogenetic analysis was conducted using MrBayes (Huelsenbeck, Ronquist, Nielsen, & Bollback, 2001). Analyses considered 10 million generations using eight chains and sampling every 2,000 generations. The mtCOI sequence of *B. atriplex* (Froggatt) (HQ457047) was included as the outgroup for *B. tabaci* analysis, while that of *T. ricini* (Misra) (AM179447) was included as the outgroup for *T. vaporariorum* phylogeny.

For *B. tabaci* sequence divergence analyses, 46 of the 47 species groups of the *B. tabaci* complex were retrieved (Table S3). A 657 nucleotides fragment of mtCOI sequence was used in this study to estimate the genetic distance within and between groups (Table S5), using the Kimura 2-parameter model (Kimura, 1980) of MEGA 6 (Tamura et al., 2013). All sequences of accepted and putative *B. tabaci* species with a divergence value higher than 3.5% were included in the phylogenetic analysis, with the exception of MEAM2 (Tay et al., 2017) and the proposed SSAF5 (Mugerwa et al., 2018), due to the short length of the available sequence.

2.4 | Statistical model

A database was produced that included whitefly species (determined by PCR-RFLP and represented as presence or absence), geographical location, production system (greenhouse or open field), plant host (sweet pepper, tomato, common bean and weed) and sampling date. The latter was referred to as sampling month, where month = 0 refers to the first sampling date. Data for the 181 *B. tabaci* and 435 *T. vaporariorum* whitefly individuals analysed by PCR-RFLP from Costa Rica populations in this study were included in the database.

To examine the influence of the different variables on the relative abundance of the whitefly species, data were arranged in contingency tables in the following manner: (a) whitefly species identified by year (2011 or 2012), by sampled plant species (tomato, sweet pepper, common bean and weeds) and by production system (greenhouses and open field), and (b) whitefly species identified in relation to region (Zarcero, Grecia and Cartago) and production type.

Multinomial regression models were constructed to examine the probability of detection of whitefly species as a function of the independent factors. Eighteen models were compared. The following factors were evaluated: whitefly species (sw), region (r), growth system (g), host plant (h), collecting date (d) and season (s). Analyses were conducted using the R software environment for statistical computing version 3.1.0 (R Core Team, 2013), using the multinom function from the nnet package. Model selection was based on model deviance and Akaike's information criterion (AIC), and biological interpretation.

3 | RESULTS

3.1 | Incidence and distribution of whiteflies

A total of 66 whitefly samples were collected from commercial crops of tomato, pepper, common bean and associated weeds in the Grecia, Zarcero and Cartago regions of the Central Valley of Costa Rica. Samples were collected during the dry and rainy seasons of 2011 and 2012 (Figure 1, Table S1). Partial mtCOI gene fragments amplified from 621 whitefly individuals (Table S1) were analysed by PCR-RFLP to differentiate whitefly types. The results indicated the presence of *T. vaporariorum*, and *B. tabaci* MED and MEAM1 types. *T. vaporariorum* was the predominant species with 435 of the 621 individuals analysed identified as *T. vaporariorum*, whereas 186 individuals correspond to *B. tabaci*. In the latter case, *B. tabaci* MED was detected quite frequently (181 individuals) and sporadic presence (only five individuals detected) of *B. tabaci* MEAM1 was observed. Both *B. tabaci* MED and *T. vaporariorum* were found widely distributed in the different sampling sites in open field and/or greenhouse crops (Table S1, Figure 1b).

Of the 621 whitefly individuals analysed, 333 correspond to individuals collected in 2011 (Table 1). The majority were identified as *T. vaporariorum* (66%), followed by *B. tabaci* MED (32.7%). Only four

TABLE 1 Relative abundance of whitefly species detected in surveys conducted in 2011 and 2012 in the Central Valley of Costa Rica[a]

		Year							
		2011				2012			
Plant species	Crop system	*B. tabaci* MEAM1	*B. tabaci* MED	*T. vaporariorum*	Total no. individuals	*B. tabaci* MEAM1	*B. tabaci* MED	*T. vaporariorum*	Total no. individuals
Sweet pepper	Greenhouse	0	40.4 (42)	59.6 (62)	104	0	13.8 (18)	86 (112)	130
	Open field	0	30 (9)	70 (21)	30	2.1 (1)	21.3 (10)	75.6 (36)	47
Tomato	Greenhouse	0	48.3 (29)	51.6 (31)	60	0	66.6 (34)	33.3 (17)	51
	Open field	0	18.3 (11)	81.6 (49)	60	0	33.3 (10)	66.6 (20)	30
Common bean	Open field	14.8 (4)	0	85.2 (23)	27	0	0	100 (20)	20
Weeds	Greenhouse	0	25 (8)	75 (24)	32	0	0	100 (10)	10
	Open field	0	50 (10)	50 (10)	20	0	0	0	0
Total		1.2 (4)	32.7 (109)	66 (220)	333	0.34 (1)	25 (72)	74.6 (215)	288

[a] Individual whiteflies were identified by means of PCR-RFLP based on a partial mitochondrial cytochrome *c* oxidase subunit 1 gene (mtCOI) fragment. Values shown are percentages; the number of individuals is indicated between parentheses.

individuals (1.2%) of *B. tabaci* MEAM1 were detected. Similar results were obtained in 2012, with 288 whitefly individuals analysed, 215 (74.6%) identified as *T. vaporariorum*, followed by *B. tabaci* MED with 72 individuals (25%), and one single individual (0.3%) of *B. tabaci* MEAM1. *B. tabaci* MEAM1 individuals were detected in field grown common bean (2011) and sweet pepper (2012) crops, whereas *B. tabaci* MED and *T. vaporariorum* were found widely distributed in the different crops and weeds sampled in both open fields and greenhouses.

As summarised in Table 1, 311 whitefly individuals from sweet pepper were analysed, with *B. tabaci* MEAM1 comprising 0.32% of those samples, *B. tabaci* MED 25.4%, and *T. vaporariorum* 74.2%. A total of 201 whitefly individuals collected from tomato were analysed, with 41.8% corresponding to *B. tabaci* MED and 58.2% to *T. vaporariorum*. In sweet pepper, *B. tabaci* MED individuals were detected at similar rates in greenhouses and in open fields (40.4% vs. 30% in 2011; 13.8% vs. 21.3% in 2012). In tomato, a higher abundance of *B. tabaci* MED was observed in greenhouses than in open fields (48.3% vs. 18.3% in 2011 and 66.6% vs. 33.3% in 2012). The abundance of *T. vaporariorum* individuals in sweet pepper plants was high both in greenhouses (59.6% in 2011; 86.0% in 2012) and open fields (70% in 2011; 75.6% in 2012). A similar situation was found in tomatoes, with 51.6% and 33.3% in greenhouses, and 81.6% and 66.6% in open fields, in 2011 and 2012, respectively.

In common beans (Table 1), sampled only from open fields, 47 whitefly individuals were analysed, of which 91.5% were identified as *T. vaporariorum* (85.2% in 2011 and 100% in 2012) and 8.5% were *B. tabaci* MEAM1. A total of 62 whitefly individuals from weeds were analysed, with 29% corresponding to *B. tabaci* MED and 71% to *T. vaporariorum*. The latter whitefly predominated among individuals from greenhouses with incidences of 75% in 2011 and 100% in 2012. The weeds most commonly found in the surveyed locations were *Ruta* spp. (family Rutaceae), *Phytolacca* spp. (Phytolaccaceae), *Plantago major* L. (Plantaginaceae), and *Brassica* spp. (Brassicaceae). Weeds were not identified at the species level.

As indicated above, *B. tabaci* MEAM1 individuals were rarely detected and were found only in the Cartago region in common bean in 2011 (four individuals, from crops cultivated close to tomato and sweet pepper production areas), and in sweet pepper in open field production in 2012 (one individual). This whitefly species was not found in the other two regions.

