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Phylo-biogeographical distribution of whitefly *Bemisia tabaci* (Insecta: Aleyrodidae) mitotypes in Ecuador

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Citation: Paredes-Montero, J. R., M. A. Ibarra, M. Arias-Zambrano, E. L. Peralta, and J. K. Brown. 2020. Phylo-biogeographical distribution of whitefly *Bemisia tabaci* (Insecta: Aleyrodidae) mitotypes in Ecuador. Ecosphere 11(6): e03154. 10.1002/ecs2.3154

Abstract. The *Bemisia tabaci* complex in Ecuador was studied with respect to phylogenetic relationships and eco-geographical distribution. Whitefly samples were collected from natural and agricultural environments in nine provinces of Ecuador (latitude, 2° N–5° S; longitude, 78°–81° W). Mitotypes were identified based on phylogenetic analysis of the 3′-mt*COI*-tRNA*leu* region (832 bp) and corrected pairwise distance analysis. The distribution of mitotypes was modeled using MaxEnt, and their predicted niches were characterized according to environmental gradients. Four *B. tabaci* mitotypes were identified, of which three are endemic, herein ECU1–3, and the other is the introduced B mitotype. Mitotypes ECU1 (44%), ECU2 (0.74%), and ECU3 (1.47%) grouped in the American Tropics (AMTROP) species and diverged by as much as 10%, which was higher than previous estimates for the AMTROP clade of 7–8.6%. Although haplotypes of ECU1 and ECU2 are known from the American Tropics, this is the first report of the ECU3 mitotype, which may possibly be restricted to southern Ecuador. The distribution of the three ECU-endemic mitotypes spanned the high-altitude niches of the western slope of the Andes, rich in microclimates with variable temperature and humidity conditions. The non-endemic B mitotype (47%) occurred only in the irrigated cropping systems located in hot and/or dry-tropical ecological niches. Of the endemic mitotypes, ECU1 occupied the most ecological niches. Among variables contributing to ECU1 and B mitotype niche range assignments, the most significant to influence ecological range was rainfall. The *B. tabaci* endemic to Ecuador were more diverse with respect to mt*COI*-tRNA*leu* sequence than previously known, and occupied distinct microclimate niches suggestive of ecological resilience.

Key words: DNA barcoding; ecology; insect vector; MaxEnt; mitochondrial *COI;* diversity niche modeling.

Received 2 January 2020; revised 31 March 2020; accepted 3 April 2020. Corresponding Editor: Robert R. Parmenter.

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Introduction

The whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is hypothesized to represent a sibling or cryptic species group (Brown 2010, Gill and Brown 2010, Hadjistylli et al. 2016). This is based on observations that despite extreme phenotypic plasticity and genetic variation

existing within the group, the fourth instar, that is, the taxonomically informative stage, lacks morphological diagnostic characters beyond the species level (Mound 1963, 1983, Gill 1992, Rosell et al. 1996, 1997, de Moya et al. 2019). The earliest study to address the taxonomy of this whitefly led to the synonymization of 20 previously recognized species and three genera into the *B. tabaci* epithet (Russell 1957, Martin and Mound 2007). Since then, studies have recognized the taxon as a sibling or cryptic species group (Brown et al. 1995*b*, Brown 2010, Esterhuizen et al. 2013, Hadjistylli et al. 2016, de Moya et al. 2019), while others have proposed dividing the group into distinct species based on a 3.5% divergence of a fragment of the mitochondrial cytochrome oxidase I gene (mt*COI*; Perring et al. 1992, Bellows et al. 1994, Dinsdale et al. 2010, De Barro et al. 2011, Boykin et al. 2012, Liu et al. 2012, Mugerwa et al. 2018).

Phylogeographic clades representing five putative and at least two additional probable cryptic species have been recognized based on phylogenetic analysis of the mt*COI* gene (Brown 2010) and high-throughput phylogenomic analyses, respectively (de Moya et al. 2019), revealing global phylogeographical distribution. The major clades and species abbreviations are as follows: (I, II) sub-Saharan Africa (SSA), (III) North Africa-Mediterranean-Middle East (NAFMEDME), (IV) Asian-Australo-Pacific (ASAUSPAC), (V) Asia 2 (ASIA), and (VI, VII) American Tropics (AMTROP), with the most recent revision including minor changes in the previously proposed nomenclature (Brown 2010, Dinsdale et al. 2010, Gill and Brown 2010, Boykin et al. 2012, Hadjistylli et al. 2016, Wosula et al. 2017, Elfekih et al. 2018, de Moya et al. 2019) to account for newly discovered variants and update the geographical context of their phylogeographical distributions. Among the mitotypes represented within these major phylogeographic (mt*COI*) clades, a relatively small number have been documented to cause damage to crop plants either by feeding or by transmission of plant viruses, and surprisingly, among these, only several have been characterized to any extent (Costa et al. 1993, Bedford et al. 1994, Rosell et al. 1997, Demichelis et al. 2005, Caballero 2007, Brown 2010), and include the mitotypes, Asia 2 (ASIA [IV]), B and Q (NAFMEDME [III]), SSA-I, and A (AMTROP [VI, and tentatively, VII]) (Brown 1992, Costa et al. 1993, Brown et al. 1995*a*, Byrne et al. 1995, Brown and Bird 1996, Legg et al. 2002, 2014, Horowitz et al. 2005, Martinez-Carrillo and Brown 2007, Dennehy et al. 2010).

The B and Q sister mitotypes of *B. tabaci* are endemic to the region encompassing the Mediterranean and Middle East, and parts of the Sahel region of Africa (Brown 2010, de Moya et al. 2019), and recently, have become widely distributed mainly through human-mediated introductions. Invasion by B and Q mitotypes has resulted in plant virus disease outbreaks in crops and, often, the displacement of endemic *B. tabaci* populations. This is thought to have occurred in part because they are polyphagous and capable of host adaptation, and because certain haplotypes have developed resistance to some commonly used insecticides (Horowitz et al. 2005).

In South America, the extent of genetic variation and geographic distributions are poorly studied for the *B. tabaci* mitotypes known to affiliate with the AMTROP species, particularly since the exotic B mitotype was introduced there during the late 1990s. Although whiteflies involved in outbreaks occurring before 1990 in South America were presumed to be endemic *B. tabaci* (i.e., belonging to the AMTROP species), non-native mitotypes could have been overlooked because identification during pest inventories has relied on morphological (and not molecular) characters, with which only species-level discrimination was possible. More recently, in nearby Colombia, a random amplified polymorphic DNA (RAPD) analysis was used to identify the invasive B mitotype in irrigated vegetable production areas, and also to recognize the presence of a presumed endemic mitotype (Wool et al. 1991, 1994, Calvert et al. 2001, Quintero et al. 2001, Rodriguez and Cardona 2001, Carabali et al. 2005). In Ecuador, a similar approach was used to identify the introduced B and its occurrence in monoculture vegetable production situation in the coastal regions of Guayas, Los Rios, and Manabí (Quintero et al. 2001). Elsewhere in South America, an endemic mitotype has been reported from Bolivia, while two endemic mitotypes and the introduced B type have been documented in cotton–vegetable cropping systems in Argentina (Viscarret et al. 2003) and

Brazil (Lourenção and Nagai 1994, Lima et al. 2002, Marubayashi et al. 2013, Barbosa et al. 2014, Queiroz et al. 2016, Quintela et al. 2016). Otherwise, information is scarce with respect to genetic diversity and distribution of *B. tabaci* in the American Tropics. Thus, sampling of representative *B. tabaci* with increased intensity and distribution has become desirable to explore and document the extent of genetic richness of this taxon among the diverse climatological niches in South America.

