MURIEL RIZENTAL

DISTRIBUIÇÃO TEMPORAL E ESPACIAL DE HAPLÓTIPOS DE *Bemisia tabaci* (GENNADIUS, 1889) NO BRASIL

Tese apresentada ao Programa de Pós-Graduação em Agronomia, da Universidade Federal de Goiás, como requisito parcial à obtenção do título de Doutora em Agronomia.

Área de concentração: Fitossanidade.

Orientadora:
Dr[a]. Eliane Dias Quintela

Coorientadora:
Dr[a]. Aluana Gonçalves de Abreu

Goiânia, GO – Brasil
2020

UNIVERSIDADE FEDERAL DE GOIÁS
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ATA DE DEFESA DE TESE

Ata Nº **132/2020** da sessão de Defesa de Tese de **Muriel Santos Rizental** que confere o título de Doutora em **Agronomia**, na área de concentração em **Agronomia.**

Ao/s **29/06/2020 - vinte e nove dias do mês de junho do ano de dois mil e vinte**, a partir das **14h:00min**, Defesa por Vídeo-conferência, realizou-se a sessão pública de Defesa de Tese intitulada "Bemisia tabaci (GENNADIUS, 1889) (INSECTA: ALEYRODIDAE) no Brasil: caracterização das espécies". Os trabalhos foram instalados pela Orientadora, **Dr.ª Eliane Dias Quintela – Embrapa Arroz e Feijão** com a participação dos demais membros da Banca Examinadora: **Dr. José Alexandre Freitas Barrigossi - Embrapa Arroz e Feijão** , membro titular externo; **Prof.ª Jaqueline Magalhães Pereira - EA/UFG**, membro titular interno, **Dr.ª Aluana Gonçalves de Abreu – Embrapa Arroz e Feijão**, membro titular interno; **Prof. Jorge Rafael Paredes Montero – Universidade do Arizona, Tucson, AZ, EUA**, membro titular externo. Durante a argüição os membros da banca **fizeram** sugestão de alteração do título do **trabalho**. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido a candidata A**provada** pelos seus membros. Proclamados os resultados pela **Dr.ª Eliane Dias Quintela – Embrapa Arroz e Feijão** Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, ao(s) **29/06/2020 - vinte e nove dias do mês de junho do ano de dois mil e vinte**.

TÍTULO SUGERIDO PELA BANCA

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DEDICATÓRIA

I dedicate these studies, with my all heart, to my beloved family members who helped me in this journey. I am deeply grateful to my mother who in her sincere and unconditional love, was always by my side, supporting me in happy and in difficult moments. Moments when I believed I would not finish this project, especially due to extreme physical pain. And, always sweetly saying to me: “Entomology is within you”.

I am grateful to my husband for his infinite patience and dedication to us, for the countless words of affection, comfort, faith and hope, always saying: “The fruits of justice are harvested in peace”. He was always at my side from Australia to the United States with a smile on his face. To my Thesis partner, my son Estêvão, the love of my life, who will be born soon, with all my heart.

Without you, I sincerely say, I would not have made it this far, thank you very much!

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Under the sun everything is vanity.
Bible, Ecclesiastes, 1: 14.

The quality of a taxonomist´s work is measured not so much by the number of new forms which she/he describes as by the percentage of synonyms among them.
Mayr, 1942, p. 16.

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RESUMO GERAL

RIZENTAL, M. **Distribuição temporal e espacial de haplótipos de *Bemisia tabaci* (Gennadius, 1889) no Brasil.** 2020. 82 f. Tese (Doutorado em Agronomia: Fitossanidade) – Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2020[1].

Bemisia tabaci é um inseto-praga polífaga estabelecida no Brasil há quase 100 anos. Esforços para mapear e identificar variantes desta espécie e as plantas hospedeiras em todo o Brasil demonstraram sucesso limitado devido à escassez de amostras de vários estados, incluindo biomas únicos Brasileiros e, à falta de coleta a longo prazo, especialmente nas regiões norte e nordeste. Neste estudo, analisamos novas amostras dos 26 estados brasileiros e do Distrito Federal coletadas de 2014 a 2019 e, também amostras de uma coleção de museu da Embrapa Recursos Genéticos e Biotecnologia de 1989 a 2005. Os objetivos foram identificar a entrada e a adaptação ambiental de haplótipos, mapear suas distribuições e plantas hospedeiras no Brasil. As amostras de moscas-brancas foram analisadas utilizando marcadores genéticos de DNA mitocondrial (mtDNA) citocromo oxidase I (COI) e DNA nuclear (nDNA) (L11). Foram geradas informações e um banco de dados de nucleotídeos (~753 pb) para ambas as coleções. Interessantemente, todas as amostras da coleção de museu analisadas pertenciam ao haplótipo B. A amostra mais antiga foi coletada em 1989, no Rio de Janeiro (região sudeste) e a segunda em 1990 no Ceará (região nordeste). Na coleção de 2014-2019 foram identificados haplótipos nativos (A) em 12 estados brasileiros. O haplótipo invasor B foi identificado em todo o país, exceto em Santa Catarina (região sul). O haplótipo invasor Q foi detectado em Alagoas, Maranhão, São Paulo, Paraná e no Rio Grande do Sul. Três possíveis novas plantas hospedeiras para *B. tabaci* foram registradas: haplótipo-A em *Acacia mangium* (Acaciaceae) em Roraima (região norte); o haplótipo B em *Spondias mombin* (Anacardiaceae) no Ceará; e em *Xanthosoma sagittifolium* (Araceae) no Rio de Janeiro. Essas descobertas sobre novas datas e locais de entrada do haplótipo B bem como novas plantas hospedeiras para os haplótipos nativos e invasores e suas dispersões no país, terão impacto nas estratégias de manejo desta praga no Brasil e nas Américas.

Palavras-chave: Mosca-branca, Aleyrodidae, mtDNA, nDNA, diversidade, distribuição geográfica

[1] Orientadora: Drª. Eliane Dias Quintela. Embrapa Arroz e Feijão, GO – Brasil
Coorientadora: Drª. Aluana Gonçalves de Abreu. Embrapa Arroz e Feijão, GO – Brasil

GENERAL ABSTRACT

RIZENTAL, M. **Temporal and spatial distribution of *Bemisia tabaci* haplotypes (Gennadius, 1889) in Brazil.** 2020. 82 f. Thesis (Doctor in Agronomy: Phytosanitary) – Agronomy School, Federal University of Goiás, Goiânia, 2020[1].

Bemisia tabaci is a polyphagous insect-pest that has been established in Brazil for almost 100 years ago. Efforts to map and identify variants of this species and host plants across Brazil have shown limited success due to the scarcity of samples from several states, including unique Brazilian biomes, and the lack of long-term collection, especially from the north and northeast regions. In this study, we analyzed new samples from the 26 states and the Federal District collected from 2014 to 2019 as well as a duplicate of the Embrapa Genetic Resources and Biotechnology museum collection from 1989 to 2005. The objectives were to identify the entry and environmental adaptation of haplotypes, map its distributions, and host plants in Brazil. The whiteflies´ samples were analyzed using mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) and nuclear DNA (nDNA) (L11) genetic markers. Information and a nucleotides database were generated (~753 bp) for both collections. Interestingly, all the analyzed samples from the museum collection belonged to B-haplotype. The oldest sample was collected in 1989, in Rio de Janeiro (southeast region), and the second in 1990 in Ceará (northeast region). In the 2014-2019 collection, native A-haplotypes were identified in 12 Brazilian states. Invasive B-haplotype was identified throughout the country, except in Santa Catarina (southern region). The invasive Q-haplotype was detected in Alagoas, Maranhão, São Paulo, Paraná, and the Rio Grande do Sul. Three possible new host plants for *B. tabaci* were registered: A-haplotype on *Acacia mangium* (Acaciaceae) in Roraima (northern region); B-haplotype on *Spondias mombin* (Anacardiaceae) in Ceará; and *Xanthosoma sagittifolium* (Araceae) in Rio de Janeiro. These discoveries of new entry dates and locations of B-haplotype, as well as new host plants for native and invasive haplotypes and their dispersions in the country will have an impact on the management strategies for this pest in Brazil and the Americas.

Keywords: Whitlefly, Aleyrodidae, mtDNA, nDNA, diversity, geographical distribution

[1]Advisor: Dr Eliane Dias Quintela. Embrapa Rice and Beans, GO – Brazil.
Co-advisor: Dr Aluana Gonçalves de Abreu. Embrapa Rice and Beans, GO – Brazil.

1 GENERAL INTRODUCTION

Bemisia tabaci (Gennadius, 1889) (Insecta: Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) is one of the most important agricultural pest worldwide (Lowe et al., 2000). It causes direct damage to plants by feeding and inducing whitefly-related physiological disorders (Byrne & Bellows, 1991). Also, indirect damages due to phytoviruses transmission (Navas-Castillo et al., 2011; Bedford et al., 1994; Peñalver-Cruz et al., 2020), and insecticide resistance (Castle et al., 2013; Basit, 2019; Horowitz et al., 2020).

The species *B. tabaci* is considered a complex of multiple genetic groups, also known as a cryptic species, formerly biotypes (Boykin, 2014), which means that contains morphologically indistinguishable, but genetically distinct individuals (Brown, 2010; Gill & Brown, 2010). Biotypes or host races were distinguished based on biological traits such as host-plant adaptability, phytotoxic symptom induction, insecticide resistance, behavior, endosymbionts, and plant virus-transmission (Brown et al., 1995; Brown et al., 1996). Later, an attempt to classify the species based also on genetic boundaries and center of origin was published (Dinsdale et al., 2010; De Barro et al., 2011).

Although efforts to name the taxa in the *B. tabaci* complex have arisen, as supra cited, the suggestions such as Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) were not adhered by the whitefly community (Boykin, 2014). That can be explained by its gaps in the species classification process including the non-observance of the International Code of Zoological Nomenclature Standards (De Barro et al., 2011). Recently, genetic groups of this species complex have been referred to as haplotypes, based also on molecular, biogeographical and population studies (Moya et al., 2019; Paredes-Montero et al., 2020).

First reported in Brazil in 1923 (Bondar, 1928) it found favorable climate conditions, and plant resources which increased its ability to adapt to native flora and propagate throughout the country (Lima et al., 2000; Lima et al., 2002). Besides its capacity to adapt to different biomes *B. tabaci* can also use its genetic plasticity to spread and displace its native populations throughout regions (Liu et al., 2007). This study started from the hypothesis that *B. tabaci* haplotypes, especially the American native (A) and the most common invader (B haplotype) would be present throughout Brazil with higher frequency of the invader.

Genetic analysis of the Brazilian *B. tabaci* identified native haplotypes (A) colonizing a more restricted plant range, including native and exotic flora (Marubayashi et al., 2013; Barbosa et al., 2014). Besides, two invasive haplotypes have been reported, B and Q, in the country (Lourenção & Nagai, 1994; Barbosa et al., 2015). In Brazil, these invasives haplotypes propagated, possibly displacing native whiteflies (Liu et al., 2007; Luan & Liu, 2012a; Luan et al., 2012b; Moraes et al., 2018; Bello et al., 2020), and adapting to environmental changes (Hoffmann & Sgrò, 2011).

Efforts to map and identify this species and host plants across Brazil have shown limited success due to the scarcity of samples from several states, including unique Brazilian biomes, and the lack of long-term collection, especially from the north and northeast regions. Here, the objectives were to provide a detailed Brazilian *B. tabaci* database using mtDNA (COI) and nDNA (L11) gene markers from a museum collection, 1989 to 2005, and a recent collection, 2014 to 2019, aiming to address these questions.

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2 WHAT A MUSEUM COLLECTION CAN TELL US ABOUT *Bemisia tabaci* B-HAPLOTYPE INTRODUCTION IN BRAZIL?

ABSTRACT

The whitefly, *Bemisia tabaci*, A-haplotype, American native, was first documented in Brazil in 1923. Later, two introductions were registered: B haplotype in 1991 in São Paulo state (southeast region), and Q haplotype in 2013 in the Rio Grande do Sul (south region). For some time now, researchers have relied almost exclusively on few and fresh whitefly samples to identify haplotypes, its richness, and variability in many countries including Brazil. Here, we analyze a museum collection of *B. tabaci* haplotypes collected from 1989 to 2005 in different regions and crops of Brazil. Thirty-six samples of *B. tabaci* adults were analyzed using mitochondrial cytochrome oxidase I (mtCOI) and one nuclear (L11) primers. Interestingly, none of the studied samples belonged to the American native *B. tabaci* haplotypes. All the analyzed individuals were identified as B haplotype. The oldest sample was collected in 1989, in Rio de Janeiro state (southeast region) and another in 1990 in Ceará state (northeast region) before the first report of this haplotype in Brazil in 1991 (southeast region). A possible host plant for *B. tabaci* was identified, *Spondias mombin*, in the 1990 sample in the Ceará state. These discoveries revealed that B haplotype was introduced before its first report in Brazil and/or that introduction occurred through multiple regions. Further studies, analyzing whether the *B. tabaci* introductions from the Old World potentially involved multiple pathways, will have significant implications for the management strategies of this pest in the New World.

Keywords: Invasive whiteflies, entomological museum collection, DNA analysis, geographical dispersion.

2.1 INTRODUCTION

Entomological museum collections represent a vast and valuable source of scientific information in richness and abundance of species around the world (Smith & Blagoderov, 2012). Likewise, ancient museum samples can represent a forensic resource of investigation to determine the approximate introduction time and location of a pest in a country (Suarez & Tsutsui, 2004).

Ancient collections can also offer economic benefits by providing information not just in agriculture but also in public health and safety especially when those invasive pests have a high capacity to adapt to environmental changes (Hoffmann & Sgrò, 2011). Once the whitefly *Bemisia tabaci* (Gennadius, 1889) (Insecta: Hemiptera: Aleyrodidae) complex started been transported and introduced among Pantropical agroecosystems it has

become one of the world´s most damaging agricultural pest through feeding and virus transmission (Brown et al., 1995; Oliveira et al., 2001; Hadjistylli et al., 2016). In Brazil, it found ecological opportunities arising from food sources, tropical climate, and three growing crop seasons allowing density increase and dissemination (Quintela et al., 2016).

Previously, entomologists have relied almost exclusively on few and fresh whitefly samples trying to solve the *Bemisia tabaci* complex conundrum not just in the light of whitefly systematic but also its biological, ecological, and molecular variability across the globe (Gill & Brown, 2010) including in Brazil. In this study, we break this vicious point by using DNA from the whitefly *B. tabaci* museum collection samples to move beyond the actual introduction timeline in Brazil.

Considered a cryptic species, *B. tabaci*, contains morphologically indistinguishable but genetically distinct specimens, pupae or adults, of widely known populations, referred to as biotypes (Costa & Brown, 1991; Brown et al., 1995; Brown et al., 1996; Perring, 2001; Brown, 2010; Boykin, 2014; Hadjistylliet al., 2016). Biotypes were distinguished based on biological traits such as host-plant adaptability, phytotoxic symptom induction, insecticide resistance, behavior, and plant virus-transmission (Brown et al., 1995; Brown et al., 1996). Recently, genetic groups of this species complex have been referred to as haplotypes, based also on molecular, biogeographical and population studies (Moya et al., 2019; Paredes-Montero et al., 2020).

The separation of these haplotypes is resolved, mainly, in the mitochondria cytochrome oxidase I (COI) phylogeny (Brown, 2010; Moya et al., 2019). Similar to other whiteflies, *B. tabaci* is a haplodiploid insect as revealed by its arrhenotokous reproductive system. Male offspring are produced by unfertilized haploid eggs, and females from fertilized diploid eggs (Byrne & Bellows, 1991; Byrne & Devonshire, 1996). From the mitochondrial perspective, maternally inherited, its markers such as COI, reflect a different history of *B. tabaci* when compared with nuclear markers that can provide the paternal and maternal history of the species (Kang et al., 2012).

Inheritance occurs differently in males and females of this whitefly. Despite being different, nuclear and mitochondrial tools can return complementary taxonomic responses (Moya et al., 2019), providing demographic information (Kang et al., 2012), being useful for long-term insect storage identification, and even with body degradation. Since this is a museum collection research, with a restricted number of samples, we analyze few whitefly individuals from different locations of Brazil.

Bemisia tabaci entered Brazil possibly in multi-introductions location points via international transports of plant material and people which have contributed to its geographical spread and related Phytoviruses worldwide (Brown et al., 1995). In Brazil, the record for *B. tabaci* dated from 1923 (Bondar, 1928) for a prevalent A-haplotype (American Tropics/Caribbean clade (AM-TROP, also known as New World [De Barro et al., 2011]) (Brown et al., 1995; Brown et al., 1996; Moya et al., 2019), American native, collected on *Euphorbia hirtella* (Euphorbiaceae). Despite this, the A-haplotype official record as a pest of various crops in Brazil, such as cotton, beans, and soybeans, came later in 1968 in the states of São Paulo, and Paraná (Costa et al., 1973), also in the Rio Grande do Sul (Barreto et al., 1980), Santa Catarina (Silva et al., 1968), Goiás (Faria, 1985), and northeast region (Beltrão et al., 1985).

In 1991, in São Paulo state, Lourenção & Nagai (1994) observed high densities of *B. tabaci* in crops of economic interest associated with whitefly-related physiological disorders named tomato irregular ripening and squash Silverleaf. Because of these symptoms, although molecular studies were not performed at that time, the authors concluded that it was an invasive B haplotype (Brown et al., 1995; Brown, 2010) (NAF-MED-ME, also known as Middle East-Asia Minor 1, MEAM1 [De Barro et al., 2011]). Then, it was suggested that the B haplotype nymphs were introduced into the country on ornamental plants, *E. pulcherrima*, from international markets (Lourenção & Nagai, 1994). Later, Oliveira et al., (2000) observed that *B. tabaci* samples from Ceará and Paraíba (northeast region) collected in 1997 showed similar patterns to haplotype B. The authors were impressed with the fast dissemination of this haplotype in Brazil; once it was introduced in the southeast region and less than six years it was already present in the northeast region (approximately 3000 km).