B. tabaci whitefly individuals were found in the three geographical regions sampled (Zarcero, Grecia and Cartago) (Table 2 and Figure 1). The abundance of this whitefly was highest (72.5%) in greenhouse tomato in Zarcero (Table 2). In contrast, the abundance of *B. tabaci* was very low (1.7%) in greenhouse-grown sweet pepper in the Cartago region (Table 2). *T. vaporariorum* was present in all regions and in all host plants surveyed with higher abundance than *B. tabaci* in most cases (Table 2 and Figure 1b).

3.2 | Genetic diversity of mtCOI whitefly sequences

Partial mtCOI gene sequences were obtained from the 56 fragments (~800 bp) amplified from whitefly individuals representing all the locations sampled (Table S1 and Table S2) and deposited in the GenBank database under the following accession numbers: *B. tabaci* MEAM1: KY441488–KY441489; *B. tabaci* MED: KY441492, KY441494–KY441503, KY441505–KY441506, KY441509, KY441512, KY441514, KY441516–KY441518, KY441520, KY441522; and *T. vaporariorum*: KY441523–KY441555. As summarised in Table S2, variable sequence fragments were obtained for the 21 *B. tabaci* MED, the two *B. tabaci* MEAM1, and the 33 *T. vaporariorum* whitefly individuals for which the mtCOI gene was analysed. Equivalent portions were then compared, with a region of 657 nucleotides for *B. tabaci* MED and *B. tabaci* MEAM1, and of 532 nucleotides for *T. vaporariorum*.

The variations observed for the sequence haplotypes deduced from mtCOI gene fragments sequenced for *B. tabaci* MED individuals from the Western Hemisphere (this work and sequences available in databases, Table S3) derived from synonymous substitutions except for position 464 in haplotype MED-vi (Table 3). Two different haplotypes were observed for sequences from individuals analysed from Costa Rica that matched with haplotype sequences previously reported from the Western Hemisphere. Haplotype MED-i found in this research occurred with the highest frequency (21 out of 22 *B. tabaci* MED Costa Rican sequences) among analysed individuals and is detected in sequences previously reported from United States, Brazil and Argentina. In addition, this haplotype matches with

TABLE 2 Relative abundance of whitefly species [*Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1), *B. tabaci* Mediterranean (MED), and *Trialeurodes vaporariorum*] collected in sweet pepper and tomato in 2011 and 2012

		Sweet pepper[a]			Tomato[a]		
		B. tabaci			*B. tabaci*		
Region	Crop system	MEAM1	MED	*T. vaporariorum*	MEAM1	MED	*T. vaporariorum*
Zarcero	Greenhouse	0	20.3 (14/69)	79.7 (55/69)	0	72.5 (58/80)	27.5 (22/80)
Grecia	Greenhouse	0	41.6 (45/108)	58.3 (63/108)	0	36.3 (4/11)	63.6 (7/11)
	Open field	0	30 (9/30)	70 (21/30)	0	28.5 (20/70)	71.4 (50/70)
Cartago	Greenhouse	0	1.7 (1/57)	98.2 (56/57)	0	5 (1/20)	95 (19/20)
	Open field	2.12 (1/47)	21.3 (10/47)	75.6 (36/47)	0	5 (1/20)	95 (19/20)
Partial abundance		0.32 (1/311)	25.4 (79/311)	74.2 (231/311)	0	41.8 (84/201)	58.2 (117/201)
Total abundance		25.7 (80/311)		74.2 (231/311)	41.8 (84/201)		58.2 (117/201)

[a] Individual whiteflies were analysed by means of PCR-RFLP based on a partial mitochondrial cytochrome *c* oxidase subunit 1 gene (mtCOI) fragment. Values are shown for abundance (%) of whitefly type (number of individuals/total number of collected individuals).

TABLE 3 Single nucleotide polymorphisms (SNPs) detected in American *Bemisia tabaci* Mediterranean (MED) species haplotypes from a sequence of 657 nt of the mitochondrial cytochrome *c* oxidase subunit 1 gene (mtCOI) fragment considered in this study

	SNP no. on the amplified mtCOI fragment[a]								
Haplotype designation	20	29	152	422	443	464	482	602	651
MED-i	A	C	T	T	T	C	C	T	C
MED-ii	A	C	C	C	C	C	T	C	T
MED-iii	A	G	C	C	C	C	T	C	T
MED-iv	G	G	C	C	C	C	T	C	T
MED-v	A	C	T	C	C	C	C	C	C
MED-vi	A	C	C	T	C	**A**	T	C	T
MED-vii	A	C	T	T	T	C	C	T	T

Note. The SNP that is nonsynonymous (nsSNP) is boxed and highlighted in a bold font to highlight when the amino acid could have been altered.
[a] Nucleotide positions refer to positions on the mtCOI gene of *B. tabaci* MED GenBank accession number KJ606633.

sequences from Cyprus, Israel, Syria and Turkey (Figure 2). This haplotype was present in individuals from all sites surveyed in the Central Valley in tomato and sweet pepper crops and weeds (Table S2). The second haplotype (named haplotype MED-ii) was found present in only one of the sequences obtained from *B. tabaci* MED Costa Rican individuals in previous studies (HQ231410) (Guevara-Coto et al., 2011). This sequence haplotype was detected from whitefly individuals collected from tomato greenhouses in Zarcero and matches Western Hemisphere *B. tabaci* MED sequences reported from United States, Guatemala and Brazil (de Moraes et al., 2017; Elfekih, Tay, Gordon, Court, & De Barro, 2018; McKenzie et al., 2009, 2012). In addition, it was detected in mtCOI sequences obtained for *B. tabaci* MED individuals from other parts of the world such as China, France, Greece, Portugal, Spain, South Korea, Tunisia and Vietnam (Figure 2). Although not present in Costa Rica, five additional haplotypes (MED-iii, MED-iv, and MED-v, MED-vi and MED-vii) were found present in whitefly individuals from the Western Hemisphere. Haplotype MED-iii was quite spread and was detected in *B. tabaci* MED individuals from North America (collected from fern, poinsettia, mint and zinnia) (McKenzie et al., 2009, 2012), Argentina (from whiteflies collected from common bean) (Alemandri et al., 2015), Brazil (from whiteflies collected from *C. annuum* and sweet potato), and Uruguay. It was also found present in sequences deduced for *B. tabaci* MED individuals from Asia (China, Japan and South Korea) and Europe (France and Spain) (Figure 2). The fourth haplotype detected in Latin American individuals (MED-iv) was found restricted to sequences from *B. tabaci* MED individuals collected from Argentina and Uruguay. This haplotype was also found present outside the Western Hemisphere, in sequences from individuals collected in France, Spain and China (Figure 2). Haplotype MED-v, MED-vi and MED-vii were detected only in individuals from Brazil (Figure 2).