Ecological niche modeling has become increasingly useful for assessing the impact of ecological processes on insect population dynamics, including determining the potential geographic distributions of species. The models of the correlative type, such as MaxEnt, have been used to predict effects of climate change on habitat loss for the leafhopper *Dalbulus maidis* in the Americas (Santana et al. 2019), and the risk of pest establishment for the mealybug *Phenacoccus solenopsis* in India (Kumar et al. 2014*a*), the whitefly *B. tabaci* in Europe (Gilioli et al. 2014), and other insect species (Kumar et al. 2014*b*, 2015). MaxEnt is a species distribution model based on a machine-learning framework that uses presence-only records and a collection of background points for the study area to estimate and compare probability densities based on a vector of environmental covariates relevant to habitat suitability (Elith et al. 2011). For example, using this approach has been pivotal in decision-making processes to establish quarantine strategies and conservation planning (Kumar and Stohlgren 2009, Costa et al. 2010, Gilioli et al. 2014, Kumar et al. 2014*a*, *b*, 2015, Santana et al. 2019), making it useful for studying the displacement of endemic *B. tabaci* by introduced mitotypes, based on ecological factors.

In this study, mitotypes of the *B. tabaci* sibling (or cryptic) species group from diverse ecological niches in Ecuador were identified from cultivated and wild plant species in nine provinces in Ecuador. Mitotypes were identified by DNA sequencing a mitochondrial 1015-base pair (bp) fragment comprising a 949-bp fragment of the mt*COI* and 66 bp of the adjacent leucine transfer RNA (tRNA*leu*) coding region. Sequences were used in phylogenetic and pairwise distance analyses. The predicted ecological niche(s) were determined for the different *B. tabaci* haplotypes based on site(s) of collection using the machine-learning algorithm MaxEnt (Phillips et al. 2017).

Materials and Methods

Whitefly sampling, and DNA isolation

Whitefly *B. tabaci* adults were collected from cultivated and uncultivated, wild eudicots from one or more locations each from nine provinces of Ecuador: Cañar, El Oro, Esmeraldas, Guayas, Loja, Los Rios, Manabí, Santa Elena, and Santo Domingo. A minimum of 10 individual whiteflies per sample were collected using a handheld aspirator and immediately transferred live into a 1.5-mL microfuge tube containing 95% ethanol and stored at −20°C.

Total DNA was purified from three or more adult whiteflies from each collection site (n = 207). Whiteflies were processed according to the method of Zhang et al. (1998), except ethanol was removed by blotting on Ahlstrom 55-mm black filter paper (Ahlstrom-Munksjö, Kaukauna, Wisconsin, USA) before incubation in 10 µL CTAB (100 mmol/L Tris-HCl pH 8.0, 20 mmol/L EDTA pH 8.0, 1.4 mol/L NaCl containing 0.2% 2-mercaptoethanol and 2% hexadecyltrimethylammonium bromide) lysis buffer (Paredes-Montero et al. 2019). Single adult whiteflies were ground in a microfuge tube with a micropestle that was washed with 170 µL of CTAB to remove whitefly fragments, and 0.005 mg/mL proteinase K was added to each tube. Samples were incubated overnight at 55°C, followed by incubation at 65°C for 15 min. One volume chloroform was added, the contents were mixed, and the preparation was centrifuged (Eppendorf Model 5415R, Eppendorf, Hamburg, Germany) at 16,000 *g* for 3 min at 4°C. The supernatant was collected, and 1 volume 100% isopropanol and 40 µg glycogen were added, followed by incubation for 10 min at 4°C. The pellet was collected by centrifugation at 16,000 *g* at 4°C for 10 min, washed with 70% ethanol, air-dried, and dissolved in 10 mmol/L Tris-HCl, pH 8.0.

Polymerase chain reaction amplification and DNA sequencing

Whitefly samples were pre-screened to identify samples containing the B mitotype by polymerase chain reaction (PCR) amplification using

the specific primers FBtBF1-5′-TATTTCACTTCAGCCACTATAA-3′ and RWfBr2-5′-GCTTAAATCTTACTAACCGCAG-3′ (Andreason et al. 2017) to yield a 550-bp fragment of the mitochondrial *COI* (mt*COI*). The PCR conditions were 95°C for 1 min, followed by 30 cycles of 94°C for 15 s, 57°C for 15 s, and 72°C for 40 s, with a final extension for 5 min at 72°C. The amplicon for 25 B-positive whitefly samples was cloned and sequenced. When a B-mitotype-specific amplicon was not obtained, the sample was subjected to PCR amplification with primers that target a 3′-fragment of the mt*COI* gene and tRNA*leu* gene, F-*COI*-628-5′-GATCGAAATTTTAATAGATCTTTTTATGATCC-3′ and R-*COI*-1629-5′-TGTTCTATTGTAAAACTAGCACTATTTTG-3′, with an expected size product of 1015 bp. The PCR cycling conditions were as follows: 95°C for 1 min, and 30 cycles of 95°C for 15 s, 52°C for 15 s, and 72°C for 60 s, with a final extension at 72°C for 5 min.

Each PCR (25 μL vol) contained 1× JumpStart REDTaq ReadyMix (Sigma-Aldrich, Saint Louis, Michigan, USA), oligonucleotide primers (0.4 μmol/L each), 20 ng whitefly DNA, and double-distilled (dd) water. The PCR product was analyzed by agarose gel (1%) electrophoresis in 1× TAE buffer, pH 8.0, containing 1× GelRed (Biotium, Hayward, California, USA), at 100 V for 50 min. Amplicons of the expected size (1015 bp) were ligated into the pGEM-T Easy plasmid vector (Promega, Madison, Wisconsin, USA) and cloned to *Escherichia coli* DH5α competent cells by heat-shock-mediated transformation (Green and Sambrook 2012). Three clones per sample were screened by colony PCR (Gussow and Clackson 1989). Clones having an insert of the expected size were sequenced using M13 primers (F-5′-TGTAAAACGACGGCCAGT-3′ and R-5′AGGAAACAGCTATGACCATG-3′; Promega). Cycling parameters were denaturation at 94°C for 10 min, followed by 35 cycles at 94°C for 60 s, 53°C for 60 s, and 72°C for 3 min, with a final extension at 72°C for 20 min. Amplicon size was confirmed by agarose gel electrophoresis, as described above.

Cloned amplicons were subjected to bidirectional capillary DNA sequencing (Sanger) on an Applied Biosystems ABI 3730XL DNA analyzer (The University of Arizona Genomics Core; http://uagc.arizona.edu/). The sequence reads were manually edited and assembled into contigs using the SeqMan Pro software, available in DNASTAR Lasergene v14.0 package (DNASTAR, Madison, Wisconsin, USA). The contigs were exported as fasta files and annotated using Blast2GO software (Conesa et al. 2005) to confirm *B. tabaci* identity.

The sequences were analyzed to identify putative NUMTS using previously established criteria (Song et al. 2008). Singletons, and sequences with stop codons, insertion–deletions (indels), and/or ambiguous bases were removed prior to sequence alignment.

The non-redundant haplotypes, defined as sequences that share 100% nucleotide (nt) identity, were removed from the analysis (collapsed) using FABOX v1.41 (https://users-birc.au.dk/~palle/php/fabox/index.php), resulting in a single sequence per haplotype.

Phylogenetic analysis

Mitotype identification was based on phylogenetic analysis using representative *B. tabaci* reference *COI* sequences available in GenBank (Benson et al. 2005) or the Brown laboratory reference *COI* database (J. K. Brown, *unpublished data*) that contains sequences for *B. tabaci* field samples initially identified based on morphological characters (Mr. Ray Gill, California Department of Food and Agriculture, Sacramento, California, USA). The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood; Brown laboratory database), was used as the outgroup to root the tree. The sequences (n = 573) were aligned using MUSCLE v3.8.31 (Edgar 2004), implemented in the Align Multiple Sequences tool available in Mesquite v2.75 (Maddison and Maddison 2018). The ends were trimmed to 832 nt in length, and the terminal gaps were treated as missing data.