The second report of an exotic introduction in Brazil of *B. tabaci*, the Q haplotype (NAF-MED-ME clade, also known as the Mediterranean, MED [De Barro et al., 2011]), was first observed in 2013 in Rio Grande do Sul, the southernmost state of Brazil (Barbosa et al., 2015), on Solanaceae, and Convolvulaceae. Later, this haplotype was observed in São Paulo and Paraná states on ornamentals (Moraes et al., 2017). Likewise, this polyphagous haplotype has been disseminated into other geographic regions of the country including Goiás state (midwestern region) (Bello et al., 2020). One adult identified as Q was later observed on ornamental plants in Mato Grosso state, also the midwestern region of Brazil (Pitta et al., 2019). These two invasive haplotypes, B and Q, are still the

most economically important worldwide due to its high adaptability and resistance to almost all chemical-synthetic compounds used for its control (Hadjistylli et al., 2016; Elfekih et al., 2018; Basit, 2019).

In this study, we aim to analyze a museum collection of *B. tabaci* haplotypes collected from 1989 to 2005 in different regions and crops of Brazil. We report that B haplotype was introduced early than reported by Lourenção & Nagai (1994) perhaps through several locations in Brazil. The implications of these new findings are discussed below.

2.2 MATERIAL AND METHODS

Museum collection samples

Thirty-six adult samples of *Bemisia tabaci* of different regions and crops, including cultivated and wild plants, of Brazil, collected from 1989 to 2005 curated in a museum collection at Embrapa Genetic Resources and Biotechnology, Brasília - Brazil were used for molecular studies. The sample selection criteria were the oldest whiteflies collection date. For all the samples a duplicate was created. These samples were stored in glass tubes containing 70% alcohol, protected from light, at room temperature.

Whitefly geographic distribution data

The *B. tabaci* museum collection geographical distribution haplotypes were plotted using the software ArcGIS™ v.10.3 (Environmental Systems Research Institute, Esri, 2014). The haplotypes spatial distributions were graphed on a cartographic base obtained from the Brazilian Institute of Geography and Statistics (IBGE, 2016) (https://www.ibge.gov.br/en/geosciences/downloads-geosciences.html). To generate the map the values of the geographic coordinates were converted into degrees and imported in the program. The layers consisted of a) altitude, in meters; b) state´s delimitation; and c) country administrative political division.

Molecular characterization of the samples

The molecular studies of the 36 adult samples of *B. tabaci* haplotypes were conducted at Plant Science Laboratory at The University of Arizona (School of Plant Sciences, Tucson, Arizona, USA). Adults from colonies held at The University of Arizona

were used as standards for *B. tabaci*. One individual per sample was transferred to a new and sterile microtube Eppendorf™ containing 100 µl Nuclease-free water and held overnight at room temperature to hydrate the whiteflies.

DNA extraction and PCR were carried out as described in Zhang et al., (1998) with modifications. One adult whitefly was placed with the Diamond™ flat wood toothpick inside a 1,5 ml clear microfuge tube, and grounded with a micro pestle that was washed with 600 µl of hexadecyltrimethylammonium bromide (CTAB) (Sigma-Aldrich, Darmstadt, Germany) lysis buffer to remove the insect fragments. Then 1.0mg⁄mL proteinase K was added to each tube. The lysis buffer contained 100 mM Tris-HCl pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl containing 0.2% 2-mercaptoethanol; 2% CTAB, followed by incubation for 10 min at 65°C mixing, 4 times. Afterward, 600 µl of cold chloroform, isoamyl-alcohol (24:1), was added, the contents were mixed by inversion producing an emulsion that was separated by centrifugation at 12.000 rpm, 4°C for 3 min. The supernatant, 400 µl was transferred to a 1.7 ml clear microtube Axygen™ with an added volume of 500 µl of cold isopropanol and 2 µl of glycogen, to improve DNA precipitation, per sample. Overnight incubation, -20°C, for yield results, proceed by centrifugation, 10 min at 12.000 rpm, at 4°C. The pellet was washed by adding 500 µl of cold 70% ethanol and centrifuge again at 12.000 rpm, 4°C, 1 min, to bring down remaining ethanol, subtracting the remaining ethanol with a pipette, always keeping the samples on ice, to increase the pellet visibility. The pellet was collected, air-dried for 15 min, and re-suspended in 30 µl of Nuclease-free water, shake and store at -20°C.

Whitefly samples were screened to identify samples containing *B. tabaci* A-haplotypes by polymerase chain reaction (PCR) amplification using the A-specific mitochondrial primers, 5′-F AM-TROP-3′ [GGGACCCTATCTTATATCAGCAC] and 5′-R AM-TROP-3′ [TTTGGTAGGCTGGGCATAA], ~1000 bp expected fragment of the mitochondrial COI (mtCOI) (Moya et al., 2019). The PCR conditions were: initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 15 sec, 48°C for 30 sec, 72°C for 1 min, with a final extension at 72°C for 3 min. Since none (n=0) of the samples amplify with this set of primers all of them were tested with B-specific mitochondrial primers (BTCOX). Samples were screened by PCR amplification using 5′-F BTCOX-3′ [GATCGAAATTTTAATAGATCTTTTTATGATCC] and 5′-R BTCOX-3′ [TGTTCTATTGTAAAACTAGCACTATTTTG], with ~900bp mtCOI fragment. PCR conditions were: initial denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 1

min, 46°C for 1 min, 72°C for 1 min, with a final extension at 72°C for 5 min.

As the B-specific mitochondrial primer tested, BTCOX, amplified only six of the Brazilian museum collection individuals, a pair of nuclear primers (L11), which amplifies A, B, and Q haplotypes were used to the rest of the collection (n=36). The selected nuclear primers were 5´-F L11-190-3´ [CACCCACTTCATCCTCACCC] and 5´-R L11-962-3´ [TCACCCCAGGCATCATAAGC], with an expected size of ~773 bp. PCR consisted of initial denaturation of 95°C for 1 min.; 30 cycles of 95°C for 15 sec; 55°C for 15 sec; 72°C for 1 min; and a final extension at 72°C for 2min.

Each PCR reactions contained (26 µl vol): 12.5 µl Jumpstart REDTaq Ready-Mix (Sigma-Aldrich, Saint Louis, MI), 0.5 µM each primer, 9.0µl Nuclease-free water, and 3.5 µlDNA. The PCR product (2.5 µl) was visualized by 1% agarose gel electrophoresis in 1X TAE buffer (pH 8.0) containing 1X GelRed (Biotium, Hayward, CA), at 100 volts for 60 min. To clone mtCOI and L11 primers, amplicons of each expected primer size were ligated into the pGEM-T Easy plasmid vector (Promega, Madison, WI), and cloned to *Escherichia coli* DH5α competent cells by heat-shock (Green & Sambrook, 2012). For each sample, two clones were screened by colony PCR (Gussow & Clackson, 1989). Amplicons of each sample expected size was confirmed by agarose gel electrophoresis (load 2.5 µl of each PCR product in a 1% agarose gel, 100 Volts, 60 min) and sequenced using M13 primers 5´-F M13-3´ [TGTAAAACGACGGCCAGT] and 5´-R M13-3´ [CAGGAAACAGCTATGAC], expected size of ~973bp (Promega, Madison, WI) (Alon et al., 2008). PCR consisted of 94°C for 10 min; 35 cycles of 94°C for 1 min; 53°C for 1 min; 72°C for 3:50 min; and a final extension at 72°C for 10 min. Reactions contained (1X): 12.5 µl Jumpstart REDTaq Ready-Mix (Sigma-Aldrich, Saint Louis, MI), 0.5 µl of each M13 primers, 9.0 µl Nuclease-free water, and 2.5 µl DNA [20 ng], to a final vol of 25 µl. Unforeseen, the minority of samples (n=5) amplified with both mtCOI and nuclear primers. To confirm the identification, all samples were sequenced (n=36): 35 samples were cloned with L11; 6 with both markers; 1 sample (40369) only with mtCOI.

Molecular data analysis

Clones, from both primers, were sequenced bi-directionally by capillary (Sanger DNA sequencing) on an Applied Biosystems ABI 3730XL DNA analyzer at The University of Arizona Genomics Core, Tucson, AZ, USA, in 2019. Sequence reads were manually assembled and edited using SeqMan Pro™ software available in DNASTAR

Lasergene v8.0 (DNASTAR, Inc., Madison, WI), exported as FASTA files, aligned using MUSCLE™ v3.8.31 (Edgar, 2004), trimmed and analyze with MEGA™ v.7 software (Pennsylvania State University, PA, USA) to confirm *B. tabaci* identity. Short sequences, singletons, and sequences containing stop codons, indels, and⁄or ambiguous bases were deleted before the alignment. The alignment was trimmed to a length of ~753bp.

The reference sequences used to reconstruct the phylogenetic tree were based on the Dr. J. K. Brown laboratory reference COI, and nuclear database (J. K. Brown, unpublished data). Sample from Ethiopia (ETH2) was added as references of B haplotype; Burkina Faso (BF13) of Q haplotype; and Argentina (ARG2) of A-haplotype. To root the tree *B. tabaci* from Namulonge, Uganda (UG232) was used as an outgroup member for the nuclear data; and for the mtCOI data, we used *Trialeurodes vaporariorum* from United States, California, Salinas (Tvap) as an outgroup member.

The best model of molecular evolution, for the nuclear primer (L11), was estimated based on a consensus of the Bayesian information criterion (BIC), Akaike information criterion (AIC), and the Maximum Likelihood value (InL) using MEGA™ v.7 (Kumar et al., 2016). The Kimura 2-parameter model (Kimura, 1980) was identified as the best-fit model of evolution. Additionally, for the mitochondrial primer (mtCOI), the best model was also estimated based on the same criteria as for the nuclear primer and the best model was Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). The phylogenetic trees were constructed with maximum likelihood, using one clone per representative samples, with one thousand bootstrap iterations (Tamura et al., 2013).

2.3 RESULTS

A total of 36 whitefly museum collection samples were used for sequencing and information was generated on the Brazilian invasive geographical distribution and host-plant data from 1989 to 2005 (Table 2.1 and 2.2). *B. tabaci* sequences were filtered by a minimum of ~753 bp, two intact clones, and sequences with low quality were discarded.

Table 2.1. Sample code, geographic location (region, state, and city), coordinates, collection dates, and host plant of *Bemisia tabaci* samples from the museum collection of Brazil used to perform the nuclear data (L11) analysis.

Code	Location	Latitude	Longitude	Collection date	Host plant
	NORTH				
	RONDÔNIA (RO)				
40345	Vilhena	-12.734487	-60.161100	2004	*Phaseolus vulgaris*
	NORTHEAST				
	BAHIA (BA)				
40399	Bom Jesus da Lapa	-13.236280	-43.400290	19/oct/1999	*Cucurbita* sp.
40420	Guanambi	-14.204061	-42.776993	20/may/1997	*Gossypium* sp.
	CEARÁ (CE)				
40359	Icapuí	-4.715230	-37.354173	20/dec/1990	*Spondias mombin*
40398	Russas	-4.937551	-37.997103	11/jun/1997	*Cucumis melo*
	MARANHÃO (MA)				
40397	São Luís	-2.611004	-44.338454	16/sep/2005	*Cucumis sativus*
	PARAÍBA (PB)				
40375	Campina Grande	-7.225.764	-35.903964	14/dec/2005	*Gossypium* sp.
	PERNAMBUCO (PE)				
40380	Petrolina	-9.325694	-40.550390	03/sep/2000	*Solanum* sp.
40395	Petrolina	-9.387342	-40.487729	30/jun/2003	*Citrullus lanatus*
40417	Petrolina	-9.387342	-40.487729	30/jun/2003	*Cucurbita* sp.
40384	Petrolina	-9.387342	-40.487729	30/jun/2003	*Solanum* sp.
40383	Recife	-8.069346	-34.922491	20/jan/1999	*Brassica oleracea*
	RIO GRANDE DO NORTE (RN)				
40339	Baraúna	-5.080659	-37.620628	05/dec/2001	*Cucumis melo*
40337	Mossoró	-5.173010	-37.345074	11/sep/2000	*Cucumis melo*
40348	Mossoró	-5.173010	-37.345074	11/sep/2000	Weed
40340	Mossoró	-5.173010	-37.345074	11/sep/2000	-
40368	Mossoró	-5.173010	-37.345074	11/sep/2000	*Cucumis melo*
	MIDWEST				
	DISTRITO FEDERAL (DF)				
40335	Brasília	-15.604142	-47.712883	14/feb/2003	*Nicotiana tabacum*
40338	Brasília	-15.604142	-47.712883	14/feb/2003	*Brassica oleracea*
40358	Brasília	-15.603499	-47.713773	13/mar/2003	*Brassica oleracea*
40363	Brasília	-15.603499	-47.713773	13/mar/2003	*Abelmoschus esculentus*
40365	Brasília	-15.603499	-47.713773	11/jun/2003	Weed
40419	Brasília	-15.603499	-47.713773	23/jul/2003	*Brassica oleracea* var.

Code	Location	Latitude	Longitude	Collection date	Host plant
					italica
40356	Brasília	-15.603499	-47.713773	19/oct/2003	*Brassica oleracea*
	GOIÁS (GO)				
40361	Jataí	-17.938925	-51.871001	08/may/2003	*Solanum* sp.
40382	Trindade	-16.645978	-49.496877	11/aug/1999	*Lycopersicon esculentum*
40400	Trindade	-16.645978	-49.496877	11/aug/1999	*Solanum* sp.
	SOUTH EAST				
	MINAS GERAIS (MG)				
40415	Belo Horizonte	-19.871055	-43.970000	28/jul/1999	*Solanum* sp.
40374	Nova Porteirinha	-15.766.366	-43.286335	12/sep/2000	*Ipomoea batatas*
40376	Viçosa	-20.758350	-42.868366	04/oct/1998	*Glycine* sp.
	RIO DE JANEIRO (RJ)				
40416	Seropédica	-22.772021	-43.689456	23/jul/2003	*Brassica oleracea*
	SÃO PAULO (SP)				
40367	Jaboticabal	-21.246029	-48.293697	08/jun/1999	*Cucumis sativus*
40381	Santo Amaro	-23.650978	-46.710598	28/mar/2000	*Brassica oleracea* var. *italica*
40418	Miguelópolis	-20.174762	-48.029275	27/jan/1998	*Glycine* sp.
	SOUTH				
	PARANÁ (PR)				
40355	-	Unknown	Unknown	14/dec/2005	Weed
	BRAZIL (BRA)				
40347	Brazil	Unknown	Unknown	02/oct/2001	Weed

Table 2.2. Sample code, geographic location (region, state, and city), coordinates, collection dates, and host plant of *Bemisia tabaci* samples from the museum collection of Brazil used to perform the mitochondrial data (mtCOI) analysis.

Code	Location	Latitude	Longitude	Collection date	Host plant
	NORTHEAST				
	PARAÍBA (PB)				
40375	Campina Grande	-7.225.764	-35.903964	14/dec/2005	*Gossypium* sp.
	PERNAMBUCO (PE)				
40383	Recife	-8.069346	-34.922491	20/jan/1999	*Brassica oleracea*
	RIO GRANDE DO NORTE (RN)				
40339	Baraúna	-5.080659	-37.620628	05/dec/2001	*Cucumis melo*
40348	Mossoró	-5.173010	-37.345074	11/sep/2000	Weed
	MIDWEST				
	GOIÁS (GO)				
40400	Trindade	-16.645978	-49.496877	11/aug/1999	*Solanum* sp.
	SOUTHEAST				
	RIO DE JANEIRO (RJ)				
40369	Campos dos Goytacazes	-21.749602	-41.308700	24/aug/1989	*Brassica oleracea* var. *italica*

The sequences were aligned to the *B. tabaci* B haplotype (n=36) (North Africa–Mediterranean–Middle East clade – NAF-MED-ME). Only five samples (40339; 40348; 40375; 40383; 40400) collected, mainly in the northeast region, were amplified with both mtCOI and L11 primers.

The phylogenetic tree, with nuclear and mtCOI data (Figure 2.1 and 2.2, respectively), were built. The sequences from one (B) of the four haplotypes present in Brazil (A1, A2, B, and Q) formed a consistent cluster, within NAF-MED-ME clade using *B. tabaci*, and *Trialeurodes vaporariorum* as outgroups, respectively.

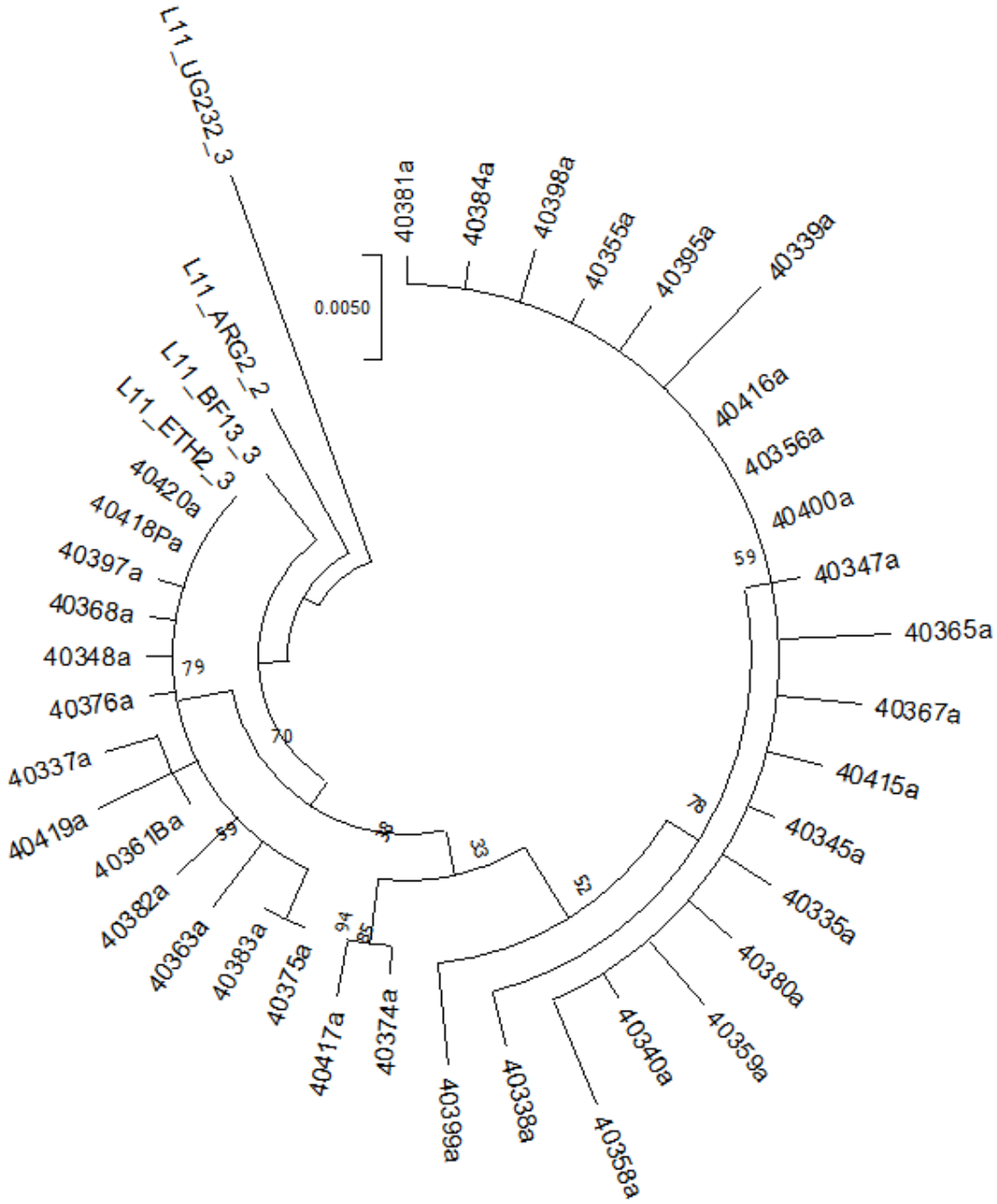

Figure 2.1. Phylogeny of *Bemisia tabaci*, from Brazil, based on 35 nuclear genes (L11) sequences. The Kimura 2-parameter model (Kimura, 1980) was identified as the best fit model. The phylogenetic tree was constructed with one clone per representative samples with one thousand bootstrap iterations in the tree-knots (Tamura et al., 2013). Sequences from Ethiopia (ETH2) were added as references of B haplotype; Burkina Faso (BF13) as Q haplotype; and Argentina (ARG2) as a representative of A-haplotype. Outgroup samples from Namulonge, Uganda (UG232).