The analysis of the mtCOI sequences identified for *B. tabaci* MEAM1 individuals from the Western Hemisphere showed the presence of 18 different haplotypes (Figure 2, Table S3) with 35 single nucleotide polymorphisms (SNPs) that occurred at different sequence locations (Table 4). Twenty-two SNPs were nsSNPs and are found present in the different haplotypes (Table 4); nsSNPs changes in coding regions such as mtCOI are important because they could determine phenotypic changes to the whitefly, owing to protein alteration. Two different haplotypes were identified from the *B. tabaci* MEAM1 individuals analysed from Costa Rica (named MEAM-i and MEAM-xviii). The MEAM1-i haplotype was identified in this work from individuals collected from sweet pepper and was found spread throughout the Western Hemisphere (Figure 2) where it has been shown in sequences from whiteflies collected from the United States (obtained from whiteflies surveyed in different crops) (McKenzie et al., 2012), Argentina (whitefly individuals collected from *Cucumis melo* L. and common bean) (Alemandri et al., 2015), Brazil, Cuba, Guatemala, Netherlands Antilles, and Trinidad and Tobago. Outside the Western Hemisphere, this haplotype was also found in sequences from *B. tabaci* MEAM1 individuals analysed from Asia (China, Japan, India, South Korea), Cyprus, France, Israel, Morocco, Réunion island, Spain, Syria,

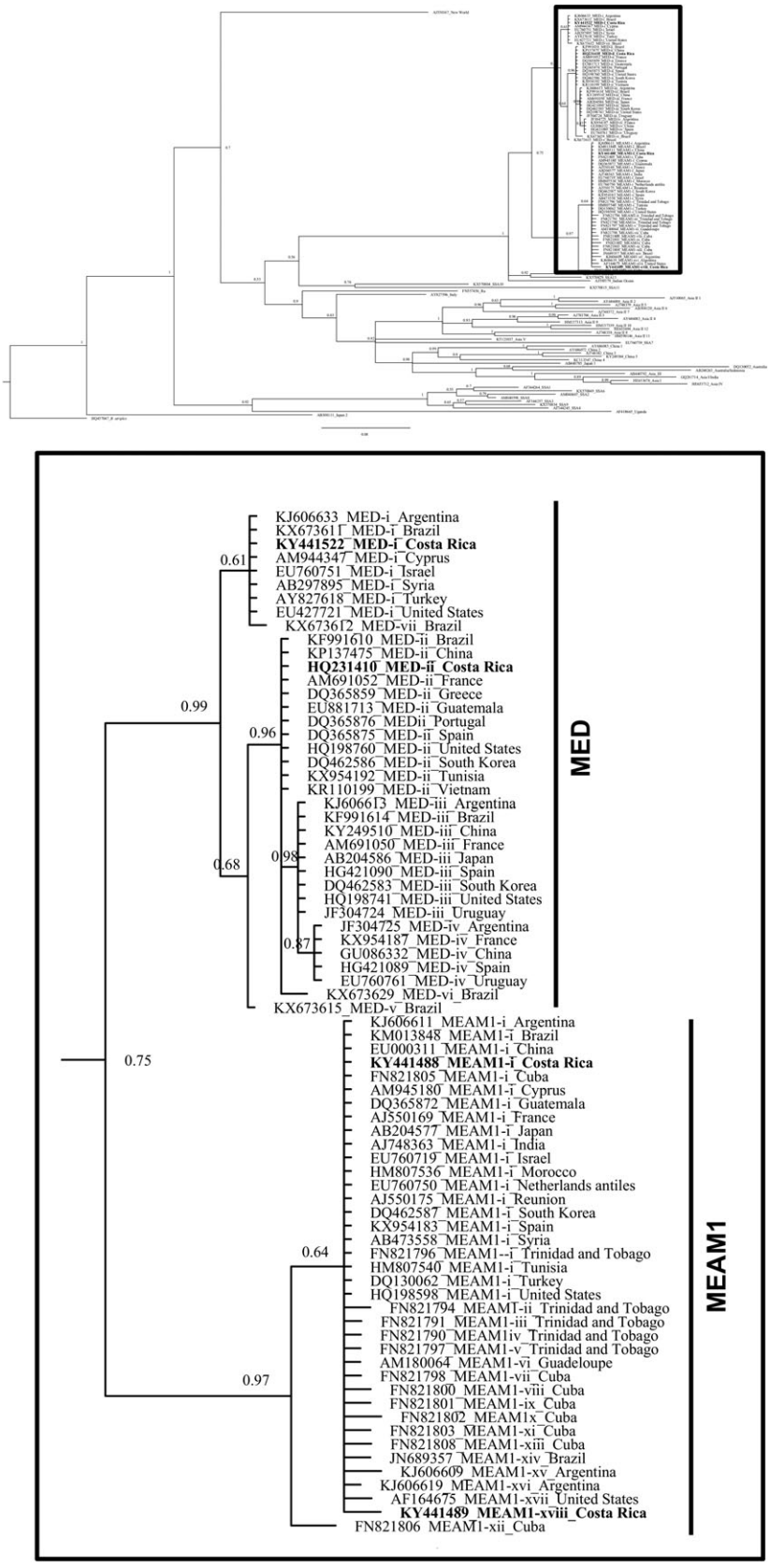

FIGURE 2 Extract from Figure S1 (shown as a small figure on the left) representing the phylogenetic tree for equivalent partial mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) sequences (657 nucleotides) of *Bemisia tabaci* Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) representing haplotypes detected in the Americas including those representative of haplotypes detected from individuals collected in Costa Rica from surveys conducted in 2011 and 2012 at the major vegetable growing regions or equivalent haplotypes available in databases from other parts of the world. Sequences were aligned (using MUSCLE) and the phylogenetic tree was estimated by using Bayesian inference. Numbers above branches represent the Bayesian posterior probabilities. The bar indicates substitutions per site. Haplotypes corresponding to those present in Costa Rica are shown in bold font. Accession number of each sequence is indicated, followed by haplotype classification, and country of origin

TABLE 4 Single nucleotide polymorphisms (SNPs) detected in American *B. tabaci* Middle East-Asia Minor 1 (MEAM1) species haplotypes from a sequence of 657 nt of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) fragment considered in this study[a]

	SNP no. on the amplified mtCOI fragment																																				
Haplotype designation	**15**	**109**	**145**	**182**	**295**	**302**	**318**	**322**	**362**	**367**	**371**	**381**	**500**	**505**	**506**	**515**	**530**	**540**	**554**	**560**	**561**	**578**	**582**	**587**	**589**	**594**	**595**	**634**	**635**	**637**	**638**	**641**	**642**	**654**	**656**
MEAM1-i	T	T	T	G	A	T	C	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-ii	T	T	T	G	A	T	C	A	A	G	T	A	G	T	T	A	T	G	C	G	G	C	**A**	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-iii	T	T	T	G	A	T	C	A	A	G	T	**G**	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-iv	T	T	T	G	A	T	**T**	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	**C**	G
MEAM1-v	T	T	T	G	A	T	C	A	A	**G**	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	**G**	G
MEAM1-vi	T	T	T	G	A	T	C	A	A	**A**	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-vii	T	T	T	G	A	T	C	A	G	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	**T**	T	A	A	A	C	**T**	C	G
MEAM1-viii	T	T	T	G	A	T	C	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	**A**	T	A	A	A	C	**C**	C	G
MEAM1-ix	**T**	T	T	G	A	T	C	A	A	G	C	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	G	C	T	C	G
MEAM1-x	**C**	T	**T**	A	**G**	T	C	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-xi	T	T	**C**	G	A	T	C	**G**	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	A	C	T	C	G
MEAM1-xii	T	T	T	G	A	T	C	A	A	G	T	A	G	T	C	G	C	G	T	A	G	C	G	A	G	T	T	T	A	A	T	T	C	T	T
MEAM1-xiii	T	**C**	T	G	A	C	C	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	T	A	A	C	C	T	C	G
MEAM1-xiv	T	T	T	G	A	T	C	A	A	G	T	A	**G**	T	T	A	T	G	C	A	G	C	G	**C**	**A**	C	T	T	A	A	C	C	T	C	G
MEAM1-xv	T	T	T	G	A	T	C	A	A	G	T	A	**C**	T	T	A	T	**C**	C	A	**A**	C	G	A	G	C	T	T	A	A	C	C	T	C	G
MEAM1-xvi	T	T	T	G	A	T	C	A	A	G	T	A	G	**T**	T	A	T	G	C	A	G	C	G	A	G	C	**C**	T	A	A	C	C	T	C	G
MEAM1-xvii	T	T	T	G	A	T	C	A	A	G	T	A	G	**C**	T	A	T	G	C	A	G	**T**	G	A	G	C	T	T	A	A	C	C	T	C	G
MEAM1.xviii	T	T	T	G	A	T	C	A	A	G	T	A	G	T	T	A	T	G	C	A	G	C	G	A	G	C	T	**A**	**T**	**T**	C	C	T	C	G

Note. The SNPs that are nonsynonymous (nsSNP) are boxed and highlighted in a bold font[a] to highlight when the amino acid could have been altered.