The best model of molecular evolution was determined using jModelTest v2.1.7 (Darriba et al. 2012), employing a majority-rule consensus of the Akaike information criterion (AIC), the corrected AIC, the Bayesian information criterion (BIC), and Decision Theory Performance-Based Selection (DT). The general time-reversible (GTR) model with invariant sites (I) and a gamma-distributed rate variation among sites (G) was identified as the best-fit model of evolution. The Bayesian analysis was carried out using MrBayes

v3.2.5 (Huelsenbeck and Ronquist 2001) consisting of four independent Markov chain Monte Carlo (MCMC) runs, each with four Markov chains, permitted to run for 1×10^7 generations. Trees were sampled every 1000th generation. Log-likelihood scores were plotted with sampled generations using Tracer v1.6 (Rambaut et al. 2018), and the effective sample size was confirmed to be >200. The MCMC runs were summarized using sump and sumt commands in MrBayes v3.2.5 (Huelsenbeck and Ronquist 2001). Trees in the first 3×10^7 generations per replicate were discarded as burn-ins. The majority-rule consensus tree was drawn using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

The within- and between-mitotype divergence was estimated by pairwise distance analysis using the PAUP v4.0a software (Swofford 2003). Distances were corrected to account for multiple substitutions at a single site based on the GTR model of evolution.

Whitefly geographic distribution

The geographical distributions of *B. tabaci* mitotypes and non-*B. tabaci* species were plotted using the biological records tool plug-in available in QGIS 3.8.1 (QGIS Development Team 2017). Mitotype distributions were plotted on map shapefiles obtained from the National Institute of Meteorology and Hydrology (INAHMI) of Ecuador (http://www.serviciometeorologico.gob.ec/) and the interactive environmental map from the Ministry of Environment of Ecuador (http://www.ambiente.gob.ec/). The layers were (1) July 2014 monthly average temperature, (2) ombrotypes, (3) altitudinal isolines, and (4) habitat structure. The ombro-type horizons consist of categorizations based on the ombrothermic index (coefficient), which is determined by dividing the sum of the average monthly precipitation for those months when the mean temperature exceeded 0°C, by the sum of monthly mean temperatures exceeding 0°C (Ingegnoli 2013). The habitat structure layer consisted of the vegetative life cycles for the dominant vegetation type and forest composition.

Niche range prediction

The climate niche range was predicted based on presence-only records for the predominant native mitotype ECU1 and introduced B mitotype. Because of the small number of records available for the ECU2 and ECU3, at 1 and 2, respectively, it was not possible to predict the distribution of these rare mitotypes. Niche range prediction analysis considered only the geographic limits of continental Ecuador with geographic range boundaries set at 82° W, 75° E, 5° S, and 2° N. All of the climate variables used for the niche range predictions were cropped to this geographic extent using the crop and extent functions of the package raster in R (Hijmans et al. 2019*a*). A set of environmental variables that define habitat suitability was downloaded from the WorldClim v2 website (https://www.worldclim.org/data/worldclim21.html). The WorldClim dataset comprises nineteen climate layers (BIO1–BIO19) with average climate predictors combined from the 1970–2000 period. The variables used for species distribution predictions were annual mean temperature (BIO1), mean diurnal temperature range (BIO2), isothermality (BIO3), temperature seasonality (BIO4), maximum temperature of the warmest month (BIO5), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of wettest quarter (BIO8), mean temperature of driest quarter (BIO9), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), annual precipitation (BIO12), precipitation of wettest month (BIO13), precipitation of the driest month (BIO14), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), and precipitation of coldest quarter (BIO19). Layers were rasterized using the band interleaved by line (BIL) format using the rasterize function of the package raster in R (Hijmans et al. 2019*a*). The geographic boundaries and coordinates of all layers were standardized, and the cell size resolution was set at 0.05×0.05 pixels. The headers of raster files were modified as needed, and the vector of bioclimatic variables was created by stacking BIL files using the stack function available in the raster package in R (Hijmans et al. 2019*a*).

Niche range predictions were carried out using the MaxEnt v3.4.1 software (Phillips et al. 2017) that estimates and compares occurrence probability densities, based on the vector relating the nineteen environmental covariates relevant to

whitefly habitat suitability (Elith et al. 2011). MaxEnt was selected because it utilizes presence-only records and produces accurate predictions for relatively small sample sizes, for example, as few as five records (Hernandez et al. 2006, Pearson et al. 2007). The niche range was predicted using the R computing environment and the maxent function available in the dismo package (Hijmans et al. 2019*b*) with Java (Urbanek 2019). The occurrence records were divided into two groups, with 75% used to train the model and 25% to test the model, randomized for 10 independent runs. To reduce the likelihood of spurious predictions potentially resulting from sampling bias, a target-group approach was used (Phillips et al. 2017), which consisted of randomly selected geographic coordinates that might or might not overlap (non-focal species records) with the focal species records were used as background records, for example, random geographic locations where a particular mitotype occurs. The non-focal species records were analyzed in R to evaluate the model using the function evaluate from the dismo package (Hijmans et al. 2019*b*).

A 10-fold cross-validation was applied to estimate variation of the area under the curve (AUC) of the receiver operating characteristic values, the accepted statistic for evaluating the prediction capability of a model. The AUC is equal to the probability that a test record is correctly differentiated from a random point in a predefined background study area and ranges from 0 to 1 (Baasch et al. 2010, Phillips et al. 2017). Models with an AUC of ≥0.7 are considered acceptable (Metz 1978), while cutoff values of 0.8 and 0.9–1.0 are indicators of excellent and outstanding models, respectively (Hosmer et al. 2013). The model values were outputted in cloglog format, with probabilities of habitat suitability ranging from 0 to 1, where 0 equals no suitability for occurrence of the focal mitotype. The continuous probabilities were plotted using the function dismo:predict (Hijmans et al. 2019*b*), and the binary maps were produced using the function threshold from the dismo package (Hijmans et al. 2019*b*), using the threshold for which the sum of the sensitivity (true positive rate) and specificity (true negative rate) was greatest. The distribution maps for the major phylogenetic clades of *B. tabaci* were exported as raster files and visualized with the Quantum Geographic Information System (QGIS) software v 3.8.1 (QGIS Development Team 2017).

The permutation importance of each variable, which equals to the contribution by each of bioclimatic variable to the model, was based on the effects a variable contributes to reducing the AUC when it is randomly permuted during training, with a large decrease indicating high reliance of the model on a particular climatic variable.

Results

Identification of mitotypes

Five hundred and seventy-three high-quality sequences were obtained by PCR amplification of a 1015-bp fragment of the mt*COI* sequence (3′-end of the mitochondrial *COI*, 949 bp) and contiguous tRNA*leu*, 66 bp. Sequences were trimmed to 832 bases and redundant haplotypes were removed, leaving 262 unique haplotypes for the analyses. The haplotype sequences are available in GenBank (Data S1).