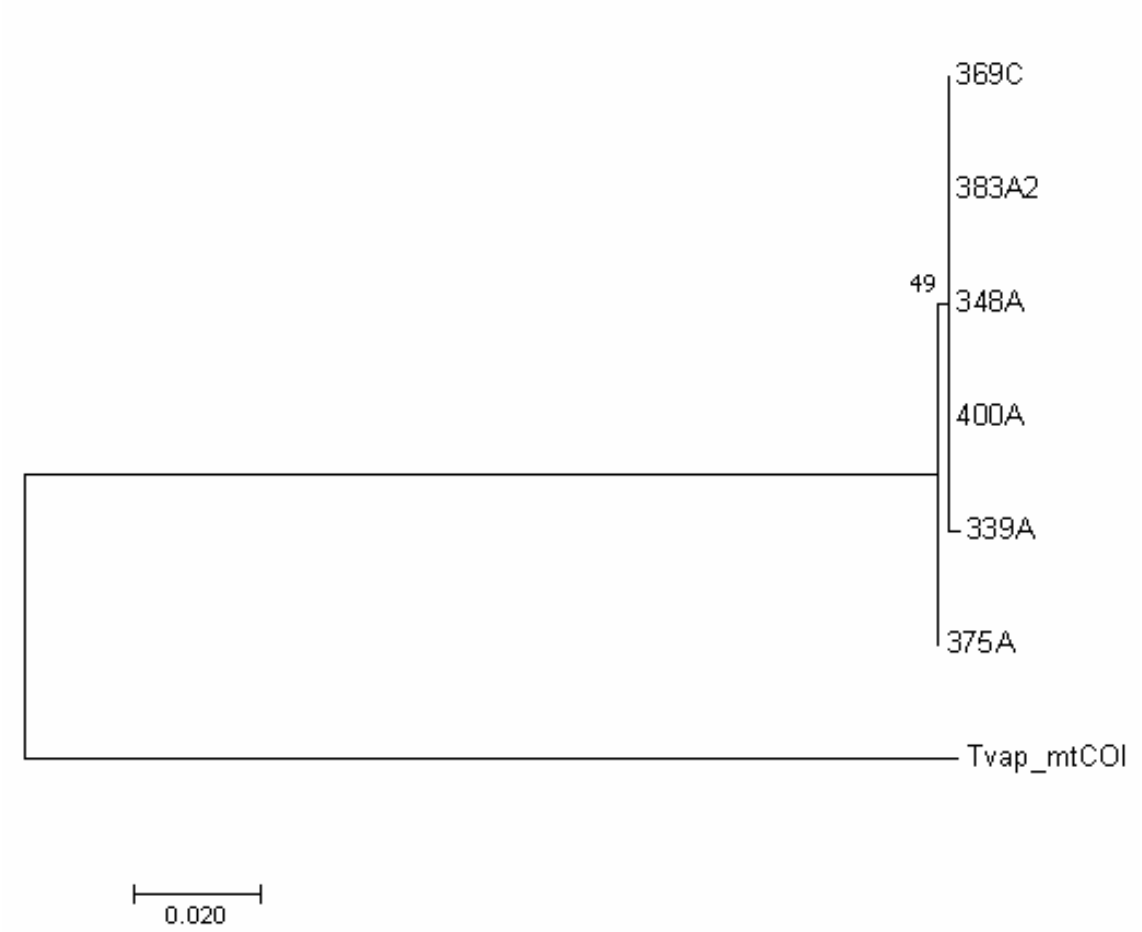

Figure 2.2. Phylogeny of *Bemisia tabaci*, from Brazil, based on 6 mitochondrial COI (mtCOI) sequences. The Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) was identified as the best fit model. The phylogenetic tree was constructed with one clone per representative samples with one thousand bootstrap iterations (Tamura et al., 2013). A sequence of *Trialeurodes vaporariorum* from the United States (Tvap) was added as an outgroup member; since it is a specific primer there is no reference sequence.

A map containing the museum collection sample´s geographical distribution and correlated haplotype was constructed (Figure 2.3). The map contains thirty-six samples of B haplotype randomly spread across the country in all five regions of Brazil. As aforementioned, the oldest museum sample, from 1989 in Rio de Janeiro state (southeast region) was collected on *Brassica oleracea* and another in 1990 in Ceará state (northeast region) on *Spondias mombin*.

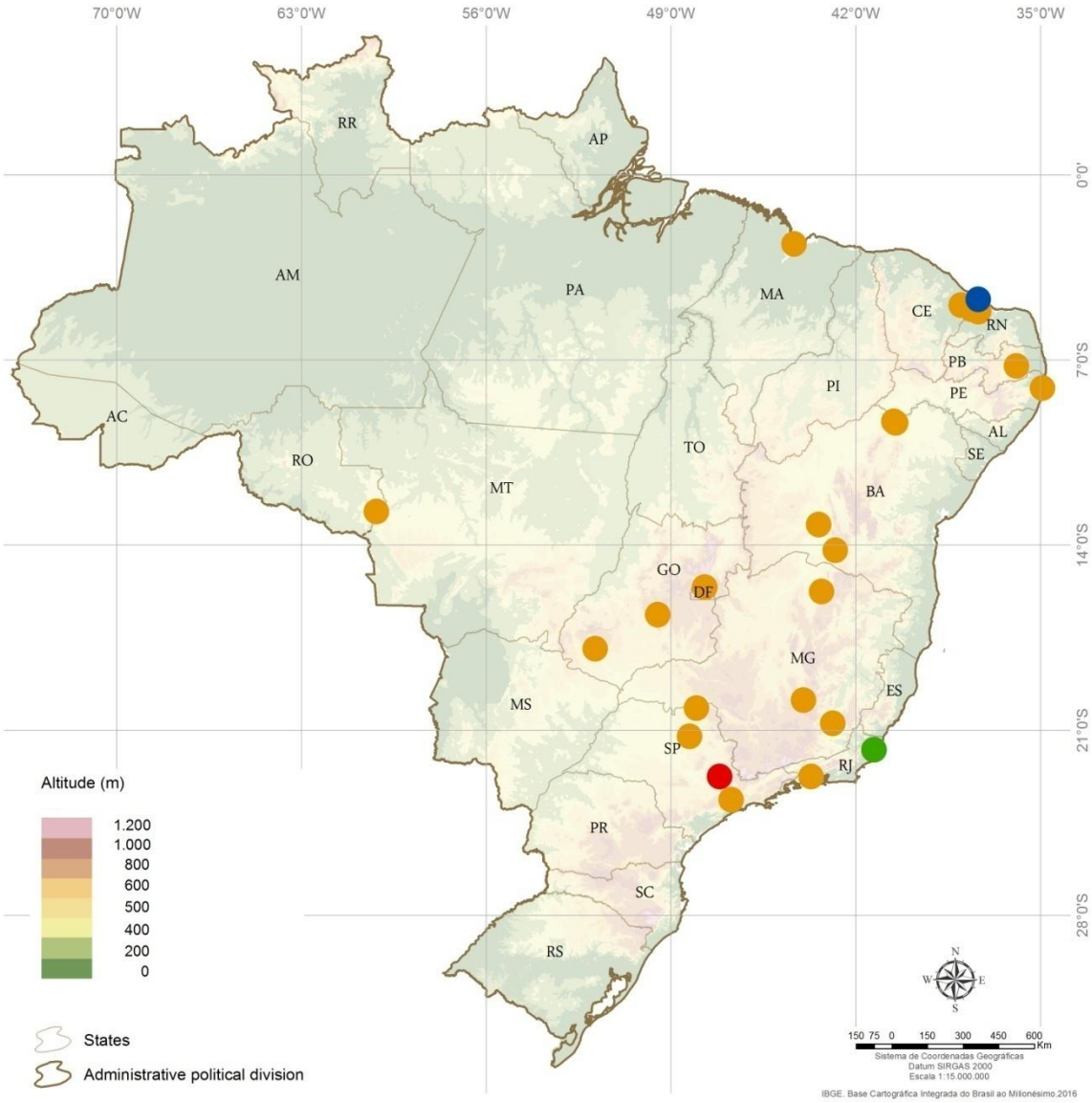

Figure 2.3. *Bemisia tabaci* museum collection samples (n = 36) locations in Brazil, 1989-2005. The red dot, in the map, indicates the previously introduction point record from Lourenção & Nagai (1994), the green dot indicates the 1989 introduction, and the blue dot the 1990 introduction as determined in our study. The yellow dots indicate the other sampling sites.

Host plants present in this study were classified according to Tropicos™ (Tropicos, 2020; <https://www.tropicos.org>). Inside B haplotypes, we identified eight most common host plants including *Brassica oleracea* (Brassicaceae) (n=5), *Solanum* sp. (Solanaceae) (n=5), *Cucumis melo* (Cucurbitaceae) (n=4), Weeds (n=4), *B. oleracea* var. *italica* (n=4), *Cucumis sativus* (n=2), *Cucurbita* sp. (Cucurbitaceae) (n=2), *Glycine* sp. (Fabaceae) (n=2), *Gossypium* sp. (Malvaceae) (n=2), followed by one observation on *Abelmoschus esculentus* (Malvaceae), *Citrullus lanatus* (Cucurbitaceae), *Lycopersicon*

esculentum (Solanaceae), *Nicotiana tabacum* (Solanaceae), *Phaseolus* sp. (Fabaceae), *P. vulgaris*, *Spondias mombin* (Anacardiaceae). The last one, *S. mombin*, as a possible new host for *B. tabaci*.

2.4 DISCUSSION

Bemisia tabaci is a unique insect-pest surrounded by controversial species boundaries delineation (Brown, 2010; Dinsdale et al., 2010). The use of mtCOI for identification, hence, haplotypes, is a genetic indicator which allows also the study of *B. tabaci* biological differences (Frohlich et al., 1999), variable in many respects, now referred to as cryptic species or perhaps sibling species if they are found to interbreed, according to classical definitions (Brown, 2010; Gill & Brown, 2010). This species shows that cut-off at the nuclear level defines far fewer variants than the mtCOI, which can be is saturated with mutations, and so resulted in sparse conclusions when used for phylogenetic predictions alone (Moya et al., 2019).

A previous study focusing on the molecular identification of the *Bemisia tabaci* and their geographical distribution across the country did not precisely ascertain when *B. tabaci* invasive B haplotypes were introduced in Brazil (Lourenção & Nagai, 1994). Over the last decades, two invasive *B. tabaci* haplotypes, B and Q, were introduced to several regions of Brazil resulting in yield loss among crops. In our studies, we analyzed entomological museum samples collected on different plants, years, and regions by relying on *B. tabaci* molecular sequences to obtain valuable historical information from introduced whiteflies in Brazil. This research allowed us to answer questions that could not be addressed before especially due to limited Brazilian ancient *B. tabaci* samples.

Our study identified 36 *B. tabaci* B-haplotypes samples consistent with previous published *B. tabaci* studies (Lima et al., 2000; Lima et al., 2002; Oliveira et al., 2000; Marubayashi et al., 2013; Barbosa et al., 2015; Moraes et al., 2018). This supports the theory of an early introduction, from exotic invaders, not only in Brazil but probably in South America as well, as revealed by the 1989 sample collected in Rio de Janeiro state, and another in 1990 in Ceará state (northeast region), before 1991 as reported by Lourenção & Nagai (1994).

The majority of the analyzed samples did not amplify with mtCOI markers probably due to the nature of the samples, some specimens with visible body tissue

disintegration, and samples conservation under 95% high-grade ethanol (Short et al., 2018). Even so, the maternally inherited marker is excellent for placing *B. tabaci* in their endemic habitats, and if combined with nuclear markers could provide a better understanding than mtCOI alone and, evidently improving the *B. tabaci* demographic study (Kang et al., 2012).

Since the first introduction of the B haplotype was reported in 1991 in São Paulo state (southeast region) (Lourenção & Nagai, 1994), we expected that most of the samples from this museum collection, sampled from 1989 to 2005 would be from A-haplotypes, American native. Unexpected, none of the samples analyzed contain any *B. tabaci* native American haplotypes (Brown, 2010; Gill & Brown, 2010) suggesting that B haplotype, invasive, was present in Brazil long before these samples were collected.

One possible explanation for the absence of the American natives' whiteflies is due to the niche displacement of natives by B haplotype. The dislocation may have occurred over time in Brazil, as a more recent collection found A-haplotypes in agricultural areas (Moraes et al., 2018). The competitive exclusion from asymmetric mating behavior, host plant suitability, and insecticide resistance may accelerate the process of displacement between these two previously allopatric haplotypes, A and B, contributing to the spread of the invasive haplotype (Luan & Liu, 2012a; Luan et al., 2012b).

Similarly, Liu et al. (2007) reported that B haplotypes seemed to override the native ones, in the 1990s, in China regions. As previously stated, factors such as ornamental importation, absence of natural enemies, and a vast number of host plants facilitate the biological invasion and displacement over natives haplotypes throughout the country. The same occurs, in a short period, under the influence of insecticide resistance and host plants with a double invasive displacement in China (Tang et al., 2019). Another explanation for the absence of A-haplotypes within the museum collection could be that samples were collected mainly in agricultural areas, excluding native plants, weeds, and nonrandom sampling sites.

Up to this moment, the first B haplotype introduction was considered in the Campinas region (São Paulo state) in 1991, a well-known region of ornamental plant production. However, our studies showed that this haplotype was also observed in the city of Campos dos Goytacazes, a coastal city of Rio de Janeiro state in 1989; and in Icapuí another coastal city of Ceará state, in 1990. Icapuí is located near the city of Mossoró (the Rio Grande do Norte state), a major melon producer region, where a high density of

whitefly is observed. Those possible multiple/new introductions can be explained by the arrival of imported plant material at the Brazilian Atlantic seaports.

Overall, the predominance of B haplotype in this museum collection suggests that it was present before these whitefly samples were collected. Additionally, since Brazil has continental dimensions, this whitefly's dispersion from São Paulo state to Ceará state, one of the most northeast points of Brazil (approximately 3000 km), in only one year (1989-1990) seems not very likely.

2.5 CONCLUSIONS

The results presented in our study revealed that *Bemisia tabaci* B haplotype was introduced before its first report in Brazil and/or that introduction may have occurred through multiple regions of Brazil.

Although the previously published samples were reported as present in many states of Brazil, possibly multiple/new introductions occurred, especially for the B and Q haplotypes, and in sympatry with American native haplotypes.

Further studies about if incursions of *B. tabaci* from the Old World potentially involved from multiple pathways will have significant implications for the study of the geographical dispersion of this pest and insecticide resistance management strategies in the New World.

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3 *Bemisia tabaci* (GENNADIUS, 1889) (INSECTA: ALEYRODIDAE) IN BRAZIL: HAPLOTYPES DISTRIBUTION

ABSTRACT

Bemisia tabaci the American native haplotype (A), American Tropics (North and Central-Caribbean, AM-TROP) clade was documented in Brazil more than 90 years ago. In the 1990s an invasive haplotype (B) from the North Africa-Mediterranean-Middle East (NAF-MED-ME) clade entered the country, and in 2013, a second introduction occurred, within NAF-MED-ME clade (Q haplotype). Attempts to map and identify its haplotypes, especially natives and its associated host plants across Brazil had limited success due to the scarcity of samples in several regions. In this study, we collected samples from all 26 states and the Federal District, identified genetically, map their geographical distribution, and host plants. A total of 204 Brazilian whitefly samples, from non-repeatable sampling sites, were analyzed using the mitochondrial cytochrome oxidase (mtCOI) and nuclear DNA (nDNA) (L11) gene markers. The American native were identified in 12 states comprising 11.3% of the samples (n=26) [34.6% A-haplotype (n=9) (nDNA); 46.15% A1-haplotype (n=12) (mtDNA); 19.2% A2-haplotype (n=5) (mtDNA)], within the American Tropics clade (North and Central-Caribbean, AM-TROP). The invasives Q (n=11) was observed in five states (4.8% of the samples) and B (n=192) in 26 states (83.8% of the samples) within North Africa-Mediterranean-Middle East clade- NAF-MED-ME. Two possible new host plants for *B. tabaci* were identified: *Acacia mangium* (Acaciaceae) at Roraima state (north region) by A-haplotype; and one by B haplotype: *Xanthosoma sagittifolium* (Araceae) at Rio de Janeiro state (southeast). B-haplotype remains prevalent throughout Brazil.