[a] Nucleotide positions refer to positions on the mtCOI gene of *B. tabaci* MEAM1 GenBank accession number KJ606611.

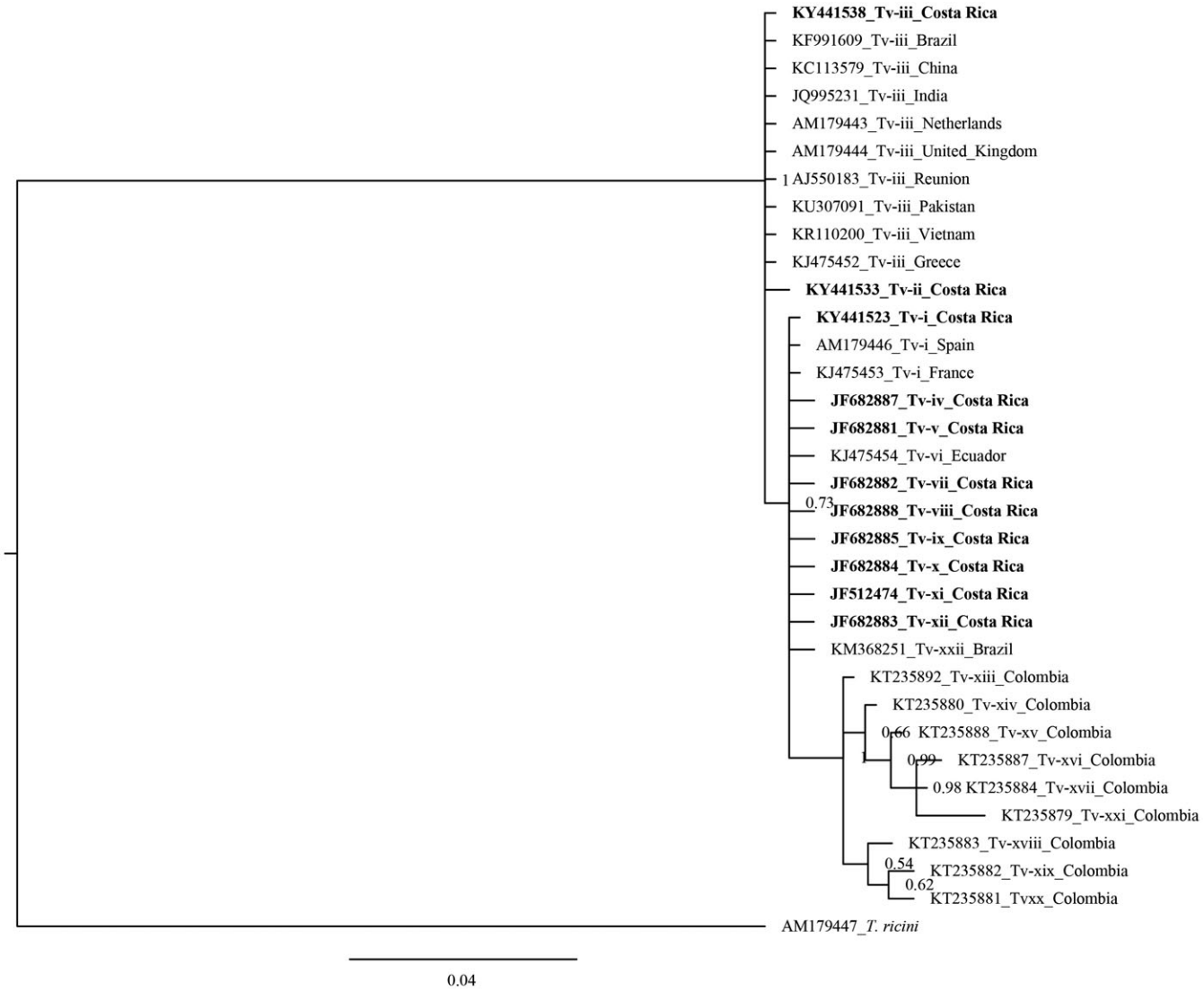

FIGURE 3 Phylogenetic tree for equivalent partial mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) sequences (532 nucleotides) of *Trialeurodes vaporariorum* individuals representing haplotypes detected in the Americas including those representative of haplotypes detected from individuals collected in Costa Rica from surveys conducted in 2011 and 2012 at the major vegetable growing regions or equivalent haplotypes available in databases from other parts of the world. Sequences were aligned (using MUSCLE) and the phylogenetic tree was estimated by using Bayesian inference. Numbers above branches represent the Bayesian posterior probabilities. The bar indicates substitutions per site. Haplotypes corresponding to those present in Costa Rica are shown in bold letters. The phylogenetic trees were rooted using an equivalent sequence from *T. ricini*. Accession number of each sequence is indicated, followed by haplotype classification, and country of origin

Tunisia and Turkey. Haplotype MEAM1-xviii was unique for a sequence characterised from an individual collected in Costa Rica from common bean crop.

Analysis of mtCOI sequences of *T. vaporariorum* individuals from the Western Hemisphere (this work and sequences available in databases, Table S3) showed the presence of 22 different haplotypes (Figure 3) with 24 SNPs that occurred at different sequence locations (Table 5). Fifteen SNPs were nsSNPs and were found in different haplotypes (Table 5). Partial mtCOI sequences deduced for *T. vaporariorum* individuals from Costa Rica showed the presence of an abundant number (n = 11) of haplotypes, three of them (Tv-i, Tv-ii and Tv-iii) deduced from sequences obtained in this work (Figure 3, Table S4). The haplotype named Tv-i was the most frequent among the sequences of individuals from Costa Rica (31 out of 43 Costa Rican sequences) and was found in individuals collected from all of the tomato and sweet pepper sites sampled in the survey of this study (Table S2). It was also found present in individuals collected from common bean and weeds. *T. vaporariorum* individuals of this haplotype were found alone or in association with *B. tabaci* MED and/or *B. tabaci* MEAM1 individuals. It matched sequences previously reported from Costa Rica (JF682886, JF682887) obtained from whiteflies present in sweet pepper crops in the Cartago region (Vargas-Asencio et al., 2013). Also, outside from Costa Rica, it matched with sequences from individuals collected in France and Spain.