Based on the phylogenetic analysis, three native mitotypes were identified in Ecuador, hereafter referred to as ECU1, ECU2, and ECU3 (Fig. 1). The ECU1–2 mitotypes clustered within one of the two previously established major clades, AMTROP VI and VII clades (AMTROP species, de Moya et al. 2019; previously, New World-1 and 2, Dinsdale et al. 2010, Boykin et al. 2012), and the most divergent mitotype, ECU3, also grouped within the AMTROP major clade. The B mitotype grouped within the NAFMEDME III major clade, representing B-type clades, which are sister to previously identified Q mitotypes. Several *COI* sequences for whiteflies collected from cassava and other wild plant species were identified as *Bemisia tuberculata* (Bondar), and belonged to a distinct clade, itself also a sister to the *B. tabaci* sibling species group. All of the main branches of the tree were well supported by having posterior probabilities (pp) of >95% (Fig. 1).

Five subclades within the AMTROP VI–VIII major clade (AMTROP species; de Moya et al. 2019) were identified. One subclade, AMTROP (VI)-Ecuador and Colombia, contained ECU1 and previously reported mitotypes from Colombia (Quintero et al. 2001). The second,

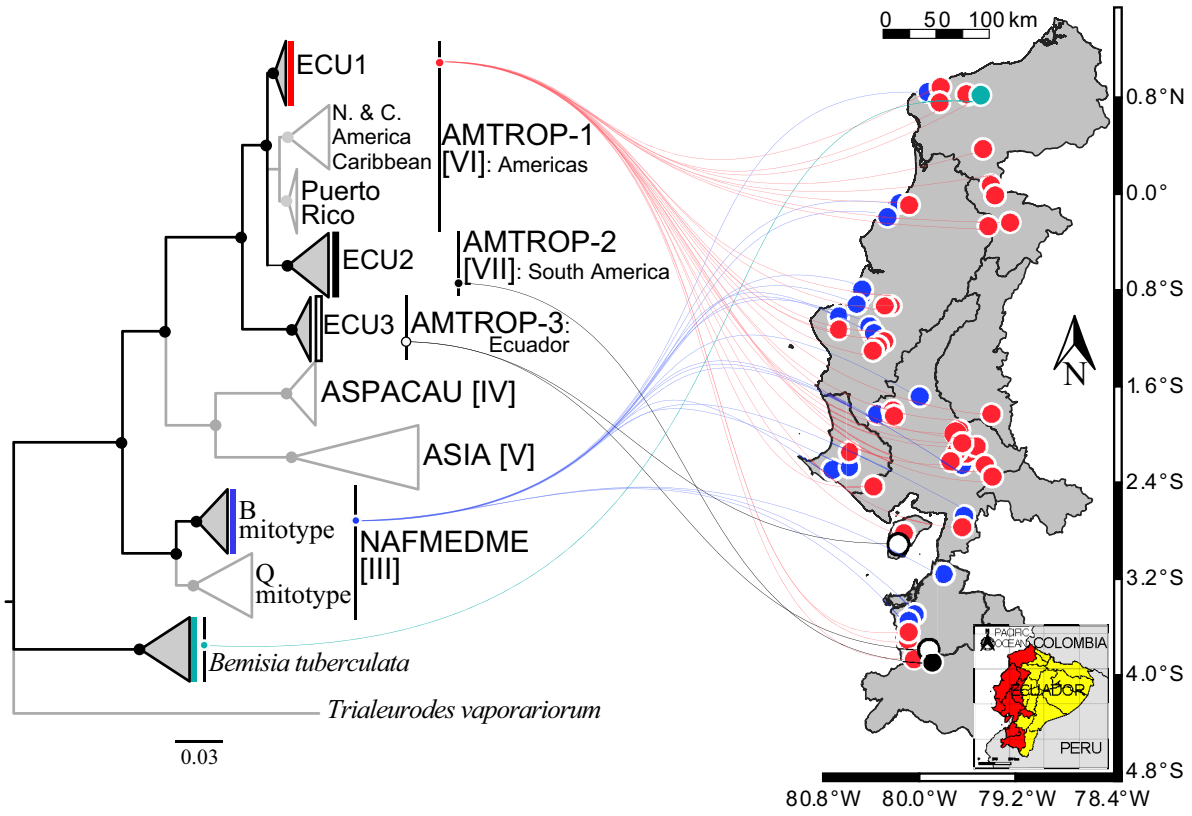

Fig. 1. Bayesian phylogeny of *Bemisia tabaci* mitotypes and other whitefly species (left) based on an 832-bp fragment comprising the 3′ half of the mitochondrial *cytochrome oxidase I* gene (mt*COI*) and the *transfer RNA leucine* region (*tRNAleu*). The tree was rooted using the sequence of the greenhouse whitefly, *Trialeurodes vaporariorum*. Highlighted clades contain sequences from Ecuador, whereas faded clusters comprise reference sequences only. Closed circles at nodes depict posterior probabilities above 95%. The roman numerals indicate major phylogeographic clades of *B. tabaci* according to Brown (2010), and acronyms refer to species designations according to Brown (2010) and de Moya et al. (2019). Colored connectors show the relationship between phylogenetic clusters and geographic distribution of *B. tabaci* mitotypes in nine provinces of coastal Ecuador (right). Black, blue, red, and white circles in map represent mitotypes ECU3, B, ECU1, and ECU2, respectively. The green circle depicts a location in Esmeraldas, where *Bemisia tuberculata* was collected from cassava plants.

AMTROP (VI)-North-Central-America/Caribbean, harbored mitotypes previously reported or archived in Brown laboratory database, from Belize, Guatemala, Honduras, Mexico, Panama, and the United States (Brown and Idris 2005), but contained no haplotypes from Ecuador. The third, AMTROP (VI)-Puerto Rico, represented a single exemplar from Puerto Rico, known as the Jatropha (race) mitotype (GenBank accession no. KX397317). The fourth, AMTROP (VII)-South America (4; Viscarret et al. 2003, Marubayashi et al. 2013), contained ECU2 and haplotypes previously reported from Argentina, Bolivia, and Brazil (Lourenção and Nagai 1994, Lima et al. 2002, Viscarret et al. 2003, Marubayashi et al. 2013, Barbosa et al. 2014, Queiroz et al. 2016, Quintela et al. 2016). The fifth subclade, AMTROP (VIII)-Ecuador, is a newly identified clade thus far represented only by the ECU3 mitotype. The three AMTROP major clades harboring the ECU–*B. tabaci* mitotypes from Ecuador therefore represent three of eight recognized groups (major clades) known thus far, worldwide. These three clades, AMTROP VI, VII, and VIII, collectively, comprise the AMTROP putative cryptic species (de Moya

et al. 2019). They are phylogeographically distributed within the tropical Americas occurring with some overlap and an apparent basis in their predicted (modeled) climate niches.

Mitotype distribution and plant species associated with B. tabaci

The ECU1 mitotype is most closely related to the *B. tabaci* mitotype previously identified in Colombia (Fig. 1). It was distributed in all nine provinces sampled in Ecuador, with a relative frequency of 44% (based on total number of samples), making it the most abundant and widespread endemic mitotype represented among the collection sites in Ecuador. In contrast, the ECU2 and ECU3 endemic types, thus far known to occur only in South America, were identified in one site in Loja (1%) and one each from Loja and Puna Island (1.5%), respectively (Fig. 1). The ECU1 mitotype was collected primarily from cassava (*Manihot esculenta* Crantz), at 45% of the associated plant species, and at a lower frequency from *Carica papaya* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumis melo* L., *Cucumis sativus* L., *Cucurbita* sp., *Glycine max* (L.) Merr., *Ipomoea* sp., *Luffa* sp., *Morinda citrifolia* L., *Mucuna pruriens* (L.) DC., *Nicotiana tabacum* L., *Physalis* sp., *Solanum lycopersicum* L., and *Wigandia crispa* (Tafalla ex Ruiz & Pav.) Kunth. By comparison, the ECU2 mitotype was collected from wild *Solanum* sp. in Loja, while ECU3 was collected from infested papaya *C. papaya* and *W. crispa*, both tropical plants commonly growing on Puna Island and in Loja, respectively. The three *B. tabaci* mitotypes from Ecuador were found associated with diverse plant families, including the Boraginaceae, Caricaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Rubiaceae, and Solanaceae.