Keywords: Whitefly, mtCOI, nuclear gene, host plants, geographical distribution.

3.1 INTRODUCTION

Bemisia tabaci (Gennadius, 1889) (Insecta: Hemiptera: Aleyrodidae), known as whitefly, is a destructive agricultural pest in Pantropical areas (Costa & Brown, 1991; Brown et al., 1995, Hadjistylli et al., 2016). It has distinguished itself among more than 1500 species of whiteflies of the world (Martin & Mound, 2007), adapted to more than 600 eudicots (Mound & Halsey, 1978; Cock, 1986; Simmons et al., 2008), damaging directly by phloem-feeding and inducing phytotoxic disorders (Costa & Brown, 1991; Oliveira et al., 2001).

Indirectly, damages by honeydew excretion, a sugar substrate for saprophytic fungi growth that reduces photosynthesis, and by trehalulose, sugar drops, into cotton fibers (Hequet & Abidi, 2002). Besides, *B. tabaci* transmits hundreds of plant viruses

including Carlaviruses, Closteroviruses, Geminiviruses, Luteoviruses, Nepoviruses, Potyviruses, and a DNA-containing rod-shaped virus (Navas-Castillo et al., 2011; Faria et al., 2016). The most economically significant are Geminiviruses, Closteroviruses and recently Carlaviruses (Navas-Castillo et al., 2011; Chen et al., 2016; Faria et al., 2016).

The species *B. tabaci* is considered a complex of multiple genetic groups, also known as a cryptic species, formerly biotypes (Boykin, 2014), which means that contains morphologically indistinguishable, but genetically distinct individuals (Brown, 2010; Gill & Brown, 2010). Biotypes or host races were distinguished based on biological traits such as host-plant adaptability, phytotoxic symptom induction, insecticide resistance, behavior, and plant virus-transmission (Brown et al., 1995; Brown et al., 1996). Later, an attempt to classify the species based also on genetic boundaries and center of origin was published (Dinsdale et al., 2010; De Barro et al., 2011).

Although efforts to name the taxa in the *B. tabaci* complex have arisen, as aforementioned, suggestions such as Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) were not adhered to by the whitefly community (Boykin, 2014). That can be explained by its gaps in the species classification process including the non-observance of the International Code of Zoological Nomenclature Standards (De Barro et al., 2011). Recently, genetic groups of this species complex have been referred to as haplotypes, based also on molecular, biogeographical, and population studies (Moya et al., 2019; Paredes-Montero et al., 2020).

Different haplotypes can also feature ecological, biological, and genetic plasticity regarding adaptability, behavior, ability to hybridize, development rate, reproduction (Byrne & Bellows, 1991; Hadjistylli et al., 2016; Moya et al., 2019), host plants interaction (Oliveira et al., 2001), endosymbionts (Chu et al., 2011), specific virus-vector transmission capabilities (Bedford et al., 1994; Peñalver-Cruz et al., 2020), and insecticide resistance (Castle et al., 2013; Basit, 2019; Horowitz et al., 2020).

In Brazil, the occurrence of *B. tabaci* was first reported in 1923 at the Bahia state on Euphorbiaceae (Bondar, 1928). Two native haplotypes, A1 and A2 haplotypes, part of the American Tropics (North and Central-Caribbean, AM-TROP) clade, also nominated New World species (Marubayashi et al., 2013), and BR biotype (Lima et al., 2000), occur on indigenous and cultivated host plants throughout the country (Lima et. al. 2000; Lima et al., 2002; Marubayashi et al., 2013; Barbosa et al., 2014; Queiroz et al., 2016). Additionally to Brazil, native haplotypes of Latin America (AM-TROP) have been

reported in Argentina, Bolivia, Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, and Venezuela hosted by wild and cultivated plants. Although native haplotypes remained low in Brazil until the 1990s some caused losses as vectors of many plant viruses especially on beans, soybean, and tomato (Costa, 1965; Costa et al., 1977; Faria, 1985).

In the 1990s, the invasive *B. tabaci* B haplotype, of the North Africa-Mediterranean-Middle East (NAF-MED-ME) clade, (also known as Middle East-Asia Minor 1, MEAM1 [De Barro et al., 2011]) was possibly introduced in Brazil via international trade of ornamental plants (Costa et al., 1993; Lourenção & Nagai, 1994). B-haplotype was first reported in São Paulo state and spread through the country causing heavy losses in beans, cabbage, cotton, melon, soybeans, tomato, watermelon, and corn (Ribeiro et al., 1998; Lima et al., 2002; Quintela et al., 2016). B-haplotype has bio-advantages over the A-haplotype with higher fecundity, a broader range of host plants (Zang et al., 2006), and a higher insecticide resistance levels (Costa et al., 1993; Coats et al., 1994; De Barro et al., 1995). Furthermore, the B-haplotype is stilling the primary vector of plant viruses in Brazil (Moraes et al., 2018).

After in 2013, a second invasion now from a Q haplotype, within NAF-MED-ME clade (also known as the Mediterranean, MED [De Barro et al., 2011]), was identified from samples collected in the Rio Grande do Sul state, south region, on *Capsicum annuum* and *Ipomoea batatas* (Barbosa et al., 2015) spreading through south and southeast region (Moraes et al., 2017). Another Q population was located at Mato Grosso state, midwestern states of Brazil, on imported ornamental plants (Pitta et al., 2019). These two invasive haplotypes, B, and Q are the most economically important worldwide (Hadjistylli et al., 2016).

Understanding the Brazilian *B. tabaci* native haplotypes' genetic basis is essential to study their evolution, interaction with invasive haplotypes, and to establish effective pest management strategies. Although studies using molecular markers as RAPD revealed genetically differences among *B. tabaci* from different locations of Brazil (Lima et al., 2000; Lima et al., 2002; Oliveira et al., 2000; Rabello et al., 2008; Silva et al., 2009; Fontes et al., 2010; Queiroz et al., 2016) the data cannot be directly compared with mitochondrial cytochrome oxidase I (mtCOI). RAPD cannot separate phylogenetic haplotypes compared to mtDNA (Brown, 2000; Hadjistylli et al., 2010).

Although mtCOI is an arthropod universal primer, in molecular identification,

it is extensively used because is one of the most conservative protein-coding genes in the mitochondrial genome of animals (Brown et al., 1995; Simon et al., 1994; Folmer et al., 1994) and provides a high divergence among members of *B. tabaci* (Gill & Brown, 2010; Brown, 2010; Frohlich et al., 1999). Also, is reproducible and informative to phylogenetic studies and biogeographical distribution analyses (Dinsdale et al., 2010; De Barro et al., 2011; Alemandri et al., 2012; Hadjistylli et al., 2016).

Difficulties within *B. tabaci* identification have occurred implying economic losses, and delay on the quarantine measurements. Adding a second diagnostic tool, a nuclear gene supports the mtCOI phylogeny (De Barro et al., 2011) validate each gene, informing the best choice to resolve divergences, reducing time and resources in the identification process. Few studies using nuclear genome data have been conducted (Hsieh et al., 2014; Hadjistylli et al., 2016; Elfekih et al., 2018; Moya et al., 2019) with worldwide whiteflies but none with Brazilian haplotypes. Although nuclear markers have lower divergences among members of *B. tabaci* (Moya et al., 2019) they complement the mtCOI published database, especially for native haplotypes.

Additionally, previously Brazilian studies, using mtCOI, are not representative, for the American indigenous haplotypes, due to the low number of analyzed samples and the extension of the country (Marubayashi et al., 2013; Barbosa et al., 2014; Moraes et al., 2018). The objectives of this study were to identify American native *B. tabaci* haplotypes, map its geographical distribution in all states of Brazil, and related host plants.

3.2 MATERIAL AND METHODS

Field collection

Adults of *Bemisia tabaci* were collected during 2014-2019 from natural fields, on cultivated and weeds, from all 27 Brazilian states with a mechanical hand-held aspirator, placed into a 1.5 ml microtube containing 95% ethanol and stored at -20°C until analysis. This collection was analyzed at Embrapa Rice and Beans, Santo Antônio de Goiás, Goiás – Brazil, from 2017-2018; and shipped to The University of Arizona, Plant Science laboratory, Arizona – the United States of America (USA), on dry ice, store at -70°C, and sequenced in 2019-2020.

Sample

A sample consisted of all whiteflies collected from a single plant, on the same day and host plant, per geographical location. The sample/sequence name (acronym) was formed by the state origin (2 letters) combined with the county initials (3 letters) and a serial number. Plants were considered hosts when it was observed normal shape and color eggs, healthy nymphs and adults on its leaves. For each state of Brazil, at least one representative sample was assessed with a distinct geographic location. At the laboratory, all specimens were visualized under the microscope and morphologically separated by the genus between *Bemisia* and Non-*Bemisia*. If positive for *Bemisia* sp. a minimum of three individuals, adults, females, per sample, were separated in a microtube and labeled. Before DNA extraction, all specimens were transferred to a quarter of No.1 Whatman™ filter paper to remove excess alcohol. Representative control samples of *B. tabaci*, A, and B haplotypes, collected from laboratory colonies in Arizona - USA, were included in each reaction as identification control. All the primers used, in this study, to segregate haplotypes, produced distinct and homogenous patterns between them. The patterns correspond with the standards haplotypes provided by The University of Arizona – US. All the materials used in this study were autoclaved follow by ultraviolet sterilization. All the samples were mapped with GPS coordinates and plotted via ArcGis™ v.10.3 (Environmental Systems Research Institute, Esri, 2014).

Molecular identification

In Brazil, the samples were analyzed at Embrapa Brazil. The DNA from four, single, *B. tabaci* adult females were extracted from each sample. Insects were individually macerated, with a glass pestle, in a 1.5 ml clear microtube Axygen™ containing 60 ml extraction buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA; 0.3% Triton X-100 and 60 µl/mL^{-1} Proteinase K). The homogenate was incubated for 15 minutes at 65°C and then at 95°C for 7 minutes, to stop Proteinase K activity, following the protocol of Silva (2006) modified, stored at -20°C. Amplification based on partial mitochondrial cytochrome oxidase I (mtCOI) sequence was performed in a 10 µl final volume containing: 1 µl of *B. tabaci* DNA; 5.0 µl of Master Mix, 0.6 µl of each Primer 5′-2195F-3′ [TTGATTTTTTGGTCATCCAGAAGT] and 5′-3014R-3′ [TCCAATGCACTAATCTGCCATATTA] and 2.8 µl of Mili Q water (Simon et al., 1994; Frohlich et al., 1999). Polymerase chain reaction (PCR) consisted of: initial denaturation of 95°C for 15 min.; 40 cycles of 30 seconds at 94°C; 90 seconds at 48°C; and 90 seconds at

72°C; and a final extension of 72°C for 5 min. Samples were screened using *Thermus aquaticus* I (Taq I) restriction enzyme to aggregate them into haplotypes. Five µl of each amplification product was cut with Taq I whose restriction site was 5'...T^CG A...3', and buffer supplied by the manufacturer (Promega™) at 65°C for 2 hours. Digestion product was visualized with a 1.5% agarose gel electrophoresis and stained with Ethidium Bromide, using LowRanger 100 bp as a molecular weight marker (Ludwig Biotec™) and run (80 Volts, 180 min). The size and pattern of homogeneous bands, obtained after cutting the fragments, were compared with the characteristics of each haplotype. For the B-haplotype, the fragment was cut in two positions yielding three fragments (~414, 162, 144 bp), for the Q haplotype, the fragment was cut in one position generating two fragments (~633, 144 bp) (Bosco et al., 2006). For the A1-haplotype, the fragment was not cut (~880 bp) and the A2-haplotype cut in one position resulting in two fragments (~414, 466) (Marubayashi et al., 2013).

In the USA, samples sent to The University of Arizona, the DNA was purified by nucleic acids extraction protocol from Zhang et al. (1998) modified. DNA was extracted from three *B. tabaci* adult females, individually. Each insect, individually, was placed with the Diamond™ flat wood toothpick inside the 1.5 ml clear safe-lock microtube Eppendorf™ containing disruption beads, Zirconium, 2 mm, RPI™. Each whitefly was grounded by bead beating homogenizer BioSpec™, 2 min at 2100 rpm, in 600 µl of CTAB lysis buffer. The lysis buffer contained 100 mM Tris-HCl pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl containing 0.2% 2-mercaptoethanol; 2% hexadecyltrimethyl-ammonium bromide (CTAB) (Sigma-Aldrich, Darmstadt, Germany), follow by incubation for 10 min at 65°C mixing, 4 times. Afterward, 600 µl of cold chloroform, isoamyl-alcohol (24:1), was added, the contents were mixed by inversion producing an emulsion that was separated by centrifugation at 12.000 rpm, 4°C for 3 min. The supernatant, 400 µl, was transferred to a 1.7 ml clear microtube Axygen™ with an added volume of 500 µl of cold isopropanol and 2 µl of glycogen, to improve DNA precipitation, per sample. Overnight incubation, -20°C, for yield results, proceeded by centrifugation, 10 min at 12.000 rpm, at 4°C. The pellet was washed by adding 500 µl of cold 70% ethanol and centrifuge again at 12.000 rpm, 4°C, 1 min, to bring down the remaining ethanol, subtracting the remaining ethanol with a pipette, always keeping the samples on ice, to increase the pellet visibility. The pellet was collected, air-dried for 15 min, and re-suspended in 30 µl of Nuclease-free water, shake, and store at -20°C. To segregate invasive *B. tabaci* from native haplotypes all the samples

were screened for B haplotype (North Africa – Mediterranean - Middle East clade - NAF-MED-ME) with mtCOI B-specific primers, 5′-BtBF1F-3′ [TATTTCACTTCAGCCACTATAA] and 5′-WfBr2R-3′ [GCTTAAATCTTACTAACCGCAG], ~550 bp expected amplicon size (Andreason et al., 2017). To amplify the fragment samples were subjected to PCR with a cycle of 95°C for 3 min, followed by 35 cycles of 94°C for 15 sec, 57°C for 30 sec, 72°C for 45 sec, with a final extension at 72°C for 3 min. PCR reactions contained (1X): 5.0 µl Jumpstart REDTaq Ready-Mix^{TM} (Sigma-Aldrich, Saint Louis, MI), 0.25 µM primers, 2.5 µl Nuclease-free water, and 2.0 µl DNA, to a final vol of 10.0 µl. Random samples were screen for Q haplotype, the primer consisted of 5′-NAF-MED-ME F1-3′ [GTGACCCTATTTTATATCAGCAT] and 5′-NAF-MED-ME R1-3′ [TCTCACATTTCTTCACATTCT], with an expected size of ~1000 bp. To amplify the fragment, tested with NAF-MED-ME, samples were subjected to PCR with a cycle of 95°C for 3 min, followed by 30 cycles of 94°C for 15 sec, 48°C for 30 sec, 72°C for 45 sec, with a final extension at 72°C for 3 min. PCR reactions contained (1X): 5.0 µl Jumpstart REDTaq Ready-Mix^{TM}, 0.25 µM primers, 2.5 µl Nuclease-free water, and 2.0 µl DNA, to a final vol of 10.0 µl. Samples that did not amplify with the B and Q primers were screened with an American Tropics (North and Central-Caribbean, AM-TROP) mitochondrial primer aiming to detect the American native haplotypes. AM-TROP consisted of 5′-AM-TROPF-3′ [GGGACCCTATCTTATATCAGCAC] and 5′-AM-TROPR-3′ [TTTGGTAGGCTGGGCATAA], ~1000 bp expected size, (Moya et al., 2019). None of the mitochondrial primers tested amplified the American native whiteflies. So, to sequence, the natives (Non-B and Non-Q haplotypes) samples were screened with a nuclear DNA (nDNA) primer (L11). Brazilian states that native´s haplotypes were not detected, all the samples, were also added in the screening. Non-native samples were also amplified with nuclear primers to build the phylogeny of *B. tabaci* in Brazil. The selected nuclear primers were 5´-L11-190F-3´ [CACCCACTTCATCCTCACCC] and 5´-L11-962R-3´ [TCACCCCAGGCATCATAAGC], with an expected size of ~773 bp. PCR consisted of: initial denaturation of 95°C for 1 min.; 30 cycles of 95°C for 15 sec; 55°C for 15 sec; 72°C for 1 min; and a final extension at 72°C for 2 min. Reactions contained (1X): 5.0 µl Jumpstart REDTaq Ready-Mix^{TM}, 0.5 µM primers, 1.5 ddH2O and 2.5 µl DNA [20 ng], to a final vol of 10 µl, and load 2.5 of each PCR product on a 1% agarose gel (100 Volts, 60 min). The amplified fragments on L11 samples were ligated containing (1X) 0.5

µl pGEM-T Easy plasmid vector (Promega™ Corp., Madison, WI), 2.5 µl T4 ligase buffer (Promega™ Corp., Madison, WI), 2 µl insert-sample (PCR product), and 0.5 µl T4 ligase (Promega™ Corp., Madison, WI), in a final volume of 5,5 µl. After ligation, the samples were transformed and planted on media. To transform each separate sample was thaw on a tube with DH5α competent cell, labeled and always kept on ice. Five microliters of ligation product added to the competent cells, mixed, and incubated on ice for 20 minutes. The cells were heat-shocked in a 42°C water bath for 1 min, placed on ice, and incubate for 10 min. SOC media, 300 µl, were added to each sample, and incubate for at least 1 hour at 37°C on 300 rpm agitation. After incubation, 150 µl of each sample was planted on each labeled plate and a final overnight incubation at 37°C. Each media plate used to incubate the samples were composed of 12.5g of Luria Broth (Tryptone 10g/l; Sodium chloride 10g/l; Yeast Extract 5g/l, RPI™), 7.5g of Bacto agar (BD™), and 500mL of deionized water. After incubation the white cells were diluted on nuclease-free water (1:10), and confirmed with universal M13 primers 5´-M13F-3´ [TGTAAAACGACGGCCAGT] and 5´-M13R-3´ [CAGGAAACAGCTATGAC], expected size of ~973 bp (Alon et al., 2008). PCR consisted of 94°C for 10 min.; 35 cycles of 94°C for 1 min; 53°C for 1 min; 72°C for 3:50 min; and a final extension at 72°C for 10 min. Reactions contained (1X): 12.5 µl Jumpstart REDTaq Ready-Mix™, 0.5 µM M13 primers, 9.0 Nuclease-free water, and 2.5 µl DNA [20 ng], to a final vol of 25.0 µl, load 2.5 of each PCR product on a 1% agarose gel (100 Volts, 60 min).