A second sequence haplotype (named Tv-iii) was found in this study only in whiteflies collected from weeds (Table S2) and matched sequences deduced from individuals collected in Brazil within the Western Hemisphere. Additionally, this sequence haplotype seems to be quite spread geographically as was found in countries such as China (from cucumber), the United Kingdom (*Primula*

TABLE 5 Single nucleotide polymorphisms (SNPs) detected in American *Trialeurodes vaporariorum* haplotypes from the sequence of 532 nucleotides of the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI) fragment considered in this study[a]

	SNP no. on the amplified mtCOI fragment																							
Haplotype designation	15	54	57	81	106	126	131	160	176	182	202	233	288	295	300	316	321	325	360	391	437	456	487	519
Tv-i	T	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	A	T	A	A	A	G
Tv–ii	T	C	C	C	A	C	T	T	C	A	T	T	G	T	C	T	T	A	A	T	A	A	A	G
Tv–iii	T	C	C	C	A	C	T	T	C	A	G	T	G	T	C	T	T	A	A	T	A	A	A	G
Tv–iv	**C**	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	A	T	A	A	A	G
Tv–v	T	C	C	C	A	C	C	T	C	A	G	T	G	C	C	T	T	A	A	T	A	A	A	G
Tv–vi	C	C	C	C	A	C	C	T	C	A	G	T	**T**	T	C	T	T	A	A	T	A	A	A	G
Tv–vii	C	C	C	C	A	C	C	T	C	A	G	**G**	G	T	C	T	T	A	A	T	A	A	A	G
Tv–viii	C	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	**C**	T	A	A	A	G
Tv–ix	C	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	A	**G**	A	A	A	G
Tv–x	C	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	A	T	A	**C**	A	G
Tv–xi	C	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	**C**	A	A	T	A	A	A	G
Tv–xii	C	C	C	C	A	C	C	C	C	A	G	T	G	T	C	T	T	A	A	T	A	A	A	G
Tv–xiii	C	C	**C**	C	G	C	C	T	C	A	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv–xiv	C	C	**T**	C	G	C	C	T	C	A	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv–xv	C	C	**T**	C	G	**A**	C	T	C	A	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv–xvi	C	C	C	C	G	**A**	C	T	C	**C**	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv–xvii	C	C	**T**	C	G	**A**	C	T	C	**C**	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv-xviii	C	**T**	**T**	C	G	C	C	T	C	A	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv-xix	C	**T**	C	C	G	C	C	T	C	A	G	T	G	T	C	T	T	G	A	T	**G**	A	G	G
Tv-xx	C	**T**	C	**G**	G	C	C	T	C	A	G	T	G	T	C	T	T	G	A	T	A	A	G	G
Tv-xxi	C	C	**T**	**G**	G	**A**	C	T	**G**	**C**	G	T	G	T	**G**	C	T	G	A	T	A	A	G	G
Tv-xxii	C	C	C	C	A	C	C	T	C	A	G	T	G	T	C	T	T	A	A	T	A	A	A	A

Note. The SNPs that are nonsynonymous (nsSNP) are boxed and highlighted in a bold font to highlight when the amino acid could have been altered.

[a] Nucleotide positions refer to positions on the mtCOI gene of *T. vaporariorum* GenBank accession number KJ475453.

spp.), the Netherlands (*Gerbera* spp.), and also in India, Pakistan, Greece, Vietnam and Réunion island (Figure 3). Other haplotypes deduced for sequences of *T. vaporariorum* individuals from Costa Rica (named Tv-ii, Tv-iv, Tv-v, Tv-vii, Tv-xviii, Tv-ix, Tv-x, Tv-xi, Tv-xii) are unique to this country (Figure 3, Table S4). These results suggest a wide variation in mtCOI sequence of *T. vaporariorum* individuals from Costa Rica.

3.3 | Models of whitefly incidence by region in Costa Rica

Eighteen models were examined to evaluate which factors best explained differences in whitefly abundance and to predict the risk of whitefly incidence in tomato, sweet pepper, weeds and common bean in the three regions of Costa Rica surveyed in this study (Table S6). The additive model of the whitefly type as a function of region (r) and host plant (h) best explained the probability of whitefly infestations in tomato, sweet pepper, weeds and common bean. The model selection was based on the lowest deviance value, AIC and biological relevance. The lowest AIC values were found when both region and host plant were considered.

4 | DISCUSSION

In this study, an analysis of the whitefly diversity present in Costa Rica was performed based on a survey conducted at the major vegetable growing regions, concentrated mostly in the Central Valley (Zarcero in the north, Grecia in the centre and Cartago in the east). *T. vaporariorum* was found to be the most widespread whitefly and was frequent in almost all regions and plant hosts surveyed. The *B. tabaci* MED species was also found in all the production areas, including the Cartago region where only *T. vaporariorum* had been reported previously (Vargas-Asencio et al., 2013). Although the prevalence of *B. tabaci* MED in Cartago was low in the present study, this finding suggests that *B. tabaci* MED is successfully colonising new areas.

In the Western Hemisphere, *B. tabaci* MED has been reported in Canada, United States, Mexico, Guatemala, and more recently Argentina, Uruguay and Brazil, mostly in protected crops (Alemandri et al., 2015; Barboza et al., 2015; Bethke et al., 2009; de Moraes et al., 2017; Martínez-Carrillo & Brown, 2007; McKenzie et al., 2009, 2012). It has also been reported in Asia and Mediterranean countries (Chu, Hao, Jun, & Brown, 2010; Horowitz, Kontsedalov, Khasdan, & Ishaaya, 2005; Hu et al., 2017; Pan et al., 2011; Pascual & Callejas, 2004). In our study, populations of *B. tabaci* MED were found for the first time in open field production of tomato and sweet pepper and associated weeds in Costa Rica. The presence of this whitefly could have a negative impact on vegetable production due to the plasticity of this species in colonising new habitats (e.g., McKenzie et al., 2012) and its previously reported propensity to develop resistance to insecticides widely used for whitefly control (Horowitz et al., 2005).

It is interesting to note the clear predominance of *B. tabaci* MED over *B. tabaci* MEAM1 in the Central Valley of Costa Rica, the latter being found only sporadically in Cartago on common bean and sweet pepper plants. This could be related to altitude, a variable that may be affecting *B. tabaci* distribution in the country, as suggested by Hilje, Cubillo, and Segura (1993). *B. tabaci* MEAM1 and New World (formerly known as biotype A) were previously reported in Costa Rica alone or in association, both in the Central Valley and Pacific regions, where the production of tomato, sweet pepper and melon (*Cucumis melo* L.) is developed (Hilje & Morales, 2008; Morales et al., 2005). The current predominance of *B. tabaci* MED in the Central Valley found in this study could reflect a change in the distribution pattern of the *Bemisia* species, especially in tomato and sweet pepper production areas. The progressive increase of MED populations in areas where intensive farming occurs has been reported in the last decades such as in China (Zheng et al., 2017) and Italy (Bertin et al., 2018). Although *B. tabaci* MED was introduced into Costa Rica only recently, it seems that this species is now widely distributed in the country. Since the first report in this country in 2011 in the Zarcero region (Guevara-Coto et al., 2011), this whitefly appears to have successfully colonised new regions such as Cartago, where originally only individuals of native (New World 2) and MEAM1 *B. tabaci* species were identified (Morales et al., 2005). This might be related to the ability of *B. tabaci* MED to successfully colonise new environments and displace native species (Chu, Tao, Zhang, Wan, & Brown, 2012; Delatte et al., 2009). Future studies of whitefly populations in other regions and crops will help determine if *B. tabaci* MED is displacing native whitefly species in Costa Rica, as shown by Chu et al. (2010) in China. In Brazil, extensive surveys have demonstrated the widespread occurrence of MEAM1 species and more recently of MED (Barboza et al., 2015; de Moraes et al., 2017; Marubayashi et al., 2013), with a low incidence of native *B. tabaci* species (Marubayashi et al., 2013). In contrast, NW2, MEAM1 and MED species occurred sympatrically in common bean in Argentina (Alemandri et al., 2015). Complex and dynamic *B. tabaci* populations seem to be present in natural infestations, suggesting that future studies to monitor the distribution and spread of these whitefly species will be needed to understand their impact on agriculture in Costa Rica.