The non-endemic *B. tabaci*, the B mitotype, was the predominant *B. tabaci* in the El Oro, Guayas, Manabí, and Santa Elena provinces along the coast of Ecuador, occurring at a relative frequency of 45%. The Ecuador coastal zone has a tropical lowland climate and deciduous and semi-deciduous forests interspersed with irrigated vegetable monocropping where insecticides are commonly used. The B type was collected from diverse families of cultivated and uncultivated (wild) plant species, including *Amaranthus spinosus* L., *Artemisia* sp., *Bauhinia aculeata* L., *Brassica* sp., *Capsicum annuum* L., *C. lanatus*, *Cordia lutea* Lam., *Cucumis dipsaceus* Ehrenb. ex Spach, *C. melo*, *C. sativus*, *Cucurbita maxima* Duchesne, *Cucurbita pepo* L., *Datura stramonium* L., *Heliotropium indicum* L., *Ipomoea* sp., *Laportea aestuans* (L.) Chew, *Luffa operculata* (L.) Cogn., *M. esculenta*, *Physalis pubescens* L., *Sida* sp., *S. lycopersicum*, and *Solanum nigrum* L., representing the plant families Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Cucurbitaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Solanaceae, and Urticaceae. Worldwide, the *B. tabaci* sibling species group has been reported to colonize or be associated (the latter defined as when reproduction is not recorded) with all of these plant families (Mound and Halsey 1978, Attique et al. 2003, Li et al. 2011, Xu et al. 2011).

In several instances, more than one mitotype was found at the same site and/or associated with the same plant species. The ECU1 and B mitotypes were the most common combination recorded, at a relative frequency of 4% (of total collection). The ECU1 mitotype was associated with weed species, while the B mitotype colonized primarily cultivated species at collection sites in Guayas, Manabí, and Santa Elena. In contrast, the ECU1, ECU2, and ECU3 mitotypes were collected from the same individual plant. The ECU1 and ECU2 occurred together on wild *Solanum* sp., while the ECU1 and ECU3 mitotypes co-associated on *W. crispa* and *C. papaya* (Data S1).

The whitefly *B. tuberculata* was represented in 8.5% of the samples (relative frequency) and was collected from cassava plants, *M. esculenta,* and to a lesser extent from non-cultivated species. It was found most often on the northwestern slopes of the Andes at an altitude of ~1500 feet. Phylogenetically, *B. tuberculata* resolved as a sister clade to *B. tabaci*, and was consistent with the results of previous studies that were based on morphological and phylogenetic differences (Bellotti and Arias 2001, Calvert et al. 2001, Gill and Brown 2010, Silva et al. 2014). The results are also in line with the proposed placement of *B. tuberculata* as a cryptic sister species to *B. tabaci* and several other difficult-to-distinguish *Bemisia* species within the proposed *Bemisia* sibling species complex (Gill and Brown 2010). The collection information, including the geographic

coordinates of the sampling sites, is available in Data S1.

Sequence divergence

The within-clade pairwise divergence for the ECU1, ECU2, and ECU3 mitotypes was 1.5%, 1.45%, and 2.27%, respectively, whereas the exotic B-type within-clade divergence was negligible. The between-mitotype divergence for the pairs ECU1 × ECU2, ECU1 × ECU3, and ECU2 × ECU3 was 5%, 8%, and 10%, respectively. With the addition of previously unstudied mitotypes from the region, the overall sequence divergence was found to be greater than previously reported, increasing from 7–8.6% to 10% (Viscarret et al. 2003, Brown and Idris 2005, Brown 2010).

Geographic distribution and habitat structure

In Ecuador, the geographic distribution of *B. tabaci* varied by mitotype. The predominant endemic, ECU1, had the widest geographical range, compared to the endemic types ECU2 and ECU3, or to the introduced B type, which were restricted to local microenvironment niches. Based on isotherm mapping, the endemic *B. tabaci* occurred in locations having temperatures ranging from 18°C to 23°C. In Ecuador, ECU1 showed a preference for the arid regions of coastal Ecuador and the tropical dry and evergreen forests near the Andean slopes (Appendix S1: Fig. S1a), and was the only one of three ECU mitotypes in Ecuador also known to occur in Central and North America. So far, the ECU2 mitotype has been identified only in Loja, Ecuador, where the temperature ranges from 18°C to 28°C and the altitude is >1600 ft above sea level. The ECU3 mitotype occupied two different habitats, one in Loja (>1600 ft) and the other at sea level on Puna Island, albeit in both locales, temperatures range from 23°C to 28°C (Appendix S1: Fig. S1a, b). In contrast, the introduced B mitotype survived only in the hot tropical coastal lowlands where temperatures range from 23°C to 28°C (Appendix S1: Fig. S1a, b).

Based on the range ombro-type horizons identified in the Ecuadorian landscape (Appendix S1: Fig. S1c), the B mitotype occupied dry, semiarid, and desertscapes where it overlapped somewhat with the most abundant endemic mitotype, ECU1. In those areas occupied solely by the B mitotype, it seems likely that it has displaced the endemic ECU1 mitotype. This hypothesis is supported by knowledge that the B mitotype is known to originate in the arid regions that span eastern North Africa, and the Middle East, and adjacent desert habitats. However, in Ecuador, the distribution of ECU1 was far more extensive than the B mitotype, and included habitats in the sub-humid and humid regions near the Andean slopes (Appendix S1: Fig. S1c). Compared to ECU1, the ECU2 mitotype was more narrowly distributed, possibly being limited to the evergreen-stationary forest of Loja. Finally, the ECU3 mitotype inhabited both deciduous and evergreen-stationary habitats occurring in Puna and Loja. In light of possible limitations imposed by the selection of sampling sites, these results should be considered relative, and serve as baseline distributions from which to guide additional cataloging of *B. tabaci* endemic to Ecuador and adjacent locales (Appendix S1: Fig. S1c).

When the distribution of whitefly mitotypes in Ecuador was considered as a function of habitat structure (Appendix S1: Fig. S1d), the B mitotype was associated with deciduous and semi-deciduous regions with plant species representing predominantly agricultural crops grown under irrigation that provided an artificial year-round food source. By comparison, the ECU1 mitotype favored the evergreen and stationary-evergreen landscapes of northeast of Esmeraldas, Manabí, and Santo Domingo (Appendix S1: Fig. S1d), but the ECU1 also overlapped in distribution with ECU2 within the stationary-evergreen forests near Loja. Finally, the ECU3 mitotype was most widely distributed with respect to climate–habitat associations, occupying both deciduous and stationary-evergreen landscapes in southwestern Ecuador.

Niche range predictions

The niche range prediction for ECU1 and B mitotypes considered 65 and 64 records, respectively. The geographic coordinates associated with each record are shown in Data S1. The probability of whitefly mitotype distribution as a function of microenvironmental conditions was analyzed to examine the potential environmental factors that could influence whitefly physiological adaptability (Costa et al. 2010, Elith et al. 2011, Kumar et al. 2015). The predicted AUC values for the B and ECU1 mitotypes were >0.9 for

the training and testing datasets (Table 1), indicating model performance was robust (Hosmer et al. 2013). The probability distribution threshold for the ECU1 and B mitotype model was 0.240 and 0.067, respectively, and maps were constructed to illustrate the distribution ranges (Fig. 2a, b).