Sequencing

Samples identified as Non-B and Non-Q haplotype, with nDNA, were sent to be sequenced. States without a native haplotype sample were also included as a state representative. For each sample, two clones per sample, amplicons were sequenced bi-directionally by capillary (Sanger DNA sequencing) at UAGC (The University of Arizona, Tucson, AZ, USA), in 2019. Posteriorly, manually assembled and edited using SeqMan Pro™ software available in DNASTAR Lasergene v8.0 (DNASTAR, Inc., Madison, WI), exported as FASTA files, aligned using MUSCLE™ v3.8.31 (Edgar, 2004), trimmed and genetic analyze with MEGA™ v.7 software (Pennsylvania State University, PA, USA). Short sequences were deleted and the alignment was trimmed to a length of ~753 bp. Moreover, the model of evolution was estimated based on a consensus of the Bayesian information criterion (BIC), Akaike information criterion (AIC), and the Maximum

Likelihood value (InL) using MEGA^{TM} v.7 (Kumar et al., 2016). The Kimura 2-parameter model (Kimura, 1980) was identified as the best fit model for all the phylogenetic trees built. All the phylogenetic trees were constructed with one clone per representative sample with one thousand bootstrap iterations (Tamura et al., 2013). The reference´s sequences used to reconstruct the phylogenetic tree were aligned with Dr. J. K. Brown´s laboratory reference for COI, and nuclear database (J. K. Brown, unpublished data). Samples from China (CHN4) and Ethiopia (ETH2) were added as references of B1 and B2 haplotypes, respectively, one from Burkina Faso (BF13) to aggregate samples related to Q haplotype, one from Argentina (ARG2) as a representative of A-haplotypes and *B. tabaci* sequence from Uganda (UG232) used as an outgroup member.

3.3 RESULTS

A total of 204 Brazilian whitefly samples, from non-repeatable sampling sites, different cities, in all 27 Brazilian states, were accessed using mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) and nuclear DNA (nDNA) (L11) gene markers (Table 3.1.). Haplotypes were classified as proposed by Brown (2010). In this light, mainly three *B. tabaci* haplotypes were identified (A, B, and Q). The American native haplotypes were identified and separated as A-haplotype, identified with a nuclear gene; and A1 and A2 haplotypes by the restriction enzyme protocol (mtCOI).

From all analyzed samples (n=229), the American native, comprised 11.35% (n=26) [34.61% A-haplotype (n=9) (nDNA); 46.15% A1-haplotype (n=12) (mtDNA); 19.24% A2-haplotype (n=5) (mtDNA)], within the American Tropics clade (North and Central-Caribbean, AM-TROP); and two invasives: Q (n=11), 4.81%; and B (n=192), 83.84%), within North Africa-Mediterranean-Middle East clade- NAF-MED-ME. B haplotype propagated across Brazil, predominantly, with the largest host plant resource.

Table 3.1. Number of samples, geographic location (region, state, and city), coordinates, code, haplotype, n. of analyzed individuals (AN), collection date, host plant, and primer of *Bemisia tabaci* samples from field collections of Brazil (2014-2019).

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
	NORTH								
	ACRE (AC)								
1	Cruzeiro do Sul	-7.7225	-72.72527778	ACCRU2	B	2	04/feb/2018	*Manihot esculenta*	UNI
2	Rio Branco	-10.01308333	-67.42194444	ACRIO1	B	4	27/nov/2017	*Lycopersicon esculentum*	UNI
3	Rio Branco	-10.01308333	-67.42194444	ACRIO1	B	3	27/nov/2017	*Lycopersicon esculentum*	Bt
4	Rio Branco	-10.01308333	-67.42194444	ACRIO1	B	1	27/nov/2017	*Lycopersicon esculentum*	L11
5	Rio Branco	-10.01308333	-67.42194444	ACRIO2	B	5	07/nov/2017	*Nicotiana tabacum*	UNI
	AMAPÁ (AP)								
6	Calçoene	2.494583	-50.948194	APCAL1	B	3	06/jun/2018	*Brassica oleracea* var. *botrytis*	Bt
7	Itaubal do Piririm	00°25´061´´N	50°50´039´´W	APITA1	B	4	11/jul/2017	*Glycine max* var. Paragominas	UNI
8	Itaubal do Piririm	00°25´061´´N	50°50´039´´W	APITA2	B	3	11/jul/2017	*Glycine max* var. Tracajá	Bt
9	Itaubal do Piririm	00°25´061´´N	50°50´039´´W	APITA2	B	1	11/jul/2017	*Glycine max* var. Tracajá	L11
10	Macapá	0.052444444	-51.05244444	APMAC1	B	5	26/feb/2018	*Citrus* sp.	UNI
11	Mazagão	-0.178194444	-51.73558333	APMAZ1	A1	1	21/feb/2018	*Psidium guajava*	UNI
12	Mazagão	-0.178194444	-51.73558333	APMAZ1	B	2	21/feb/2018	*Psidium guajava*	UNI
13	Oiapoque	3.811461	-51.864006	APOIA1	B	1	30/apr/2018	*Brassica oleracea* var. *botrytis*	Bt
14	Oiapoque	3.811472	-51.864000	APOIA4	B	2	30/apr/2018	*Brassica oleracea* var. *botrytis*	Bt
15	Oiapoque	3.822472	-51.805861	APOIA5	B	3	18/apr/2018	*Lactuca sativa*	Bt
16	Oiapoque	3.811472	-51.864	APOIA6	B	1	30/apr/2018	*Brassica oleracea* var. *botrytis*	UNI
17	Oiapoque	3.811472	-51.864	APOIA6	B	4	30/apr/2018	*Brassica oleracea* var. *botrytis*	Bt
18	Oiapoque	3.811472	-51.864	APOIA6	B	1	30/apr/2018	*Brassica oleracea* var. *botrytis*	L11
19	Santana	-0.152	-51.57447222	APSAN1	B	3	03/mar/2018	*Brassica oleracea*	UNI
	AMAZONAS (AM)								
20	Careiro da Várzea	-3.116861111	-59.85811111	AMCAR1	B	5	05/apr/2018	*Brassica oleracea*	UNI
21	Careiro da Várzea	-3.116861111	-59.85811111	AMCAR1	B	3	05/apr/2018	*Brassica oleracea*	Bt
22	Careiro da Várzea	-3.116861111	-59.85811111	AMCAR1	B	1	05/apr/2018	*Brassica oleracea*	L11

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
23	Itacoatiara	0.433611111	-50.84416667	AMITA1	B	2	06/mar/2018	*Brassica oleracea* var. *italica*	Bt
24	Iranduba	-3.146444444	-60.332	AMIRA1	B	6	27/nov/2017	*Solanum melongena*	UNI
25	Iranduba	-3.146444444	-60.332	AMIRA1	B	2	27/nov/2017	*Solanum melongena*	Bt
26	Iranduba	-3.146444444	-60.332	AMIRA1	B	1	27/nov/2017	*Solanum melongena*	L11
27	Manaus	-3.101972222	-59.97586111	AMMAN1	B	6	24/nov/2017	*Brassica oleracea*	UNI
28	Manaus	-3.101972	-59.975833	AMMAN2	B	3	09/apr/2018	*Brassica oleracea*	Bt
29	Manaus	-3.101972	-59.975833	AMMAN2	B	1	09/apr/2018	*Brassica oleracea*	L11
	PARÁ (PA)								
30	Marabá	-12.19805556	-51.04138889	PAMAR1	B	5	13/may/2018	*Abelmoschus esculentus*	UNI
31	Marabá	-12.19805556	-51.04138889	PAMAR1	B	2	13/may/2018	*Abelmoschus esculentus*	Bt
32	Marabá	-12.19805556	-51.04138889	PAMAR1	B	1	13/may/2018	*Abelmoschus esculentus*	L11
33	Marabá	-12.46416667	-50.37388889	PAMAR2	B	5	13/may/2018	*Lactuca sativa*	UNI
34	Paragominas	-3.658333333	-47.68666667	PAPAR1	B	5	20/apr/2018	*Glycine max*	UNI
35	Paragominas	-3.658333333	-47.68666667	PAPAR1	B	1	20/apr/2018	*Glycine max*	Bt
36	Paragominas	-3.658333333	-47.68666667	PAPAR1	B	1	20/apr/2018	*Glycine max*	L11
	RONDÔNIA (RO)								
37	Buritis	-10.26277778	-63.74429167	ROBUR1	B	2	01/oct/2017	*Brassica oleracea*	UNI
38	Buritis	-10.26277778	-63.74429167	ROBUR1	B	3	01/oct/2017	*Brassica oleracea*	Bt
39	Rolim de Moura	-11.582500	-61.772500	ROROL1	B	3	15/sep/2017	*Brassica oleracea* var. *italica*	Bt
40	Seringueiras	-11.72138889	-63.13416667	ROSER1	B	5	28/sep/2017	*Brassica oleracea*	UNI
41	Seringueiras	-11.72138889	-63.13416667	ROSER1	B	3	28/sep/2017	*Brassica oleracea*	Bt
	RORAIMA (RR)								
42	Boa Vista	2°42'30.0"N	47°38'54.0"W	RRBOA1	A	1	21/may/2014	*Acacia mangium*	L11
43	Boa Vista	2.756333333	-60.73186111	RRBOA2	B	1	11/sep/2014	*Glycine max*	UNI
44	Boa Vista	2°42'30.0"N	47°38'54.0"W	RRBOA3	B	1	08/apr/2014	*Musa* spp.	L11
	TOCANTINS (TO)								
45	Formoso do Araguaia	-11.975556	-49.606667	TOFOR1	B	2	18/feb/2017	*Glycine max*	Bt
46	Formoso do Araguaia	-11.975556	-49.606667	TOFOR1	A	1	18/feb/2017	*Glycine max*	L11

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
47	Formoso do Araguaia	-11.97359722	-49.89663333	TOFOR3	B	4	01/may/2017	*Brassica oleracea* var. *botrytis*	UNI
48	Lagoa da Confusão	-10.86986111	-49.60394444	TOLAG1	B	4	01/jul/2017	*Glycine max*	UNI
49	Lagoa da Confusão	-10.86986111	-49.60394444	TOLAG1	B	2	01/jul/2017	*Glycine max*	BQ
50	Lagoa da Confusão	-10.86986111	-49.60394444	TOLAG1	B	1	01/jul/2017	*Glycine max*	L11
51	Lagoa da Confusão	-10.80416667	-49.63672222	TOLAG2	A1	3	01/jul/2017	*Manihot esculenta*	UNI
52	Pedro Afonso	-9.293333	-48.015000	TOPED2	B	3	23/jan/2019	*Solanum gilo*	Bt
53	Pedro Afonso	-9.158055	-48.186111	TOPED1	B	1	23/jan/2019	*Glycine max*	BQ
54	Pedro Afonso	-9.293333	-48.015000	TOPED2	B	1	23/jan/2019	*Solanum gilo*	L11
55	Pedro Afonso	-9.293333	-48.015000	TOPED3	B	3	23/jan/2019	*Brassica oleracea*	Bt
56	Pedro Afonso	-9.306944	-48.007222	TOPED4	B	3	23/jan/2019	*Glycine max*	Bt
57	Pedro Afonso	-9.306944	-48.007222	TOPED4	B	3	23/jan/2019	*Glycine max*	BQ
58	Pedro Afonso	-9.308611	-48.016944	TOPED5	B	3	23/jan/2019	*Glycine max*	Bt
59	Pedro Afonso	-9.308611	-48.016944	TOPED5	B	2	23/jan/2019	*Glycine max*	BQ
60	Pedro Afonso	-8.979167	-48.165833	TOPED6	B	3	24/jan/2019	*Cucurbita* sp.	Bt
	NORTHEAST								
	ALAGOAS (AL)								
61	Arapiraca	-9.751666667	-36.66027778	ALARA1	B	5	11/apr/2018	*Brassica oleracea*	UNI
61	Arapiraca	-9.751666667	-36.66027778	ALARA1	B	1	11/apr/2018	*Brassica oleracea*	Bt
63	Arapiraca	-9.751666667	-36.66027778	ALARA1	B	1	11/apr/2018	*Brassica oleracea*	L11
63	Limoeiro de Anadia	-9.738055556	-36.50305556	ALLIM1	B	5	11/apr/2018	*Brassica oleracea*	UNI
64	Limoeiro de Anadia	-9.738055556	-36.50305556	ALLIM1	B	2	11/apr/2018	*Brassica oleracea*	Bt
65	Limoeiro de Anadia	-9.738055556	-36.50305556	ALLIM1	Q	1	11/apr/2018	*Brassica oleracea*	L11
66	Rio Largo	-9.45	-35.45	ALRIO1	B	5	01/mar/2018	*Brassica oleracea*	UNI
67	Rio Largo	-9.45	-35.45	ALRIO1	B	3	01/mar/2018	*Brassica oleracea*	Bt
	BAHIA (BA)								
68	Barreiras	-11.83805556	-45.79205556	BABAR1	B	5	07/mar/2018	*Glycine max*	UNI
69	Barreiras	-11.83805556	-45.79205556	BABAR1	B	1	07/mar/2018	*Glycine max*	Bt
70	Barreiras	-11.83805556	-45.79194444	BABAR2	B	3	08/mar/2019	*Glycine max*	Bt

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
71	Barreiras	-11.83805556	-45.79194444	BABAR2	B	1	08/mar/2019	*Glycine max*	L11
72	Juazeiro	-9.575055556	-40.60736111	BAJUA1	A1	2	28/mar/2017	*Lycopersicon esculentum*	UNI
73	Juazeiro	-9.575055556	-40.60736111	BAJUA1	B	10	28/mar/2017	*Lycopersicon esculentum*	UNI
74	Juazeiro	-9.575055556	-40.60736111	BAJUA1	B	3	28/mar/2017	*Lycopersicon esculentum*	Bt
75	Juazeiro	-9.575055556	-40.60736111	BAJUA1	B	2	28/mar/2017	*Lycopersicon esculentum*	BQ
76	Juazeiro	-9.575055556	-40.60736111	BAJUA1	B	1	28/mar/2017	*Lycopersicon esculentum*	L11
77	Luiz Eduardo Magalhães	-12.09783333	-45.70491667	BALEM6	B	4	03/mar/2017	*Glycine max*	UNI
78	Luiz Eduardo Magalhães	-12.09786111	-45.70477778	BALEM7	B	4	03/mar/2017	*Phaseolus vulgaris*	UNI
79	Luiz Eduardo Magalhães	-12.09788889	-45.70494444	BALEM8	B	4	03/mar/2017	*Gossypium hirsutum*	UNI
80	Luiz Eduardo Magalhães	-12.09788889	-45.70494444	BALEM8	B	3	03/mar/2017	*Gossypium hirsutum*	Bt
81	Luiz Eduardo Magalhães	-12.09791667	-45.70488889	BALEM9	B	2	03/mar/2017	*Cucumis melo*	UNI
82	Luiz Eduardo Magalhães	-12.09780556	-45.70494444	BALEM10	B	4	03/mar/2017	*Citrullus lanatus*	UNI
83	Luiz Eduardo Magalhães	-12.12938889	-45.91811111	BALEM11	B	5	19/mar/2018	*Gossypium hirsutum*	UNI
84	Luiz Eduardo Magalhães	-12.67586111	-45.96741667	BALEM12	B	5	21/mar/2018	*Gossypium hirsutum*	UNI
85	Luiz Eduardo Magalhães	-12.09775	-45.70783333	BALEM13	B	5	15/mar/2018	*Gossypium hirsutum*	UNI
86	Luiz Eduardo Magalhães	-12.05511111	-45.42277778	BALEM14	B	5	23/mar/2018	*Phaseolus vulgaris*	UNI
87	Luiz Eduardo Magalhães	-12.05511111	-45.42277778	BALEM14	B	2	23/mar/2018	*Phaseolus vulgaris*	Bt
88	Luiz Eduardo Magalhães	-12.05511111	-45.42277778	BALEM14	B	1	23/mar/2018	*Phaseolus vulgaris*	L11
89	Luiz Eduardo Magalhães	-12.05580556	-45.42252778	BALEM15	B	5	23/mar/2018	*Cucumis melo*	UNI
90	Luiz Eduardo Magalhães	-12.09844444	-45.70852778	BALEM16	B	5	16/mar/2018	*Glycine max*	UNI
91	Luiz Eduardo Magalhães	-11.83783333	-45.79216667	BALEM17	B	3	23/mar/2018	*Lycopersicon esculentum*	UNI
92	Luis Eduardo Magalhães	-12.098992	-45.705969	BALEM18	B	2	08/mar/2019	*Cucumis melo*	Bt
93	Rosário	-13.94777778	-46.23583333	BAROS1	A1	2	10/jan/2017	*Glycine max*	UNI
94	Rosário	-13.94777778	-46.23583333	BAROS1	B	10	10/jan/2017	*Glycine max*	UNI
95	Rosário	-13.94777778	-46.23583333	BAROS1	B	1	10/jan/2017	*Glycine max*	L11
96	Rosário	-13.953723	-46.237579	BAROS2	B	1	01/feb/2018	*Gossypium* sp.	L11
97	São Desidério	-13.03972222	-46.26083333	BASAD1	A1	4	12/jan/2017	*Glycine max*	UNI
98	São Desidério	-13.03972222	-46.26083333	BASAD1	B	4	12/jan/2017	*Glycine max*	Bt