T. vaporariorum was found to be the predominant whitefly in all surveyed regions of the Central Valley of Costa Rica irrespective of the sampled plant species, which was expected due to the altitude at which most of the commercial fields were found. It was present at high abundance in greenhouses and in open fields and seems to be well adapted to tropical highland conditions (Manzano & van Lenteren, 2009). Our results indicate that conditions in greenhouses and open field farms in Costa Rica are adequate for the survival and reproduction of this species alone or in association with *B. tabaci* species. *T. vaporariorum* has been found present for a long time in the country (Hilje, Lastra, et al., 1993), and populations well adapted to the range of climate and cultivation conditions might have been selected. *T. vaporariorum* is an agricultural pest of global importance (Wainaina et al., 2018) and its emergence can be associated with the spread of viruses that cause severe threats to vegetable production (Wintermantel, 2004). Therefore, the results presented here suggest that a special attention should be given to this whitefly and associated viruses in Costa Rica.

Costa Rican *B. tabaci* mtCOI sequences showed limited variation for *B. tabaci* MED with presence of only two haplotypes that might represent independent introductions. MED-i haplotype was not found

associated with a particular region. In contrast, MED-ii was present only in the Zarcero region, which could suggest a recent introduction. Additional *B. tabaci* sequences should be obtained to confirm these results. McKenzie et al. (2012), also found MED-i (named as Q1) associated with MED-ii (Q2) in a low proportion. Two additional haplotypes, MED-iii and MED-iv, were also frequently found in sequences available from individuals collected from the Western Hemisphere. The SNPs observed in MED-i, MED-ii and MED-iii sequences were almost the same found by McKenzie et al. (2009) in the equivalent haplotypes Q1, Q2 and Q3, respectively. Similar correspondence was found with haplotype 6 (MED-iii) and 7 (MED-i) reported in Argentina by Alemandri et al. (2015). Furthermore, these haplotypes also correspond to Hap3 (MED-i), Hap1 (MED-ii), Hap2 (MED-iii) and Hap7 (MED-iv) proposed by De Barro and Ahmed (2011), that were obtained for sequences deduced from individuals from Turkey and China, and other parts of the world. Regarding its distribution in the Western Hemisphere, MED-i is found in North, Central and South America, as well as MED-ii. Although MED-iii was not identified in Costa Rican sequences, it matched with sequences from individuals collected in North and South America. MED-iv haplotype was found present in South America. Then, there are three haplotypes (MED-v, MED-vi and MED-vii) that were reported only from Brazil. We hypothesize that there have been several independent introductions of *B. tabaci* MED into the Western Hemisphere. Except for MED-v to MED-vii that were only detected in Brazil (de Moraes et al., 2017), the same *B. tabaci* MED variants were also found spreading in other parts of the world (Figure 2). Tsagkarakou, Tsigenopoulous, Gorman, Lagnel, and Bedford (2007) mention that the origin of MED-i (named Q1) is the eastern Mediterranean region (Israel), whereas the origin of MED-ii and MED-iii (named Q2 and Q3) may be the Western Mediterranean regions, for example, Spain or Morocco. All these data suggest that wide geographical movement of *B. tabaci* MED and that a global emergence seems to be occurring. Additional efforts should be made to obtain more sequences from *B. tabaci* MED individuals from the Western Hemisphere to determine if other haplotypes are present and to better understand the emergence of this species at the continental scale.

B. tabaci MEAM1 has been reported in the Western Hemisphere since early 1980s, affecting mainly ornamental plants. According to Brown, Frohlich, and Rosell (1995), it seems that this whitefly arrived in the Caribbean area through imported ornamental plants. Here we showed that 18 different sequence haplotypes were found for this species in the Western Hemisphere (Figure 2), with MEAM1 being represented in several areas of this hemisphere in addition to other areas in the Old World. This haplotype seems to be widely distributed worldwide and corresponds to the ancestral haplotype mentioned by De Barro and Ahmed (2011), named Jap1, and also with haplotype BH1 described in Asia (Hu et al., 2017). It is important to mention that the other haplotypes are unique or present in few whitefly individuals from the Western Hemisphere. The high variation of haplotypes found in sequences of *B. tabaci* MEAM1 from the Western Hemisphere is notable. The presence of this species for more than 30 years in the country, where it can reproduce at high speed, might have favoured wide diversification through point mutations. No such high genetic diversity was observed in other geographical areas in which presence of this whitefly has also been reported for long time. Therefore, this is an aspect that merits further study.

A significant number of haplotypes is observed in the *T. vaporariorum* population present in Costa Rica with at least 11 different haplotypes detected for the analysed mtCOI region suggesting a high diversification of the population. Nevertheless, the mtCOI phylogeny reconstruction showed that *T. vaporariorum* haplotypes are closely related. These results are similar to those found by Wainaina et al. (2018) for samples collected from Kenya that showed that the obtained sequences grouped in a single clade. Similarly, Kapantaidaki et al. (2015) also revealed a remarkably low diversity in *T. vaporariorum* populations, including mtCOI sequences from individuals representative of 18 countries around the world showing a phylogeny reconstruction with only two clades with limited sequence divergence. Thus, it seems that low levels of genetic variation exist for mtCOI sequence data available for *T. vaporariorum* from different parts of the world. The SNPs and the presence of nonsynonymous variation in sequences from the Western Hemisphere might suggest that a relevant diversification of the *T. vaporariorum* population is occurring in the region which should be taken into account.

The results obtained in this work provide basic knowledge that contributes to our understanding of the ecology of whitefly pests in Costa Rica. We analysed which factors best explained differences in whitefly species abundance and showed that the host plant species and the region appear to affect the distribution of whiteflies in the country. Other variables such as temperature, altitude, crop management regime, precipitation should be evaluated in future studies to obtain a more precise picture of whitefly distribution. Seedling production location is another aspect that should be taken into consideration; growers buy seedlings from providers located in different parts of the Central Valley, which could determine the spread of whitefly species in cultivated areas. Pest species are ideal for studying how different agroecosystems affect the population genetic structure within a species at different climatic extremes (Prijović et al., 2014). We showed here that *B. tabaci* MED may be invading new areas of Costa Rica, whereas *B. tabaci* MEAM1 was nearly absent in tomato and sweet pepper production areas in the Central Valley of this country. A wider survey is needed to determine if the native *B. tabaci* species NW2 is still present in the country (Morales et al., 2005) or if it has been displaced by the invasive species *B. tabaci* MED and MEAM1. We showed that *T. vaporariorum* is widely distributed, frequently associated with *B. tabaci*, and exhibits a genetically uniform population. Tomato and sweet pepper were the major crops surveyed in the present work. Sampling of other crops may help a more comprehensive understanding of whiteflies distribution in the country.

ACKNOWLEDGEMENTS

We thank all the growers and land owners for providing us access to survey the sites. The work was supported by a grant from FEES-CONARE (VI-801-b1-650) and University of Costa Rica. EM was supported by Ministerio de Economía y Competitividad and Ministerio de Economía, Industria y Competitividad, Spain with assistance from the European Regional Development Fund (ERDF) and the European Social Fund (ESF), Grant/Award Number: AGL2016-75819-C2-2. A.K.I.N. is a CNPq fellow.

CONFLICTS OF INTEREST

The authors declare no potential conflict of interests.

REFERENCES

Alemandri, V., De Barro, P., Bejerman, N., Argüello-Caro, E. B., Dumón, A. D., Mattio, M. F., … Truol, G. (2012). Species within the *Bemisia tabaci* (Hemiptera: Aleyrodidae) complex in soybean and bean crops in Argentina. *Journal of Economic Entomology*, *105*, 48–53.

Alemandri, V., Vaghi, C. G., Dumón, A. D., Argüello, E. B., Mattio, M. F., García, S., … Truol, G. (2015). Three members of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species complex occur sympatrically in Argentina horticultural crops. *Journal of Economic Entomology*, *18*, 405–413.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410.

Barboza, L. D. F., Yuki, V. A., Marubayashi, J. M., De Marchi, B. R., Perini, F. L., Pavan, M. A., … Krause-Sakate, R. (2015). First report of *Bemisia tabaci* Mediterranean (Q biotype) species in Brazil. *Pest Management Science*, *71*, 501–504.