The ECU1 and B mitotype had a predicted geographic range spanning 60,801.91 and 27,120 km^2, respectively (Fig. 2), which is consistent with previous reports of plasticity among members of the *B. tabaci* cryptic species, conducive to a near-pantropical distribution and a broad range of hosts within (Cock 1993, Brown 2007, Delatte et al. 2009; Fig. 2c). Compared to the B mitotype, the range of ECU1 indicated adaptation to wider range of habitats including hotspots localized in the desert environments of Esmeraldas, Manabí, and El Oro, and the humid regions of Esmeraldas, north Guayas, Los Rios, and Santo Domingo. The observation is supported by the distribution of the B mitotype, which was limited to the coastal lowlands, and specifically to the hotspot created artificially by the irrigated agroecosystems along the coast where Guayas, Manabí, El Oro, and Santa Elena are located (Fig. 2a, b). Unfortunately, the small sample sizes available for the ECU2 and ECU3 mitotypes precluded the prediction of their distribution range, underscoring the need for additional studies to more completely understand the extent of plasticity among *B. tabaci* endemic to Ecuador and the rest of South America.

The raster layers used for modeling the niche ranges were represented by microclimate, topography, and vegetation structure. Among the raster layers, the variable defined by the precipitation of the driest quarter, considered a reliable indicator for microclimate, was identified as the greatest contributor to the ECU1 model, at 64.8%. This variable was followed by another microclimate indicator, the mean temperature of wettest quarter, at 25.1%. With these two variables explaining 90% of total variation, the ECU1 mitotype distribution is importantly influenced by the amount of summer rainfall in each given niche. Based on summer rainfall as a predominant indicator of habitat suitability, it may be inferred that ECU1 possesses

Table 1. Estimates of the relative contribution (percent permutation importance) of environmental variables to the MaxEnt distribution models for the ECU1 and B mitotypes in Ecuador, and area under the receiver operator characteristic curve (AUC) for the training and testing datasets.

Bioclimatic variables	ECU1 mitotype	B mitotype
Annual mean temperature	0.0	0.6
Mean diurnal range	0.0	0.1
Isothermality	0.0	0.0
Temperature seasonality	1.0	0.1
Max temperature of warmest month	0.0	0.0
Min temperature of coldest month	0.1	2.1
Temperature annual range	0.0	0.2
Mean temperature of wettest quarter	25.1	44.3
Mean temperature of driest quarter	0.0	0.0
Mean temperature of warmest quarter	0.3	0.5
Mean temperature of coldest quarter	2.2	3.1
Annual precipitation	0.9	38.3
Precipitation of wettest month	0.0	0.0
Precipitation of driest month	0.3	9.0
Precipitation seasonality	0.0	0.0
Precipitation of wettest quarter	3.7	0.5
Precipitation of driest quarter	64.8	1.0
Precipitation of warmest quarter	1.0	0.1
Precipitation of coldest quarter	0.7	0.0
Mean training AUC	0.9708 ± 0.0006	0.9881 ± 0.0005
Mean testing AUC	0.9460 ± 0.0111	0.9781 ± 0.0054

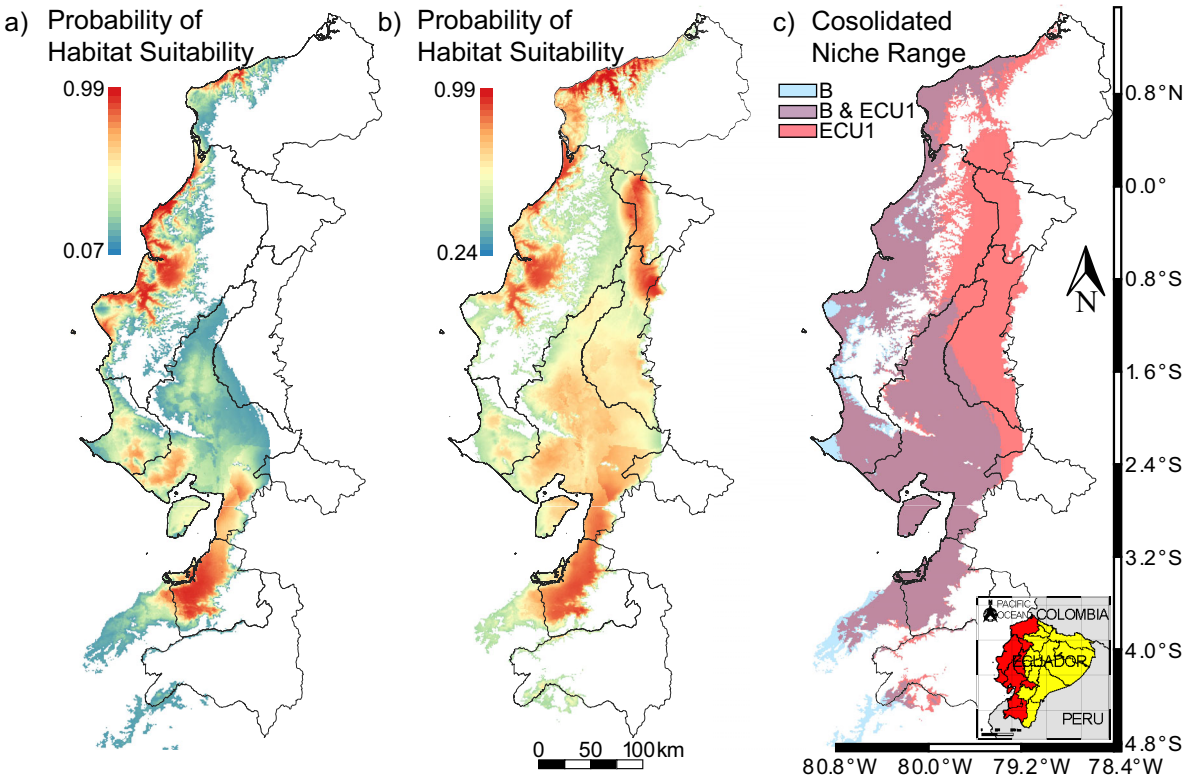

Fig. 2. Niche range predictions based on occurrence records of the B (a) and ECU1 (b) mitotypes of *Bemisia tabaci* in nine provinces of coastal Ecuador. Heatmap color scale shows the cloglog probability of habitat suitability starting at optimal thresholds with maximum of the sum of sensitivity and specificity (max TPR + TNR) values of 0.07 and 0.24 for the B and ECU1 mitotypes, respectively. The consolidated distribution ranges (c) show the B and ECU1 mitotypes exist in sympatry, and the niche range of the ECU1 is the largest in Ecuador.

certain unique adaptations required for survival in temperate humid niches that are widely prevalent in much of Ecuador. By comparison, the raster layer variables found to contribute the most to the niche range model for the B mitotype were mean temperature of wettest quarter and annual precipitation, at 44.3% and 38.3%, respectively, which is consistent with its limited range tightly linked to annual rainfall, in this instance, contributed by irrigated crops grown in an otherwise rain-limited microniche. In this light, survival of the B mitotype was restricted to the dry coastal areas in Ecuador, which receive the lowest annual precipitation in the country. In its native desert habitats, the host plants of the B mitotype are localized along flowing rivers and washes (wadis) that expand and contract seasonally, forcing short- and long-distance dispersal to identify suitable resources. This predicted adaptation to annual rainfall is consistent with the long-distance dispersal and highly polyphagous behaviors noted for the B mitotype, and its propensity for thriving in irrigated, desert agricultural settings occurring worldwide in the subtropics and dry tropics (Mound 1962, 1963, Cock 1993, Bernays 1999, Bird and Krüger 2006, Delatte et al. 2009).