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99	São Desidério	-13.03972222	-46.26083333	BASAD1	B	4	12/jan/2017	*Glycine max*	BQ
100	São Desidério	-13.03972222	-46.26083333	BASAD1	B	1	12/jan/2017	*Glycine max*	L11
101	São Desidério	-12.822375	-46.13837	BASAD3	B	2	09/apr/2019	*Gossypium* sp.	Bt
	CEARÁ (CE)								
102	Fortaleza	-3.717222222	-38.54305556	CEFOR1	B	5	08/jun/2015	*Brassica oleracea*	UNI
103	Fortaleza	-3.725783	-38.499339	CEFOR2	B	2	10/apr/2017	*Calopogonium mucunoides*	Bt
104	Icapui	-4.86775	-37.33838889	CEICA1	B	5	01/feb/2018	*Cucumis melo*	UNI
105	Mulungu	-4.285833333	-38.98527778	CEMUL1	B	4	20/mar/2017	*Brassica oleracea* var. *italica*	UNI
106	Quixeré	-5.185083333	-37.92288889	CEQUI1	B	5	01/feb/2018	*Cucumis melo*	UNI
	MARANHÃO (MA)								
107	Balsas	-7.533056	-46.035000	MABAL1	B	3	12/apr/2017	*Vigna unguiculata*	Bt
108	Balsas	-7.814027778	-46.01944444	MABAL2	A2	4	12/apr/2017	*Vigna unguiculata*	UNI
109	Balsas	-7.814027778	-46.01944444	MABAL2	B	2	12/apr/2017	*Vigna unguiculata*	Bt
110	Balsas	-7.814027778	-46.01944444	MABAL2	B	1	12/apr/2017	*Vigna unguiculata*	L11
111	Sambaíba	-7.655972	-45.746.944	MASAL1	B	3	30/jan/2017	*Glycine max*	Bt
112	Sambaíba	-7.619722222	-45.76166667	MASAL2	B	4	30/jan/2017	*Glycine max*	UNI
113	Sambaíba	-7.6075	-45.76416667	MASAL3	A1	3	30/jan/2017	*Zea mays*	UNI
114	Sambaíba	-7.6075	-45.76416667	MASAL3	B	9	30/jan/2017	*Zea mays*	UNI
115	Sambaíba	-7.6075	-45.76416667	MASAL3	B	1	30/jan/2017	*Zea mays*	Bt
116	Sambaíba	-7.6075	-45.76416667	MASAL3	B	1	30/jan/2017	*Zea mays*	L11
117	São Domingos do Azeitão	-6.840833333	-44.50361111	MASAO1	A1	4	03/feb/2017	*Glycine max*	UNI
118	São Domingos do Azeitão	-6.840833333	-44.50361111	MASAO1	B	3	03/feb/2017	*Glycine max*	Bt
119	São Domingos do Azeitão	-6.840833333	-44.50361111	MASAO1	Q	4	03/feb/2017	*Glycine max*	BQ
120	São Domingos do Azeitão	-6.840833333	-44.50361111	MASAO1	Q	1	03/feb/2017	*Glycine max*	L11
121	Tasso Fragoso	-8.468055556	-45.75722222	MATAS1	B	5	12/apr/2017	*Glycine max*	UNI
122	Tasso Fragoso	-8.468055556	-45.75722222	MATAS1	B	3	12/apr/2017	*Glycine max*	Bt
	PARAÍBA (PB)								
123	Alagoa Nova	-7.052388889	-35.74819444	PBALA1	B	4	22/mar/2017	*Brassica oleracea* var. *itálica*	UNI

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
124	Areia	-7.04925	-35.75941667	PBARE1	B	5	22/mar/2017	*Brassica oleracea*	UNI
125	Campina Grande	-7.226166667	-35.90630556	PBCAM1	B	5	22/mar/2017	*Gossypium hirsutum*	UNI
126	Itabaiana	-7.337277778	-35.35408333	PBITA1	B	5	29/mar/2017	*Abelmoschus esculentus*	UNI
127	Itabaiana	-7.305028	-35.362889	PBITA2	B	1	03/apr/2017	*Cucurbita* sp.	Bt
128	Lagoa Seca	-7.135278	-35.841944	PBLAG1	B	2	22/mar/2017	*Brassica oleracea*	Bt
129	Lagoa Seca	-7.135277778	-35.84194444	PBLAG2	B	5	22/mar/2017	*Brassica oleracea*	UNI
130	Mogeiro	-7.277583333	-35.52622222	PBMOG1	B	4	29/mar/2017	*Phaseolus lunatus*	UNI
	PERNAMBUCO (PE)								
131	Oricuri	-7.881278	-40.101500	PEORI1	B	3	16/feb/2018	*Lactuca sativa*	Bt
132	Bebedouro	-9.134694444	-40.30761111	PEPET2	B	4	20/feb/2017	*Cucumis anguria*	UNI
133	Bebedouro	-9.134694444	-40.30761111	PEPET3	B	1	28/mar/2017	*Cucurbita* sp.	UNI
134	Petrolina	-9.070583333	-40.31822222	PEPET4	B	5	27/feb/2018	*Brassica oleracea*	UNI
135	Recife	-8.016111	-34.944611	PEREC1	B	3	02/feb/2018	*Brassica oleracea*	Bt
	PIAUI (PI)								
136	Baixa Grande do Ribeiro	-7.850417	-45.213611	PIBAI1	B	3	11/apr/2017	Weed	Bt
138	Baixa Grande do Ribeiro	-7.850417	-45.213611	PIBAI2	A	1	11/apr/2017	Weed	L11
139	Baixa Grande do Ribeiro	-7.850416667	-45.21361111	PIBAI3	A2	4	11/apr/2017	*Glycine max*	UNI
140	Baixa Grande do Ribeiro	-7.850416667	-45.21361111	PIBAI3	B	3	11/apr/2017	*Glycine max*	Bt
141	Bom Jesus	-9.252777778	-44.84722222	PIBOM1	B	4	02/feb/2017	*Glycine max*	UNI
142	Bom Jesus	-9.252777778	-44.84722222	PIBOM1	B	3	02/feb/2017	*Glycine max*	Bt
143	Currais	-9.025250	-44.389222	PICUR2	B	4	8/nov/2018	*Lycopersicon* sp.	Bt
144	Currais	-9.025250	-44.389222	PICUR2	B	1	8/nov/2018	*Lycopersicon* sp.	BQ
145	Currais	-9.025250	-44.389222	PICUR2	B	1	8/nov/2018	*Lycopersicon* sp.	L11
146	Monte Alegre do Piaui	-9.407777778	-45.12133333	PIMON1	B	4	31/jan/2017	*Glycine max*	UNI
147	Monte Alegre do Piaui	-9.407777778	-45.12133333	PIMON1	B	3	31/jan/2017	*Glycine max*	Bt
148	Ribeiro Gonçalves	-7.558333333	-45.24222222	PIRIB1	B	4	11/apr/2017	*Cucurbita* sp.	UNI
149	Uruçui	-7.233888889	-44.55388889	PIURU1	B	4	10/apr/2017	*Glycine max*	UNI
	RIO GRANDE DO NORTE								

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
	(RN)								
150	Baraúna	-4.919416667	-37.50741667	RNBAR1	B	5	01/feb/2018	*Cucumis melo*	UNI
151	Baraúna	-5.075	-37.63166667	RNBAR2	B	5	01/mar/2018	*Cucurbita* sp.	UNI
152	Baraúna	-5.075	-37.63166667	RNBAR2	B	3	01/mar/2018	*Cucurbita* sp.	Bt
153	Baraúna	-5.075000	-37.631667	RNBAR3	B	3	01/mar/2018	*Anacardium occidentale*	Bt
154	Mossoró	-4.97985	-37.43285278	RNMOS1	B	5	01/feb/2018	*Cucumis melo*	UNI
155	Mossoró	-4.9075	-37.40138889	RNMOS2	B	5	01/mar/2018	*Cucumis* sp.	UNI
	SERGIPE (SE)								
156	Itabaiana	-10.685000	-37.425282	SEITA1	B	4	01/nov/2018	*Brassica oleracea* var. *italica*	Bt
157	Itabaiana	-10.685000	-37.425282	SEITA1	B	1	01/nov/2018	*Brassica oleracea* var. *italica*	BQ
	MIDWEST								
	DISTRITO FEDERAL (DF)								
158	Brasília	-15.78	-47.92916667	DFBRA1	B	4	11/mar/2015	*Phaseolus vulgaris* RMD	UNI
159	Brasília	-15.78	-47.92916667	DFBRA1	B	3	11/mar/2015	*Phaseolus vulgaris* RMD	Bt
160	Brasília	-16.011500	-47.556083	DFBRA2	B	3	03/may/2018	*Brassica oleracea* var. *botrytis*	Bt
161	Planaltina	-15.60313333	-47.71213611	DFPLA1	B	5	21/mar/2017	*Glycine max*	UNI
162	Planaltina	-15.60313333	-47.71213611	DFPLA1	B	3	21/mar/2017	*Glycine max*	Bt
	GOIÁS (GO)								
163	Anápolis	-16.49694444	-49.57277778	GOANA1	B	5	09/mar/2017	*Phaseolus vulgaris*	UNI
164	Brazabrantes	-16.42972222	-49.38694444	GOBRA1	B	5	19/mar/2014	*Phaseolus vulgaris* RMD	UNI
165	Cabeceiras	-15.603889	47.117222	GOCAB 1	B	2	18/jan/2017	*Glycine max*	Bt
166	Cabeceiras	-15.60611111	-47.13805556	GOCAB 2	B	5	18/jan/2017	*Phaseolus vulgaris*	UNI
167	Cristalina	-16.76861111	-47.61361111	GOCRI1	B	5	15/feb/2017	*Glycine max*	UNI
168	Formosa	-15.55277778	-47.19277778	GOFOR 2	B	6	15/feb/2017	*Cucurbita pepo* var. *cylindrica*	UNI
169	Formosa	-15.55277778	-47.19277778	GOFOR 2	B	1	15/feb/2017	*Cucurbita pepo* var. *cylindrica*	Bt
170	Formosa	-15.55277778	-47.19277778	GOFOR 2	B	1	15/feb/2017	*Cucurbita pepo* var. *cylindrica*	L11
171	Goiânia	-16.59888889	-49.30222222	GOGYN1	B	1	06/apr/2016	*Cucumis melo*	UNI
172	Goiânia	-16.59888889	-49.30222222	GOGYN3	B	4	14/feb/2017	*Lycopersicon esculentum*	UNI

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173	Goiânia	-16.59888889	-49.30222222	GOGYN4	B	4	14/feb/2017	*Citrullus lanatus*	UNI
174	Goiânia	-16.59888889	-49.30222222	GOGYN5	B	4	14/feb/2017	*Cucumis melo*	UNI
175	Goiânia	-16.59166667	-49.29166667	GOGYN7	B	5	30/mar/2018	*Solanum tuberosum*	UNI
176	Goiânia	-16.592434	-49.288636	GOGYN8	B	3	27/feb/2019	*Lycopersicon* sp.	Bt
177	Goiânia	-16.592434	-49.288636	GOGYN8	B	1	27/feb/2019	*Lycopersicon* sp.	L11
178	Goiânia	-16.592434	-49.288636	GOGYN9	B	2	27/feb/2019	*Solanum tuberosum*	Bt
179	Goiânia	-16.592434	-49.288636	GOGYN9	B	1	27/feb/2019	*Solanum tuberosum*	BQ
180	Goiânia	-16.592434	-49.288636	GOGYN10	B	4	27/feb/2019	*Cucumis melo*	Bt
181	Jataí	-17.87772222	-51.73358333	GOJAT1	B	4	26/feb/2017	*Cucumis melo*	UNI
182	Jataí	-17.87772222	-51.73358333	GOJAT1	B	3	26/feb/2017	*Cucumis melo*	Bt
183	Jaraguá	-15.91783333	-49.50469444	GOJAR1	B	5	11/feb/2017	*Phaseolus vulgaris*	UNI
184	Maurilandia	-17.95472222	-50.35916667	GOMAU 1	B	4	05/jan/2017	*Glycine max*	UNI
185	Pires do Rio	-17.161.778	-48.227.917	GOPIR1	B	2	23/dec/2017	*Lycopersicon esculentum*	Bt
186	Rio Verde	-17.78333333	-51.00555556	GORIV2	B	5	15/feb/2017	*Glycine max*	UNI
187	Rio Verde	-17.78333333	-51.00555556	GORIV2	B	3	15/feb/2017	*Glycine max*	Bt
188	Rio Verde	-17.9225	-51.14530556	GORIV3	B	4	24/apr/2018	*Glycine max*	UNI
189	Rio Verde	-17.4988	-50.628	GORIV5	B	1	04/apr/2019	*Gossypium* sp.	BQ
190	Rio Verde	-17.4988	-50.628	GORIV5	B	1	04/apr/2019	*Gossypium* sp.	L11
191	Santa Helena de Goiás	-17.72416667	-50.60972222	GOSAH 1	B	5	05/jan/2017	*Glycine max*	UNI
192	Santa Helena de Goiás	-17.83055556	-50.59527778	GOSAH2	B	5	14/feb/2017	*Phaseolus vulgaris*	UNI
193	Santa Helena de Goiás	-17.83055556	-50.59527778	GOSAH2	B	3	14/feb/2017	*Phaseolus vulgaris*	Bt
194	Santo Antônio de Goiás	-16.50638889	-49.30138889	GOSAN1	B	1	22/sep/2016	*Phaseolus vulgaris*	UNI
195	Santo Antônio de Goiás	-16.50638889	-49.30138889	GOSAN11	B	1	05/oct/2016	*Phaseolus vulgaris*	UNI
196	Santo Antônio de Goiás	-16.513162	-49.295390	GOSAN22	B	3	30/feb/2014	*Phaseolus vulgaris* RMD	Bt
197	Santo Antônio de Goiás	-16.50672222	-49.28394444	GOSAN23	B	4	19/apr/2017	*Glycine max*	UNI
198	Santo Antônio de Goiás	-16.49841667	-49.28016667	GOSAN24	B	12	21/feb/2017	*Phaseolus vulgaris*	UNI
199	Santo Antônio de Goiás	-16.49841667	-49.28016667	GOSAN28	B	5	08/jun/2018	*Glycine max*	UNI
200	Silvânia	-16.65888889	-48.60805556	GOSIL 1	B	5	26/jan/2017	*Glycine max*	UNI

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201	Turvelândia	-17.85277778	-50.30222222	GOTUR 1	B	4	05/jan/2017	*Glycine max*	UNI
	MATO GROSSO (MT)								
202	Campo Novo dos Parecis	-13.70616667	-57.94927778	MTCAM1	B	5	03/may/2018	*Gossypium hirsutum*	UNI
203	Campo Novo dos Parecis	-13.70616667	-57.94927778	MTCAM1	B	1	03/may/2018	*Gossypium hirsutum*	L11
204	Água Boa	-13.65611111	-52.38055556	MTCAN1	B	5	05/apr/2017	*Glycine max*	UNI
205	Água Boa	-13.65611111	-52.38055556	MTCAN1	B	1	05/apr/2017	*Glycine max*	Bt
206	Água Boa	-13.65611111	-52.38055556	MTCAN1	B	1	05/apr/2017	*Glycine max*	L11
207	Lucas do Rio Verde	-13.00425	-55.97169444	MTLUC1	B	4	28/jan/2017	*Glycine max*	UNI
208	Lucas do Rio Verde	-13.00269444	-55.97058333	MTLUC2	B	5	06/feb/2018	*Glycine max*	UNI
209	Primavera do Leste	-15.53222222	-54.40944444	MTPRI 1	B	4	05/jan/2017	*Glycine max*	UNI
210	Primavera do Leste	-15.46630278	-54.26085556	MTPRI2	B	4	03/apr/2017	*Gossypium hirsutum*	UNI
211	Primavera do Leste	-15.46130278	-54.26188889	MTPRI3	B	5	06/feb/2018	*Glycine max* M7739 IPRO	UNI
212	Primavera do Leste	-15.461067	-54.261917	MTPRI5	B	4	10/jan/2019	*Glycine max*	Bt
213	Primavera do Leste	-15.461067	-54.261917	MTPRI5	B	1	10/jan/2019	*Glycine max*	BQ
214	Rondonópolis	-16.47083333	-54.63555556	MTRON1	B	5	21/may/2015	*Gossypium hirsutum* TMG 47 B2RF	UNI
215	Rondonópolis	-16.47083333	-54.63555556	MTRON1	B	1	21/may/2015	*Gossypium hirsutum* TMG 47 B2RF	L11
216	Várzea Grande	-15.68191667	-56.10977778	MTVAR1	B	5	19/may/2017	*Brassica oleracea*	UNI
	MATO GROSSO DO SUL (MS)								
217	Chapadão do Sul	-18.779.861	-52.645.185	MSCHA1	B	1	01/jul/2016	*Glycine sp.*	UNI
218	Chapadão do Sul	-18.779.861	-52.645.185	MSCHA1	B	1	01/jul/2016	*Glycine sp.*	L11
219	Maracaju	-21.6185	-55.12022222	MSMAR1	B	4	22/feb/2017	*Glycine max*	UNI
220	Maracaju	-21.61944444	-55.12083333	MSMAR2	B	5	04/feb/2018	*Phaseolus vulgaris*	UNI
221	Maracaju	-21.61944444	-55.12083333	MSMAR2	B	1	04/feb/2018	*Phaseolus vulgaris*	Bt
222	Maracaju	-21.61944444	-55.12083333	MSMAR2	B	1	04/feb/2018	*Phaseolus vulgaris*	L11
	SOUTH EAST								
	ESPÍRITO SANTO (ES)								
223	Alegre	-20.76833333	-41.54305556	ESALE1	B	5	29/dec/2017	*Brassica oleracea*	UNI