Bellows, T. S., Perring, T. M., Gill, R. J., & Headrick, D. H. (1994). Description of a species of *Bemisia* (Homoptera: Aleyrodidae) infesting North American agriculture. *Annals of the Entomological Society of America*, *87*, 195–206.

Bertin, S., Luigi, M., Parrella, G., Giorgini, M., Davino, S., & Tomassoli, L. (2018). Survey of the distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Lazio region (Central Italy): A threat for the northward expansion of *Tomato leaf curl New Delhi virus* (Begomovirus: Geminiviridae) infection. *Phytoparasitica*, *46*, 171–182. https://doi.org/10.1007/s12600-018-0649-7

Bethke, J. A., Byrne, F. J., Hodges, G. S., McKenzie, C. L., & Shatters, R. G. (2009). First record of the Q biotype of the sweet potato whitefly, *Bemisia tabaci*, in Guatemala. *Phytoparasitica*, *371*, 61–64.

Bonfield, J. K., Smith, K. F., & Staden, R. (1995). A new DNA sequence assembly program. *Nucleic Acids Research*, *23*, 4992–4999.

Bosco, D., Loria, A., Sartor, C., & Cenis, J. L. (2006). PCR-RFLP identification of *Bemisia tabaci* biotypes in the Mediterranean basin. *Phytoparasitica*, *34*, 243–251.

Boykin, L. M. (2014). *Bemisia tabaci* nomenclature: Lessons learned. *Pest Management Science*, *7*, 1454–1459.

Boykin, L. M., & De Barro, P. J. (2014). A practical guide to identifying members of the *Bemisia tabaci* species complex: And other morphologically identical species. *Frontiers in Ecology and Evolution*, *2*, 45.

Boykin, L. M., Shatters, R. G., Jr, Rosell, R. C., McKenzie, C. L., Bagnall, R. A., De Barro, P. J., & Frohlich, D. R. (2007). Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Molecular Phylogenetics and Evolution*, *44*, 1306–1319.

Boykin, L. M., Savill, A., & De Barro, P. (2017). Updated mtCOI reference dataset for the *Bemisia tabaci* species complex. *F1000Research*, *6*, 1835. https://doi.org/10.12688/f1000research.12858.1

Brown, J. K., Frohlich, D. R., & Rosell, R. C. (1995). The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Annual Review of Entomology*, *40*, 511–534.

Chowda-Reddy, R. V., Kirankumar, M., Seal, S. E., Muniyappa, V., Valand, G. B., Govindappa, M. R., & Colvin, J. (2012). *Bemisia tabaci* phylogenetic groups in India and the relative transmission efficacy of *Tomato leaf curl Bangalore virus* by an indigenous and an exotic population. *Journal of Integrative Agriculture*, *11*, 235–248.

Chu, D., Hao, F., Jun, Y., & Brown, J. (2010). Change in the biotype composition of *Bemisia tabaci* in Shandong province of China from 2005 to 2008. *Environmental Entomology*, *39*, 1028–1036.

Chu, D., Tao, Y. L., Zhang, Y. J., Wan, F. H., & Brown, J. K. (2012). Effects of host, temperature and relative humidity on competitive displacement of two invasive *Bemisia tabaci* biotypes [Q and B]. *Insect Science*, *19*, 595–603.

Chu, D., Zhang, Y. J., Brown, J. K., Cong, B., Xu, B. Y., Wu, Q. J., & Zhu, G. R. (2006). The introduction of the exotic Q biotype of *Bemisia tabaci* from the Mediterranean region into China on ornamental crops. *Florida Entomologist*, *89*, 168–174.

R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Dalton, R. (2006). The Christmas invasion. *Nature*, *443*, 898–900.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*, 772.

De Barro, P. (2012). The *Bemisia tabaci* species complex, questions to guide future research. *Journal of Integrative Agriculture*, *11*, 187–196.

De Barro, P., & Ahmed, M. A. (2011). Genetic networking of the *Bemisia tabaci* cryptic species complex reveals pattern of biological invasions. *PLoS One*, *6*, e25579. https://doi.org/10.1371/journal.pone.0025579

De Barro, P. J., Liu, S. S., Boykin, L. M., & Dinsdale, A. B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, *56*, 1–19.

de Moraes, L. A., Marubayashi, J. M., Yuki, V. A., Ghanim, M., Bello, V. H., de Marchi, B. R., … Pavan, M. A. (2017). New invasion of *Bemisia tabaci* Mediterranean species in Brazil associated to ornamental plants. *Phytoparasitica: Israel Journal of Plant Protection Sciences*, *45*, 1–9.

Delatte, H., Duyck, P. F., Triboire, A., David, P., Becker, N., Bonato, O., & Reynaud, B. (2009). Differential invasion success among biotypes: Case of *Bemisia tabaci*. *Biological Invasions*, *11*, 1059–1070.

Dinsdale, A., Cook, L., Riginos, C., Buckley, Y. M., & De Barro, P. J. (2010). Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Annals of the Entomological Society of America*, *103*, 196–208.

Elfekih, S., Tay, W., Gordon, K., Court, L., & De Barro, P. (2018). Standardized molecular diagnostic tool for the identification of cryptic species within the *Bemisia tabaci* complex. *Pest Management Science*, *74*, 170–173.

Firdaus, S., Vosman, B., Hidayati, N., Supena, E. D. J., Visser, R. G. F., & van Heusden, A. W. (2013). The *Bemisia tabaci* species complex: Additions from different parts of the world. *Insect Science*, *20*, 723–733.

Frohlich, D. R., Torres-Jerez, I., Bedford, I. D., Markham, P. G., & Brown, J. K. (1999). A phylogeographical analysis of *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology*, *8*, 1683–1691.

Gilbertson, R. L., Batuman, O., Webster, C. G., & Adkins, S. (2015). Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annual Review of Virology*, *2*, 67–93.

Gorman, K., Devine, G., Bennison, J., Coussons, P., Punchard, N., & Denholm, I. (2007). Report of resistance to the neonicotinoid insecticide imidacloprid in *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Pest Management Science*, *63*, 555–558.

Grille, G., Gauthier, N., Buenahora, J., Basso, C., & Bonato, O. (2011). First report of the Q biotype of *Bemisia tabaci* in Argentina and Uruguay. *Phytoparasitica*, *39*, 235–238.

Guevara-Coto, J., Barboza-Vargas, N., Hernández-Jiménez, E., Hammond, R., & Ramírez-Fonseca, P. (2011). *Bemisia tabaci* Biotype Q is present in Costa Rica. *European Journal of Plant Pathology*, *131*, 167–170.

Hilje, L., Cubillo, D., & Segura, L. (1993). Observaciones ecológicas sobre la mosca blanca *Bemisia tabaci* (Gennadius) en Costa Rica. *Manejo Integrado de Plagas*, *30*, 24–30.

Hilje, L., Lastra, R., Zoebisch, T., Calvo, G., Segura, L., Barrantes, L., … Amador, R. (1993). Las moscas blancas en Costa Rica. In L. Hilje & O. Arboleda (Eds.), *Informe Técnico No. 205 Serie Técnica* (pp. 58–63). Turrialba, Costa Rica: CATIE.

Hilje, L., & Morales, F. J. (2008). Whitefly bioecology and management in Latin America. In J. L. Capinera (Ed.), *Encyclopedia of entomology* (pp. 117–128). Dordrecht, The Netherlands: Springer Science Business Media BV.

Horowitz, A. R., Kontsedalov, S., Khasdan, V., & Ishaaya, I. (2005). Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Archives of Insect Biochemestry Physiology*, *58*, 216–225.