Discussion

Phylogenetic analysis and genetic diversity

The phylogenetic analyses of whitefly mt*COI*-tRNA*leu* sequences (832 nt) resolved three divergent native mitotypes among *B. tabaci* from Ecuador that grouped within the AMTROP species (de Moya et al. 2019), with robust support (>0.95 pp). Phylogenetically, the exotic B mitotype clustered with its closest relatives belonging to the NAFMEDME species (Fig. 1). Among the

B. tabaci endemic to Ecuador, ECU1 was phylogenetically most closely related to a mitotype previously identified in Colombia, herein members of the ECU1 sister group (Fig. 1). Among available mt*COI* sequences, the ECU2 sister group was most closely related to *B. tabaci* haplotypes from Argentina and Brazil, identified more than fifteen years ago by mt*COI* sequence analysis (Fig. 1; Viscarret et al. 2003). Finally, a previously unrecognized mitotype, the ECU3 sister group, was identified here for the first time in the Americas, from southern Ecuador (Fig. 1).

Pairwise distance analysis indicated that the sister groups ECU1, ECU2, and ECU3 diverged by ~2%, 5%, and 8% from their AMTROP prototype AZ-A biotype (Costa and Brown 1991), results that are in agreement with the Bayesian phylogeny (Fig. 1). Strikingly, the ECU3 sister group, thus far known to occur only in Ecuador (South America), diverged by 8% and 10% from haplotypes that grouped in one of the two recognized sister clades of *B. tabaci* that are endemic to North and Central America, the Caribbean Basin, and South America. Thus, the endemic haplotypes known previously from South America were identified here as belonging to the ECU1 mitotype group (Brown and Idris 2005, Barbosa et al. 2014) and whose occurrence has been previously documented in South America (Viscarret et al. 2003, Barbosa et al. 2014). The relatively low divergence between ECU1 and its close relatives extant in the American Tropics, that is, North and Central America and Caribbean Basin, suggests their derivation from ancestral sympatric populations. In this scenario, the spread of ECU1 into Central and North America (major clade AMTROP, VI) could have occurred after the closure of the isthmus of Panama that created the Panama land bridge ~2.4–4.2 million years ago (mya; Keller et al. 1989, Haug et al. 2001), making it of great interest to determine the extent of gene flow between ECU1 and its divergent ECU2 and ECU3 (AMTROP species) relatives (Fig. 1).

The pairwise distances among the *B. tabaci* AMTROP mitotypes were estimated at 0–10%. This value is greater than previously published estimates of 7.0–8.6% (Brown and Idris 2005, Brown 2010), because of the discovery of a previously unreported sister clade, represented here by the ECU3 mitotype, identified for the first time (albeit a very small sample size). Previously published estimates have been based on *B. tabaci* collections from North and Central America, and the Caribbean region, because only a small number of sequences have been available for South American exemplars. The results from this study suggest that the diversity of *B. tabaci* on the South American continent is likely far richer than presently realized. This gap underscores the need for additional studies of *B. tabaci* representing the diverse ecosystems of South America.

Whitefly–plant associations

Worldwide, about 600 plant species representing 80 families have been reported as hosts of *B. tabaci* (Mound and Halsey 1978, Cock 1993, Gelman et al. 2005, Li et al. 2011). Among *B. tabaci* mitotypes from Ecuador, eight and ten botanical families were associated with the ECU1–3 endemic and B exotic mitotypes. Although only adults were collected from these species, it is tentatively presumed that they are utilized by *B. tabaci* for both feeding and reproduction. Among these plant families, only ECU1 and ECU3 were associated with host species within the Caricaceae, and only ECU1 was associated with a species in the Rubiaceae. The B mitotype occurred on cultivated and wild species belonging to the Solanaceae and Boraginaceae, whereas ECU2 and ECU3 occurred on wild *Solanum* sp. and *W. crispa*, also belonging to the latter families, respectively. The various host preferences observed provide support for the hypothesis that mitotypes in the AMTROP clade have diversified in part by adaptation to potentially previously unsuitable host plants, and are consistent with the previously reported phenotypic plasticity among *B. tabaci* variants worldwide (Mound 1963, Bedford et al. 1994).

Host specialization and/or adaptation to new host plants is expected to result in genetic divergence and at times even reproductive isolation, serving as a mechanism for the diversification of new strains or races (Bird 1957, Drès and Mallet 2002), cryptic species, or perhaps new species altogether (Elias et al. 2012, de Vienne et al. 2013). Indeed, the monophagous Jatropha race (now, mitotype) from Puerto Rico (Fig. 1) was recognized as a race distinct from the polyphagous Sida race (mitotype). The Sida race was widely distributed on the island, colonizing species in many plant families, while the Jatropha

race colonized *Jatropha gossypifolia* L. (Euphorbiaceae) and was restricted to dry-tropical niches where *J. gossypifolia* was abundant (Bird 1957). In Ecuador, the range of hosts from which ECU2 and ECU3 mitotypes were collected suggests these two mitotypes are (minimally) oligophagous. If host specialization is found to be greater for ECU2 and ECU3, the combined host range and niche range(s) could explain their thus far narrow geographic distribution in Ecuador (and perhaps throughout the continent), compared to putative polyphagous, more widely prevalent ECU1, which occurs widely distributed occupying multiple niches and having an apparent broad host range (Marubayashi et al. 2013, Barbosa et al. 2014, Alemandri et al. 2015).

Although cassava, *M. esculenta,* was previously reported as a non-host of *B. tabaci* in Brazil (Costa and Russell 1975), 45% of the ECU1 samples were collected from this crop, which was notably co-colonized by *B. tabaci* ECU1 and *B. tuberculata*. Although the report that *B. tabaci* in Brazil did not colonize cassava was based on very limited sampling, the finding formed the basis for this extant paradigm (Costa and Russell 1975). The widespread association of ECU1 with cassava in Ecuador is consistent with the ability of certain *B. tabaci* mitotypes to colonize cassava in Africa. Cassava, endemic to South America (EL-Sharkawy 2003), was introduced into sub-Saharan Africa several hundred years ago, thereafter becoming a major staple (Legg and Fauquet 2004). The long-standing coevolution between cassava and at least one AMTROP *B. tabaci* mitotype may in part explain the absence of begomoviruses infecting cassava in the American Tropics even though cassava is widely grown, whereas upon the introduction of cassava to Africa, it was found to be susceptible to begomoviruses endemic there. These observations, and others, also illustrate the capacity of *B. tabaci* to adapt to new host plant species, including cassava, reported recently as a B mitotype host in Colombia (Carabali et al. 2005), and maize, as a host of the B mitotype in Brazil (Quintela et al. 2016), where following its introduction in the 1990s, it was found colonizing soybean and vegetable crops. After the institution of a soybean–maize rotation there about 10 yr ago, the B mitotype surprisingly host-adapted to maize, the first monocot known to be colonized by a member of the *B. tabaci* sibling species group (Quintela et al. 2016). In Ecuador, cassava is an important crop in the Esmeraldas, Los Rios, and Santo Domingo provinces, making the widespread colonization of *B. tabaci* in cassava a possible new concern, if it reaches pest status.