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
224	Alegre	-20.76833333	-41.54305556	ESALE1	B	1	29/12/2017	*Brassica oleracea*	Bt
225	Castelo	-20.53163889	-41.30280556	ESCAS1	B	5	09/jan/2018	*Brassica oleracea*	UNI
226	Castelo	-20.53163889	-41.30280556	ESCAS1	B	1	09/jan/2018	*Brassica oleracea*	Bt
227	Castelo	-20.53163889	-41.30280556	ESCAS1	B	1	09/jan/2018	*Brassica oleracea*	L11
	MINAS GERAIS (MG)								
228	Arinos	-15.94444444	-46.31138889	MGARI 1	B	5	19/jan/2017	*Glycine max*	UNI
229	Arinos	-15.94444444	-46.31138889	MGARI 1	B	3	19/jan/2017	*Glycine max*	Bt
230	Uberlândia	-18.89519444	-48.29036111	MGUBE1	B	4	12/feb/2017	*Ornamental*	UNI
231	Uberlândia	-18.89519444	-48.29036111	MGUBE1	B	3	12/feb/2017	*Ornamental*	Bt
232	Uberlândia	-18.90811111	-48.29375	MGUBE2	B	4	12/feb/2017	*Brassica oleracea*	UNI
233	Uberlândia	-18.92766667	-48.17130556	MGUBE3	B	4	04/mar/2017	*Glycine max*	UNI
234	Uberlândia	-18.92633333	-48.17302778	MGUBE4	B	2	04/mar/2017	*Gossypium hirsutum*	UNI
235	Uberlândia	-18.92633333	-48.17302778	MGUBE4	B	2	04/mar/2017	*Gossypium hirsutum*	Bt
236	Uberlândia	-18.92633333	-48.17302778	MGUBE4	B	1	04/mar/2017	*Gossypium hirsutum*	L11
237	Uberlândia	-18.92775	-48.16591667	MGUBE5	B	3	04/mar/2017	*Phaseolus vulgaris*	UNI
238	Uberlândia	-18.92728611	-48.1735	MGUBE6	B	5	01/mar/2018	*Phaseolus vulgaris*	UNI
239	Uberlândia	-18.92778056	-48.1735	MGUBE7	B	5	01/mar/2018	*Solanum tuberosum*	UNI
240	Uberlândia	-18.92763889	-48.1754	MGUBE8	B	5	01/mar/2018	*Solanum tuberosum*	UNI
241	Uberlândia	18°55'63"S	48°09'69"W	MGUBE9	B	4	26/feb/2019	*Cucumis melo*	Bt
242	Uberlândia	18°55'63"S	48°09'69"W	MGUBE9	B	1	26/feb/2019	*Cucumis melo*	BQ
243	Uberlândia	-18.931667	-48.165833	MGUBE10	B	4	26/feb/2019	*Solanum tuberosum*	Bt
244	Uberlândia	-18.939444	-48.173889	MGUBE11	B	4	26/feb/2019	*Lycopersicon* sp.	Bt
245	Jaíba	-15.13333333	-43.96666667	MGJAI1	A1	4	14/jul/2017	*Manihot esculenta*	UNI
246	Viçosa	-20.75944444	-42.86916667	MGVIÇ2	A2	4	27/may/2017	*Lycopersicon esculentum*	UNI
247	Viçosa	-20.75944444	-42.86916667	MGVIÇ2	B	4	27/may/2017	*Lycopersicon esculentum*	Bt
	RIO DE JANEIRO (RJ)								
248	Campos dos Goytacazes	-21.79208333	-41.27805556	RJCAM1	B	5	14/mar/2018	*Brassica oleracea*	UNI
249	Campos dos Goytacazes	-21.79208333	-41.27805556	RJCAM2	B	4	14/mar/2018	*Abelmoschus esculentus*	UNI

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
250	Campos dos Goytacazes	-21.79208333	-41.27805556	RJCAM2	B	2	14/mar/2018	*Abelmoschus esculentus*	Bt
251	Rio de Janeiro	-23.012306	-43.322500	RJRIO1	B	1	22/aug/2018	Ornamental	Bt
252	Seropédica	-22.75580556	-43.67438889	RJSER1	B	5	06/apr/2018	*Xanthosoma sagittifolium*	UNI
	SÃO PAULO (SP)								
253	Atibaia	-23.1171	-46.5502	SPATI1	Q	1	nov/2018	*Gerbera jamesonii*	L11
254	Conchal	-22.36785556	-47.17681389	SPCON1	A1	1	06/apr/2018	*Lycopersicon esculentum*	UNI
255	Conchal	-22.36785556	-47.17681389	SPCON1	B	4	06/apr/2018	*Lycopersicon esculentum*	UNI
256	Conchal	-22.36785556	-47.17681389	SPCON1	B	2	06/apr/2018	*Lycopersicon esculentum*	Bt
257	Conchal	-22.36785556	-47.17681389	SPCON2	A1	1	06/apr/2018	*Lycopersicon esculentum*	UNI
258	Conchal	-22.36785556	-47.17681389	SPCON2	B	4	06/apr/2018	*Lycopersicon esculentum*	UNI
259	Conchal	-22.36785556	-47.17681389	SPCON2	B	3	06/apr/2018	*Lycopersicon esculentum*	Bt
260	Holambra	-22.38577	-47.04536	SPHOL1	B	1	20/feb/2019	*Solanum tuberosum*	L11
261	Iperó	-23.392056	-47.644500	SPIPE1	B	3	09/feb/2018	*Lycopersicon* sp.	Bt
262	Itaberá	-23.86194444	-49.13722222	SPITA1	B	4	01/mar/2016	*Glycine max*	UNI
263	Itaberá	-23.86194444	-49.13722222	SPITA1	B	2	01/mar/2016	*Glycine max*	Bt
264	Itaberá	-23.86194444	-49.13722222	SPITA2	B	3	07/mar/2016	*Glycine max*	UNI
265	Itaberá	-23.86194444	-49.13722222	SPITA3	B	1	23/mar/2016	*Glycine max*	UNI
266	Jaboticabal	-21.23472222	-48.28583333	SPJAB1	B	4	01/may/2017	*Brassica oleracea*	UNI
267	Jaboticabal	-21.23472222	-48.28583333	SPJAB1	B	3	01/may/2017	*Brassica oleracea*	Bt
268	Jaboticabal	-21.26772222	-48.41436111	SPJAB2	B	5	28/feb/2018	*Lycopersicon esculentum*	UNI
269	Jaboticabal	-21.26772222	-48.41436111	SPJAB2	B	3	28/feb/2018	*Lycopersicon esculentum*	Bt
270	Jaguariúna	-22.72708333	-47.017125	SPJAG1	B	5	22/jan/2018	*Lycopersicon esculentum*	UNI
271	Jaguariúna	-22.72708333	-47.017125	SPJAG1	B	3	22/jan/2018	*Lycopersicon esculentum*	Bt
272	Morro Alto	-23.48908333	-47.94177778	SPMOR1	Q	5	20/jan/2018	*Lycopersicon esculentum*	UNI
273	Morro Alto	-23.48908333	-47.94177778	SPMOR1	B	1	20/jan/2018	*Lycopersicon esculentum*	L11
274	Piracicaba	-22.70833333	-47.63333333	SPPIR1	B	5	18/apr/2017	*Brassica oleracea*	UNI
275	Piracicaba	-22.70833333	-47.63333333	SPPIR1	B	3	18/apr/2017	*Brassica oleracea*	Bt
276	São José do Rio Preto	-20.820.278	-49.379.722	SPSAO1	B	2	22/jul/2018	*Lycopersicon esculentum*	Bt

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
277	Taquarivaí	-23.94652778	-48.71613333	SPTAQ1	B	3	04/apr/2017	*Capsicum annuum*	UNI
278	Taquarivaí	-23.94652778	-48.71613333	SPTAQ1	Q	2	04/apr/2017	*Capsicum annuum*	UNI
279	Taquarivaí	-23.94652778	-48.71613333	SPTAQ1	B	1	04/apr/2017	*Capsicum annuum*	Bt
280	Taquarivaí	-23.94652778	-48.71613333	SPTAQ1	B	1	04/apr/2017	*Capsicum annuum*	L11
	SOUTH								
	PARANÁ (PR)								
281	Cascavel	-24.989583	-53.510639	PRCAS18	B	2	18/jan/2017	*Phaseolus vulgaris*	Bt
282	Cascavel	-24.989583	-53.510639	PRCAS18	A	1	18/jan/2017	*Phaseolus vulgaris*	L11
283	Cascavel	-24.95555556	-53.45527778	PRCAS2	B	1	25/may/2016	*Glycine max*	UNI
284	Cascavel	-24.95555556	-53.45527778	PRCAS3	B	5	25/may/2016	*Phaseolus vulgaris*	UNI
285	Céu Azul	-25.14802778	-53.84144444	PRCEU19	B	5	18/jan/2017	*Glycine max*	UNI
286	Céu Azul	-25.14802778	-53.84144444	PRCEU19	B	3	18/jan/2017	*Glycine max*	Bt
287	Coronel Vivida	-25.94280556	-52.70011944	PRCOR1	B	5	02/mar/2017	*Glycine max*	UNI
288	Faxinal	-23.95022222	-51.37588889	PRFAX1	B	2	17/jun/2017	*Lycopersicon* sp.	UNI
289	Faxinal	-23.95022222	-51.37588889	PRFAX1	B	1	17/jun/2017	*Lycopersicon* sp.	L11
290	Guarapuava	-25.393.33\3	-51.488.611	PRGUA1	B	1	15/may/2017	*Solanum lycopersicum*	L11
291	Londrina	-23.19134444	-51.18376667	PRLON1	Q	4	25/may/2017	*Glycine max*	UNI
292	Londrina	-23.19134444	-51.18376667	PRLON1	B	3	25/may/2017	*Glycine max*	Bt
293	Londrina	-23.19114167	-51.18278333	PRLON2	Q	1	25/may/2017	*Glycine max*	UNI
294	Londrina	-23.19114167	-51.18278333	PRLON2	B	3	25/may/2017	*Glycine max*	UNI
295	Londrina	-23.19063889	-51.18233333	PRLON16	B	4	17/jan/2017	*Glycine max*	UNI
296	Londrina	-23.19433333	-51.18258333	PRLON17.1	B	3	17/jan/2017	*Phaseolus vulgaris*	UNI
297	Londrina	-23.19433333	-51.18258333	PRLON17.1	Q	7	17/jan/2017	*Phaseolus vulgaris*	UNI
298	Primeiro de Maio	-22.80055556	-51.06305556	PRPRI01i	B	4	17/jan/2017	*Glycine max*	UNI
299	Realeza	-25.76436111	-53.55491667	PRREA20	A2	3	18/jan/2017	*Phaseolus vulgaris* – black seeds	UNI
300	Realeza	-25.76436111	-53.55491667	PRREA20	B	2	18/jan/2017	*Phaseolus vulgaris* – black seeds	UNI
301	Realeza	-25.76436111	-53.55491667	PRREA20	A	1	18/jan/2017	*Phaseolus vulgaris* - black seeds	L11
302	Tibagi	-24.50944444	-50.41361111	PRTIB1	B	2	04/mar/2016	*Phaseolus vulgaris*	UNI

N	Region, State and City	Latitude	Longitude	Code	Haplotype	NA	Collection date	Host	Primer *
303	Tupãssi	-24.58777778	-53.51166667	PRTUP1	B	5	25/may/2016	*Phaseolus vulgaris*	UNI
	RIO GRANDE DO SUL (RS)								
304	Barra do Quaraí	-30.20511111	-57.53736111	RSBAR32	Q	4	21/jan/2017	*Capsicum annuum*	UNI
305	Barra do Quaraí	-30.20511111	-57.53736111	RSBAR32	Q	1	21/jan/2017	*Capsicum annuum*	L11
306	Bento Gonçalves	-29.17416667	-51.44911111	RSBEN41	A1	1	23/jan/2017	*Solanum muricatum*	UNI
307	Bento Gonçalves	-29.17416667	-51.44911111	RSBEN41	B	1	23/jan/2017	*Solanum muricatum*	UNI
308	Bento Gonçalves	-29.17416667	-51.44911111	RSBEN41	Q	1	23/jan/2017	*Solanum muricatum*	UNI
309	Bento Gonçalves	-29.17416667	-51.44911111	RSBEN41	B	1	23/jan/2017	*Solanum muricatum*	Bt
310	Cristal do Sul	-27.423917	-53.273361	RSCRI27	A	1	19/jan/2017	*Glycine max*	L11
311	Iraí	-27.20213889	-53.25544444	RSIRA26	Q	4	19/jan/2017	*Ipomoea batatas*	UNI
312	Iraí	-27.20213889	-53.25544444	RSIRA26	Q	1	19/jan/2017	*Ipomoea batatas*	L11
313	Santana do Livramento	-30.85311111	-55.49183333	RSSAN35	A1	1	21/jan/2017	*Solanum lycopersicum*	UNI
314	Santa Cruz do Sul	-29.821389	-52.378889	RSSAT1	B	3	30/mar/2018	*Glycine max*	Bt
315	Uruguaiana	-29.742028	-56.982028	RSURU30	A	1	20/jan/2017	*Physalis* sp.	L11
	SANTA CATARINA (SC)								
316	Caçador	-26.81844444	-50.98705556	SCCAÇ48	A2	2	25/jan/2017	*Lycopersicon esculentum*	UNI
317	Caçador	-26.81844444	-50.98705556	SCCAÇ48	A	1	25/jan/2017	*Lycopersicon esculentum*	L11
318	Iraceminha	-26.782361	-53.282944	SCIRA25	A	1	19/jan/2017	*Phaseolus vulgaris*	L11

Primers*- Bt = BtBF1F/WfBr2R (mtCOI); BQ = NAF-MED-ME F1/NAF-MED-ME R1 (mtCOI); UNI = 2195F/3014R (mtCOI); L11 = L11-190F/L11-962 (nDNA).

Whiteflies were randomly distributed in Brazil (Figure 3.1.). All three haplotypes (A, B, and Q) were found in four states: Maranhão (MA – northeast region), São Paulo (SP – southeast region), Paraná (PR) and the Rio Grande do Sul (RS – south region). A1-haplotype samples were found in open fields on several cultivated and non-cultivated plants mainly on soybeans *Glycine* sp. (TO, BA, MA, and RS state), cassava (*Manihot esculenta* - Euphorbiaceae) (TO and MG state), tomato (BA, SP, SC, and RS state), Acacia (*Acacia mangium* – Acaciaceae), the first report of this haplotype on this host plant, and the fruit bush *Physalis* sp. (Solanaceae). The last one, *Physalis* sp., was exclusively colonized by this native haplotype, in the north and south regions of Brazil. A1-haplotype was also collected in corn (*Zea mays* - Poaceae), common beans, weeds, guava (*Psidium* sp. - Myrtaceae), and andine melon in an organic farm.

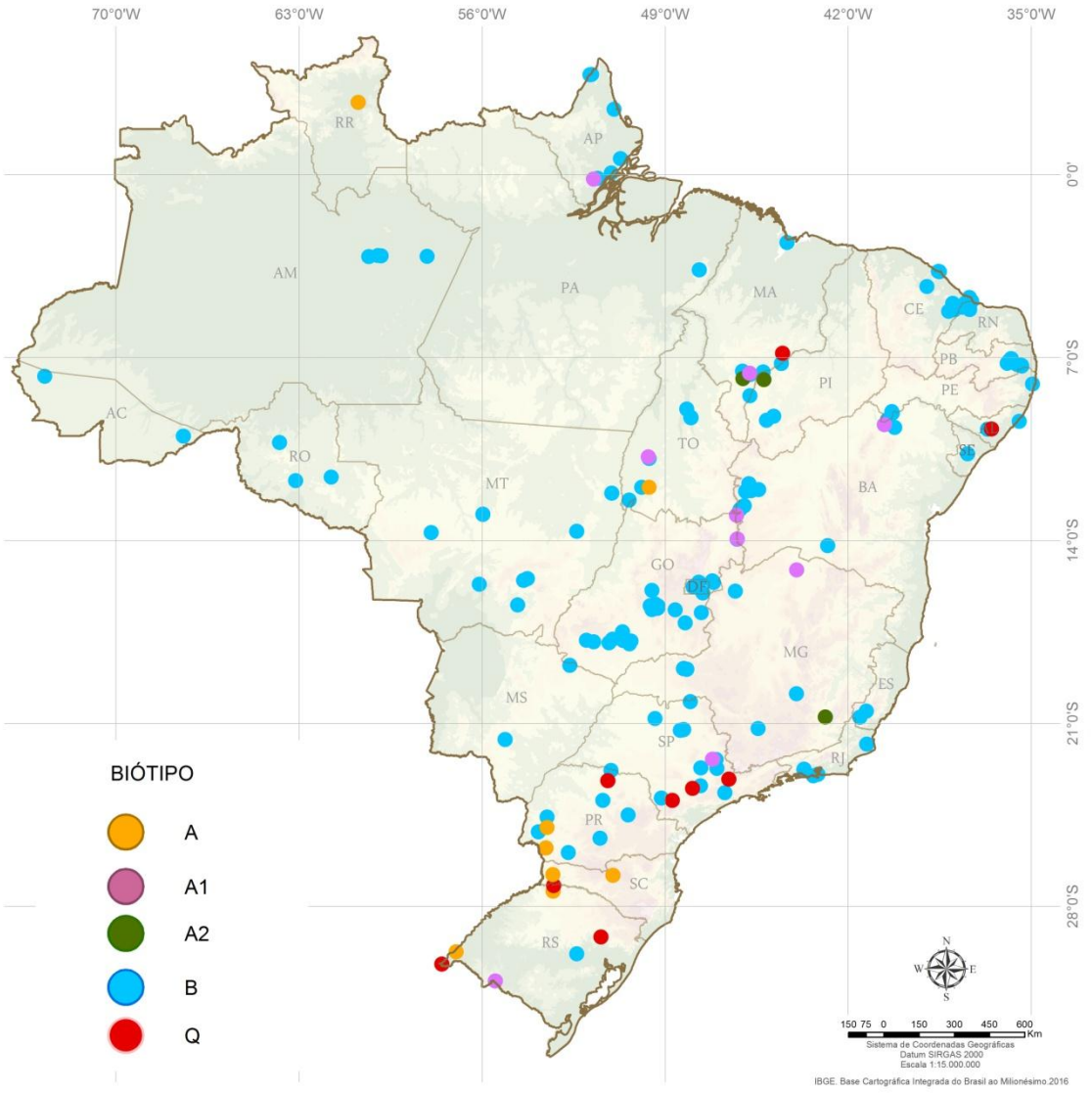

Figure 3.1. *Bemisia tabaci* haplotypes in Brazil collected from 2014 to 2019.

A2-haplotype whiteflies were present in five states (northeast, southeast, and south regions) of Brazil, except for the northern states, as expected, since it is the region with higher botanical richness. Also, in the northeast region, A2 was collected on Fabaceae: cowpea (*Vigna unguiculata*) and soybeans (*Glycine* sp.). In the southeast-south region was collected on tomatoes (*Lycopersicon* sp. - Solanaceae), and common beans (*Phaseolus vulgaris* - Fabaceae). Samples from south regions were collected by interstates routes. A-haplotype samples, also identify with nDNA, were collected in the northmost state of Brazil, Roraima (RR) on Acacia (*Acacia mangium* – Acaciaceae), *Physalis* sp., common beans (brown and black seeds), soybeans, tomatoes, and weed.