Hu, J., De Barro, P., Zhao, H., Wang, J., Nardi, F., & Liu, S. S. (2011). An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. *PLoS ONE*, *6*, e1606. https://doi.org/10.1371/journal.pone.0016061

Hu, J., Zhang, X., Jiang, Z., Zhang, F., Liu, Y., Li, Z., & Zhang, Z. (2017). New putative cryptic species detection and genetic network analysis of *Bemisia tabaci* (Hempitera: Aleyrodidae) in China based on mitochondrial COI sequences. *Mitochondrial DNA Part A*, *29*, 474–484. https://doi.org/10.1080/24701394.2017.1307974

Huelsenbeck, J. P., Ronquist, F., Nielsen, R., & Bollback, J. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, *294*, 2310–2314.

Kapantaidaki, D., Ovčarenko, I., Fytrou, N., Knott, K. E., Bourtzis, K., & Tsagkarakou, A. (2015). Low levels of mitochondrial DNA and symbiont diversity in the worldwide agricultural pest, the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Journal of Heredity*, *106*, 80–92.

Karatolos, N., Pauchet, Y., Wilkinson, P., Chauhan, R., Denholm, I., Gorman, K., … Williamson, M. S. (2011). Pyrosequencing the transcriptome of the greenhouse whitefly, *Trialeurodes vaporariorum* reveals multiple transcripts and detoxifying enzymes. *BMC Genomics*, *12*, 56.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120.

Lee, W., Park, J., Lee, G. S., Lee, S., & Akimoto, S. I. (2013). Taxonomic status of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. *PLoS One*, *8*, e63817. https://doi.org/10.1371/journal.pone.0063817

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452.

Liu, S. S., Colvin, J., & De Barro, P. J. (2012). Species concepts as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? *Journal of Integrative Agriculture*, *11*, 176–186.

Manzano, M., & van Lenteren, J. (2009). Life history parameters of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) at different environmental conditions on two bean cultivars. *Neotropical Entomology*, *38*, 452–458.

Martínez-Carrillo, J., & Brown, J. K. (2007). First report of the Q biotype of *Bemisia tabaci* in southern Sonora, Mexico. *Phytoparasitica*, *35*, 282–284.

Marubayashi, J. M., Yuki, V. A., Rocha, K. C., Mituti, T., Pelegrinotti, M., Ferreira, F. Z., … Krause-Sakate, R. (2013). At least two indigenous species of *Bemisia tabaci* complex are present in Brazil. *Journal of Applied Entomology*, *137*, 113–121.

McKenzie, C., Bethke, J. A., Byrne, F., Chamberlin, J. R., Dennehy, T., Dickey, A. M., … Shatters, R. G., Jr. (2012). Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in North America after the Q invasion. *Journal of Economic Entomology*, *105*, 753–766.

McKenzie, C. L., Hodges, G., Osborne, L. S., Byrne, F., & Shatters, R. G. (2009). Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Florida–investigating the Q invasion. *Journal of Economic Entomology*, *102*, 670–676.

Morales, F. J., Hilje, L., Vallejos, J., Sibaja, G., Araya, C., & Araya, R. (2005). Whitefly and whitefly-borne viruses in the tropics: Building a knowledge base for global action. In P. K. Anderson & F. J. Morales (Eds.), *CIAT Publication 341* (pp. 217–221). Valle del Cauca, Colombia: CIAT.

Mugerwa, H., Seal, S., Wang, H. L., Patel, M. V., Kabaalu, R., Omongo, C. A., … Colvin, J. (2018). African ancestry of New World, *Bemisia tabaci*-whitefly species. *Scientific Reports*, *8*, 2734.

Navas-Castillo, J., López-Moya, J. J., & Aranda, M. A. (2014). Whitefly-transmitted RNA viruses that affect intensive vegetable production. *Annals of Applied Biology*, *165*, 155–171.

Pan, H., Chu, D., Ge, D., Wang, S., Wu, Q., Xie, W., … Zhang, Y. (2011). Further spread and domination by *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotype Q on field crops in China. *Journal of Economic Entomology*, *104*, 978–985.

Parrella, G., Scassillo, L., & Giorgini, M. (2012). Evidence for a new genetic variant in the *Bemisia tabaci* species complex and the prevalence of the biotype Q in southern Italy. *Journal of Pest Science*, *85*, 227–238.

Pascual, S., & Callejas, C. (2004). Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bulletin of Entomological Research*, *94*, 369–375.

Prijović, M., Skaljac, M., Drobnjaković, T., Zanić, K., Perić, P., Marčić, D., & Puizina, J. (2014). Genetic variation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), among populations from Serbia and neighboring countries, as inferred from COI sequence variability. *Bulletin of Entomological Research*, *104*, 357–366.

Qin, L., Pan, L. L., & Liu, S. S. (2016). Further insight into reproductive incompatibility between putative cryptic species of the *Bemisia tabaci* whitefly complex. *Insect Science*, *23*, 215–224.

Simón, B., Cenis, J., & De la Rúa, P. (2007). Distribution patterns of the Q and B biotypes of *Bemisia tabaci* in the Mediterranean Basin based on microsatellite variation. *Entomologia Experimentalis et Applicata*, *124*, 327–336.

Simon, C., Frati, F., Beckenbach, B., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, *87*, 651–704.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Tay, W. T., Elfekih, S., Court, L. N., Gordon, K. H., Delatte, H., & De Barro, P. J. (2017). The trouble with MEAM2: Implications of pseudogenes on species delimitation in the globally invasive *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species complex. *Genome Biology and Evolution*, *9*, 2732–2738.

Tsagkarakou, A., Tsigenopoulous, C. S., Gorman, K., Lagnel, J., & Bedford, I. D. (2007). Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: Mitochondrial DNA and microsatellites. *Bulletin of Entomological Research*, *97*, 29–40.

Vargas-Asencio, J. A., Hernández, E., Barboza, N., Hammond, R., Mora, F., & Ramírez, P. (2013). Detection of *Tomato chlorosis virus* and its vector *Trialeurodes vaporariorum* in greenhouse-grown tomato and sweet pepper in the Cartago province, Costa Rica. *Journal of Plant Pathology*, *95*, 627–630.

Wainaina, J. M., De Barro, P., Kubatko, L., Kehoe, M. A., Harvey, J., Karanja, D., & Boykin, D. (2018). Global phylogenetic relationships, population structure and gene flow estimation of *Trialeurodes vaporariorum* (greenhouse whitefly). *Bulletin of Entomological Research*, *108*, 5–13.

Wang, X. W., Luan, J. B., Li, J. M., Su, Y. L., Xia, J., & Liu, S. S. (2011). Transcriptome analysis and comparison reveal divergence between two invasive whitefly cryptic species. *BMC Genomics*, *12*, 458.

Wintermantel, W. M. (2004). Emergence of greenhouse whitefly (*Trialeurodes vaporariorum*) transmitted criniviruses as threats to vegetable and fruit production in North America. *APSnet Feature Story*. Retrieved from https://doi:10.1094/APSnetFeature-2004-0604

Zhang, G. F., Li, D. C., Liu, T. X., Wan, F. H., & Wang, J. J. (2011). Interspecific interactions between *Bemisia tabaci* Biotype B and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Environmental Entomology*, *40*, 140–150.

Zheng, H., Xie, W., Wang, S., Wu, Q., Zhou, X., & Zhang, Y. (2017). Dynamic monitoring (B versus Q) and further resistance status of Q-type *Bemisia tabaci* in China. *Crop Protection*, *94*, 115–122.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Barboza NM, Esker P, Inoue-Nagata AK, Moriones E. Genetic diversity and geographic distribution of *Bemisia tabaci* and *Trialeurodes vaporariorum* in Costa Rica. *Ann Appl Biol*. 2019;174:248–261. https://doi.org/10.1111/aab.12490