The plant families and species with which the ECU1–3 and B mitotypes were found to associate in Ecuador were consistent with host range previously reported for this sibling species group, worldwide. It should be noted that the host range of the majority of *B. tabaci* mitotypes in the region is still not known because only recently has it been possible to identify mitotypes by molecular analysis (Calvert et al. 2001). For the B and ECU1 mitotypes identified here from Ecuador, several were collected from previously unreported plant species, including the shrub-like species, *W. crispa and C. lutea* (family Boraginaceae), respectively. The *W. crispa* is endemic to the Andes Mountain range that spans Colombia, Ecuador, and Peru (Jorgensen and León-Yánez 1999, Cornejo 2006), at 350–9800 feet above sea level, characterized as temperate humid niches. The niche where *C. lutea* was identified as a putative host occurs in hot, dry region of Ecuador and Peru at 0–800 feet above sea level (www.tropicos.org), and the Galapagos Islands. The other plant species that were associated with *B. tabaci* mitotypes in this study are endemic to the Andean tropics of Ecuador and Peru. Although immature *B. tabaci* stadia were observed on many of the plant species sampled for this study, whitefly adults were more readily accessible because they are not sessile. Thus, it is only possible to state with certainty that the whitefly adults were associated with these various plant host species at the study sites, and so, whether the same mitotype colonized the host for reproduction is not known. Nonetheless, these observations provide important baseline records for additional studies to revisit suspected host plants to document the presence or absence of immature instars and/or adult specimens.

These observations are consistent with the plasticity in host range, long recognized for *B. tabaci* as a species, which has been extensively documented in the historical literature (Mound and Halsey 1978, Costa and Brown 1991, Cock 1993, Bedford et al. 1994). The host range

plasticity of the collective *B. tabaci* group has been instrumental in facilitating its adaptation to a wide range of host plants (Mound 1963, Cock 1993, Quintela et al. 2016), which in some instances has led to the concomitant diversification of whitefly-transmitted geminiviruses (Brown 2007; genus *Begomovirus*; family Geminiviridae), with some of the most damaging outbreaks involving the B mitotype due to its ability to thrive in agricultural settings (Brown 1992, 2010). Indeed, with the expansion of irrigation in agriculture over the last seventy years, some mitotypes of *B. tabaci* have become recognized as one of the most important insect vectors of plant viruses in the subtropics/tropics, and mild temperate regions of the world.

In Ecuador and elsewhere in the Andean Tropics, a better understanding of the role of endemic whitefly mitotypes in the spread of begomoviruses in crops would provide useful insights for management of a number of recently emergent begomoviruses, such as *tomato leaf deformation virus* (ToLDeV; Ibarra-Matamoros 2012, Paz-Carrasco et al. 2014) and *Rhynchosia golden mosaic Yucatan virus* (RhGMYuV; Paz-Carrasco et al. 2014), which have been associated with outbreaks in crops grown where ECU-endemic mitotypes prevail (Melgarejo et al. 2013). By comparison, the whitefly-transmitted virus outbreaks reported recently in irrigated tomato and other vegetable crops grown in Guayas, Loja, Manabi, and Santa Elena (Melgarejo et al. 2013, Paz-Carrasco et al. 2014) are associated with the B mitotype vector, which thrives in irrigated hotspots along the otherwise arid coastal lands of Ecuador.

Periodically, the B mitotype and/or ECU-endemic mitotypes overlapped at collection sites where they occurred on the same plant species, alone or in mixtures. The most frequent mixed infestations consisted of the B mitotype with ECU1 in irrigated crops along the Ecuadorian coast. Even so, in this scenario the B mitotype was found colonizing the cultivated host, while ECU1 was found on wild host plants. The ability of the B mitotype to preferentially thrive under hot, dry conditions associated with large-scale irrigated cropping systems compared to the more versatile ECU1 offers a plausible explanation for the apparent displacement of ECU1 and the observed displacement of other New World endemic mitotypes (Zang et al. 2006, Liu et al. 2007). Because there are few distribution records available for *B. tabaci* endemic to Ecuador and most of South America, where the B mitotype has become established post-introduction, it is not possible to determine with certainty where displacement of endemic mitotypes has occurred. Based on the results of this study, the ECU1 is the most widely distributed mitotype, occupying the most environmental niches in Ecuador, in comparison with the other two recognized ECU mitotypes. However, its extant distribution may actually reflect a retreat of ECU1 from the local gardens and native plants nurtured only by natural rainfall along the coast, now occupied by the irrigated monoculture agroecosystems more suitable for B mitotype colonization, a scenario that is also consistent with the low occurrence of endemic ECU1 in Colombia where *B. tabaci* have been sampled primarily from irrigated crops (Quintero et al. 2001).

Niche range predictions

Based on nineteen environmental variables, the distribution ranges for ECU1 and B mitotype were predicted by niche modeling analysis (Phillips et al. 2017). Hotspots were identified for ECU1 in the dry and desert lowlands of Esmeraldas, Manabí, El Oro, and in regions of high humidity, characteristic of the Esmeraldas, north Guayas, Los Rios, and Santo Domingo, including the highlands near the western slope of the Andes. Each of the mitotypes endemic to Ecuador showed adaptation to unique and overlapping niches. These niches were associated with different climatic variables, primarily the annual precipitation, the mean temperature of wettest quarter, and the precipitation of the driest quarter. Because this study was limited to specific collection sites, it seems likely that other unidentified, uniquely adapted mitotypes prevail in the diverse ecosystems in Ecuador, and throughout South America, a continent that remains largely unexplored. In stark contrast, the B mitotype was confined to irrigated agroecosystems of Guayas, Manabí, El Oro, and Santa Elena located in the hottest, driest lowland areas. These distributions were consistent with rainfall as the most significant variable contributing to the distribution models for both the ECU1 and B mitotypes (Table 1).

Within the agricultural and natural ecosystems, the B and ECU1 were the predominant mitotypes, at 47% and 44%, respectively. However, the ECU1 nicherange was more than twice as large as that of the B mitotype, at 60,801.91 km^2, and overlapped with that which was uniquely occupied by the B mitotype. Although the small sample sizes available for ECU2 and ECU3 precluded environmental niche analysis, they were each associated with distinct environments, overlapping with each other and the ECU1, but not with the B mitotype (Appendix S1: Fig. S1). Among the three AMTROP mitotypes, ECU1 was found predominantly in monocrop-associated weed species and cassava in dry and humid microenvironments, respectively, whereas ECU2 was only associated with a *Solanum* sp. in a humid niche at South Ecuador, and ECU3 was collected from *C. papaya* and *W. crispa* in dry and humid microniches, respectively.

Niche range analyses predicted that the endemic ECU1 was well adapted, or resilient, to the extreme conditions (for whiteflies) associated with the Andes Mountain range in Ecuador, tolerating a wide range of dry and humid characteristics equated with natural and disturbed landscapes of the coastal and western slopes. In contrast, the B mitotype did not establish in the humid environments, suggesting that this limitation precluded expansion beyond the agricultural areas in Ecuador where the microniche is hot and dry, and beneficiary of rainfall through the use of irrigation. This type of unnatural habitat in Ecuador also occurs in many other tropical locales where irrigated agriculture is practiced and the B mitotype has become established, often displacing endemic *B. tabaci* mitotype(s) (Delatte et al. 2009, Brown 2010). Not surprisingly, the latter conditions mimic the hot, dry arid (desert) niche where the B mitotype sister clades have evolved. Adaptation of *B. tabaci* to the stark deserts of Saharan Africa, and the Middle East, where host plants are seasonal and ephemeral, is consistent with high fecundity, associated with its invasive reputation in tropical/subtropical irrigated agroecosystems worldwide (Bedford et al. 1994, Brown et al. 1995*b*, Bird and Krüger 2006, Brown 2010). Despite its recognition as invasive, the B mitotype is limited by the requirement for conditions associated with its ecological endemism. In contrast, the ECU1–3 groups harbor resilience required for survival in the fluctuating environments of the Andes and adjacent coastal habitats, which are quite distinct from the arid deserts in Africa, the Middle East, and Arabian Peninsula.

Acknowledgments

The first author is supported by a fellowship provided by SENESCYT: Convocatoria Abierta 2011 (Ecuador), scholarship program 2011. This research was funded in part through the International Foundation for Science (IFS) grant C 5485-1 to JRP-M and ELP.

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Supporting Information

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