Q haplotype samples were found, in open fields and greenhouses, in five states (northeast, south, and southeast region) of Brazil. Also, two new records for the northeast region at Alagoas (AL) state on kale, *B. oleracea*, and MA on *Glycine* sp. It was exclusively collected on *Gerbera jamesonii* (Asteraceae), in São Paulo (SP) state (southeast region). Additionally, it was found in the following hosts: common beans, tomatoes, peppers (*Capsicum annuum* - Solanaceae), soybeans, sweet potato (*Ipomoea batatas* - Convolvulaceae), and on andine melon *Solanum muricatum* (Solanaceae), this last one in an organic farm.

B-haplotype was the most abundant and distributed haplotype across the country. Except for the SC state (south region) B-haplotype was present in all Brazilian states. The majority of samples were identified on soybeans, followed by kale, common beans, cotton (*Gossypium hirsutum* - Malvaceae), and tomatoes. Elephant ear leaf (*Xanthosoma sagittifolium* – Araceae) and the fruit trees *Spondias mombin* (Anacardiaceae) are possibly new host plants for this haplotype. Therefore, Lady´s finger, *Abelmoschus esculentus* (Malvaceae), was exclusively hosted by B-haplotype, and present in all regions of Brazil, except the south. Among an extensive host plant list, more than 10 botanical families, from cultivated and non-cultivated crops such as banana, broccoli, cabbage, cassava, citrus, corn, eggplant, guava, jilo, kale, lettuce, melon, okra, potato, pumpkin, tobacco, tomato, ornamentals, and watermelon hosted by haplotype.

Sympatric haplotypes were observed in twelve locations/samples in Brazil. Although observed in many regions none were identified in the Midwest region of the country. Most of the samples from the Midwest region were found in Leguminosae and Solanaceae plants.

Another interesting point is half of those sympatric haplotypes samples were composed by the native A1 and B hosted mainly by Solanaceae and Poaceae. Besides, A2 and B were found cohabiting in a single common beans plant (black seeds), *Phaseolus vulgaris*, in Realeza, PR state, a border city between Brazil, Argentina, and Paraguay.

B and Q haplotypes were also found cohabiting the same niche in northeast and south-southeast regions of Brazil, hosted by kale, green pepper, common beans (brown, and black seeds), and soybeans. A case of multiple sympatric haplotypes (A, B, and Q) was identified on, andine melon, near Bento Gonçalves, RS, the southernmost state of Brazil, in an organic farming system.

Phylogenetic trees of the Brazilian whitefly samples are shown in Figures 3.2, 3.3, and 3.4. First tree (Figure 3.2) shows the major clades obtained in this research: American Tropics (AM-TROP) (including A1 and A2 haplotypes) and North Africa-Mediterranean-Middle East (B, and Q haplotypes). Second tree (Figure 3.3), is an expanded version of the first one but also shows B haplotype as a consistent clade. As expected, the older clade, American-native, A-haplotype, appears first in the tree followed by B and Q haplotype. The third tree (Figure 3.4) groups all the native samples collected in Brazil from 2014-2019. All A-haplotypes samples presented low diversity among them.

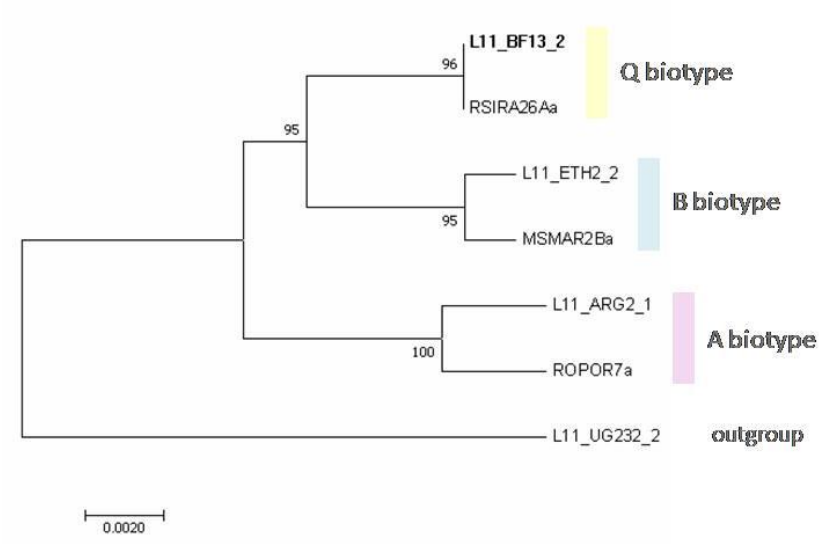

Figure 3.2. Phylogeny of Brazilian *Bemisia tabaci* based on 7 nuclear genes (L11)

sequences. The Kimura 2-parameter model (Kimura, 1980) was identified as the best fit model. The phylogenetic tree was constructed with one clone per representative samples with one thousand bootstrap iterations (Tamura et al., 2013). Samples were added as references: Burkina Faso (BF13) as Q haplotype; Ethiopia (ETH2) for B haplotype; Argentina (ARG2) as A-haplotypes, and *B. tabaci* sequence from Uganda (UG232) as an outgroup member.

Figure 3.3. Phylogeny of Brazilian *Bemisia tabaci* based on 24 nuclear genes (L11) sequences. The Kimura 2-parameter model (Kimura, 1980) was identified as the best fit model. The phylogenetic tree was constructed with one clone per representative samples with one thousand bootstrap iterations (Tamura et al., 2013). Samples from China (CHN4) and Ethiopia (ETH2) were added as references of B1 and B2 haplotypes, respectively; Burkina Faso (BF13) as Q haplotype; Argentina (ARG2) as A-haplotype, and *B. tabaci* sequence from Uganda (UG232) as an outgroup member.

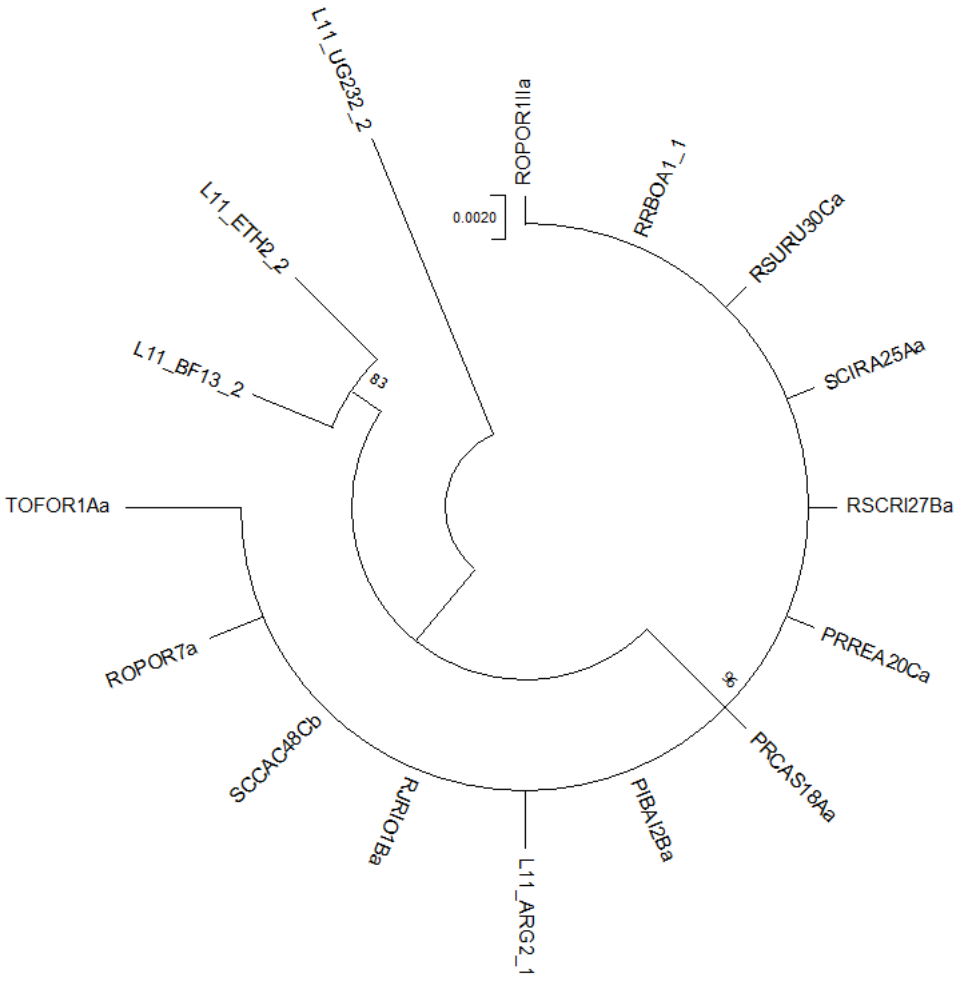

Figure 3.4. Phylogeny of American native *Bemisia tabaci*, A-haplotype (American Tropics, AM-TROP clade), collected in Brazil, based on 16 nuclear genes (L11) sequences. The Kimura 2-parameter model (Kimura, 1980) was identified as the best fit model. The phylogenetic tree was constructed with one clone per representative samples with one thousand bootstrap iterations (Tamura et al., 2013). One sample from Burkina Faso (BF13) was added as references as Q haplotype; Ethiopia (ETH2) for B haplotype; Argentina (ARG2) as A-haplotype, and *B. tabaci* sequence from Uganda (UG232) as an outgroup member.

3.4 DISCUSSION

Species of agronomical and economical importance such as *B. tabaci* can be taxonomically characterized by morphological and ecological features but better classified with molecular tools. Besides, they can be best represented and grouped in molecular databases (Brown, 2010). The observed haplotypes A1, A2, B, and Q (Figure 3.1) from different host plants, locations, and niches indicate the adaptation in broader botanical hosts

in Brazil.

Whereas mtDNA barcoding is cheaper and faster than nDNA, it also outnumbers the genomic studies based on mtDNA in insects (Roy, 2003). Although more expensive than mtDNA, the nDNA technique was worthwhile in this study due to the questions that we could address this large collection. Another advantage of analyzing an entire country whitefly survey is the study possibilities of geographically haplotypes distribution, genotypic and phenotypic variability, and to exclude the possibility of irregularities with barcodes (Zakharov et al., 2009).

The nuclear genome is the largest contributor of genetic information within an insect (Roy, 2003). So to try to recover all haplotypes (A, B, and Q) a nuclear tree was built (Figure 3.2.). *B. tabaci* grouped as a monophyletic group, with ≥ 95% bootstrap, support (Figure 3.2.). For *B. tabaci*, there is evidence that nDNA genes recover similar relationships to those resolved previously by COI, indicating that both have co-evolved over evolutionary time, and are appropriate for identifying haplotypes boundaries in these taxa (De Barro et al., 2011).

Although the supra cited haplotypes were described in the literature, as also present in many states of Brazil (Lima et al., 2000; Moraes et al., 2018), possibly multiple/new introductions occurred, especially for the B and Q invasive haplotypes, as revealed by the B haplotype collected at Bahia, Maranhão and Pará state, north-northeast regions (BAROS1, MABAL2, PAPAR1, respectively; Figure 3.3.) that were genetically different from other samples in the collection. Those possible multiple/new introductions can be explained by the frequent importation of ornamentals and other exotic plants at the Brazilian Atlantic seaports.

As expected, the number of native haplotypes (A1 and A2) collected in Brazil is reduced compared with the outsider B-haplotype (Table 3.1.). One reason for the reduced number of A-haplotypes samples may be the collection site, mainly in agricultural areas, excluding some native plants, weeds, and nonrandom sampling sites. Another reason can be by the ability, especially of B haplotype, to rapid disperse across long distances seeking new niches and resistance to insecticides (Liu et al., 2007; Chu et al., 2010; Sun et al., 2013) especially neonicotinoids (Horowitz & Ishaaya, 2014) commonly used in Brazil. On the other hand, native haplotypes do not disperse fast and are also adapt to indigenous plants that can provide niches year-round (Frohlich et al., 1999). Besides, B and Q haplotypes can displace indigenous haplotypes by competitive bio-advantage like increasing the offspring

production of the invader female, and consequently reducing the number of native´s female progeny, change of behavioral mechanisms, and mating interactions (Liu et al., 2007; Hadjistylli et al., 2016). The expansion of B-haplotype can also be explained by the broader botanical feeding resource, such as *Abelmoschus*, *Brassica*, *Citrullus*, *Citrus*, *Cucumis*, *Cucurbita*, *Glycine*, *Gossypium*, *Lycopersicon*, *Manihot*, *Nicotiana*, *Phaseolus*, *Psidium*, *Solanum*, *Xanthosoma*, *Zea*, ornamentals, weed, among others. Besides, the same occurred in other countries of the American continent (McKenzie et al., 2012; McKenzie & Osborne, 2017).

One question that remain is if the recently introduced invader Q haplotype, first collected at the Rio Grande do Sul (Barbosa et al., 2015), second in São Paulo (Moraes et al., 2017) and later in Mato Grosso (Pitta et al., 2019), will displace the natives (A1 and A2 haplotypes) and the already established B haplotype that is still dominant in Brazil since its introduction (Lourenção & Nagai, 1994; Lima et al., 2000; Lima et al., 2002).

Our results are similar to the first extensive survey in Brazil whose predominance was B haplotype (Lima et al., 2000; Lima et al., 2002). However, as analyses were done by RAPD to differentiate between whitefly biotypes (Lima et al., 2000; Lima et al., 2002; Rabello et al., 2008; Silva et al., 2009; Fontes et al., 2010; Queiroz et al., 2016) the comparison is not straightforward (Hadjistylli et al., 2010).

Even with a high number of samples, was not possible to observe a significant shift in the haplotype ratios. We expected a large number of A-haplotype samples, in the early 1990s, instead of the invasive B haplotype. A-haplotype was most likely competitively displaced during the invasion of B in Brazil. These haplotypes were only found in twelve states. This can be explained due to the adaptation to native plants, the low sampling sites in preserved areas with non-cultivated fields and weeds, or even by its absence within regions. The B haplotype has come to predominate over the other two haplotypes ranging up to more than 80% in Brazilian fields.

Q-haplotype recent introductions will be determined in long term outcome since environmental changes and overlapping niches will occur (Moraes et al., 2017; Pitta et al., 2019). Migrations to the Midwest regions of Brazil indicate an adaptation to higher temperatures and other plants suggesting that it may spread predominantly in agricultural areas. More data will be needed to monitor the adaptation and dispersion of those important worldwide invasive pests in the Brazilian agriculture system.

Our data support the theory that Q haplotypes, already present in Brazil for seven

years (Barbosa et al., 2015), may not displace native whiteflies and the well-established invasive B haplotype. This can be asserted by the velocity of propagation and adaptation through the regions. In the same frame time since its first introduction report (Lourenção & Nagai, 1994), the B-haplotype has spread in twenty Brazilian states (Lima et al., 2000).

Results in this research will contribute to an understanding of *Bemisia tabaci* complex, its relations to botanical families, and geographic distribution on the agricultural regions of Brazil and South America, also contributing to Phytovirus containment strategies, especially Geminiviruses, Closteroviruses, and Carlavirus.

3.5 CONCLUSIONS

American native haplotypes (A) were identified in 12 Brazilian states, and two invasives haplotypes, B and Q, in 26 and 5 states, respectively. The B haplotype was geographic randomly distributed across the country. We identified more than 15 botanical families associated with the collected whiteflies from cultivated to wild plants.

B-haplotype was predominant in all Brazil possibly due to its environmental adaptation, resistance to insecticides, and the agricultural system, with three annual crops, which maintain plants all year-long in fields' areas. These haplotypes possibly have been dislocating A-haplotypes in cultivated and non-cultivated systems.

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4 FINAL CONSIDERATIONS

This study started due to a lack of *Bemisia tabaci* mapping and identification in several Brazilian regions, mainly north and northeast. Other interesting factors that drew our attention were the possible dates and geographical entry points of this quarantine pest in Brazil.

Therefore, the research had as its general objective the collection and identification of *B. tabaci* samples in all 27 Brazilian states, host plants, mapping and genetic analysis, randomly collected, between 1989 to 2019. The general objective was achieved because we effectively analyzed all the *B. tabaci* haplotypes described in the literature and present in several regions until nowadays.

The specific objectives of the first study were to identify *B. tabaci* haplotypes and to map its distribution from samples collected between 1989 to 2005 maintained by Embrapa Genetic Resources and Biotechnology, Brasília – Brazil, in an entomological museum collection. The objectives were achieved using nuclear (nDNA) and mitochondrial (mtDNA) molecular markers in the identification as well as using cartographic software. We identified only one haplotype in the entomological collection, B haplotype, present in thirteen Brazilian states.

The specific objectives of the second study were to identify *B. tabaci* haplotypes and to map its distribution from samples collected in all 27 Brazilian states from 2014 to 2019, in cultivated and non-cultivated host plants. The objectives were achieved using nuclear (nDNA) and mitochondrial (mtDNA) molecular markers in the identification as well as using cartographic software. We identified three haplotypes (A, B, and Q) in the collection. B-haplotype was found in all Brazilian states, except in Santa Catarina, with the largest number of associated host plants. A-haplotype, the American native, was registered in 12 states followed by Q haplotype present in 5 states of the country.

The research started from the hypothesis that the native and the most common invasive haplotype (B) would be present throughout Brazil because this whitefly has been present in Brazil for almost 100 years. During the study we found that the haplotypes aforementioned were present in several Brazilian regions, randomly distributed, however, they were not present in all Brazilian states, thus refuting the study hypothesis.

We answered the question proposed by the study with the proposed methodology. The methodology included collections, identification, and mapping of *B.*

tabaci throughout Brazil, in different years, and host plants. Quantitative data were generated with the assistance of a research team from Brazil and the United States of America in two laboratories, Embrapa Rice and Beans, in Brazil, and The University of Arizona, in The United States of America.

We noticed limitations in this project/study. The Project could have as one of its central objectives sampling in the north and northeast regions, due to the scarcity of publications with whiteflies data in these regions.

We recommend additional sampling and genetic analysis in the north and northeast regions, emphasizing the native haplotypes as well as comparing their genetic variability with other whiteflies present in Latin America. We also suggest the analysis of other entomological collections present in several Federal Universities to create a DNA barcoding database (nuclear and mitochondrial) including several whiteflies species as a source of knowledge for students, teachers, and researchers worldwide. This database would assist in pest management strategies due to its agricultural and economic importance.