Morphological and Mitochondrial DNA Marker Analyses of Whiteflies (Homoptera: Aleyrodidae) Colonizing Cassava and Beans in Colombia

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ABSTRACT Morphology of the third antennal segment and compound eyes of adults of the whitefly species *Bemisia tabaci* (Gennadius), *B. argentifolii* Bellows & Perring, *B. tuberculata* (Bondar), *Trialeurodes vaporariorum* (Westwood), *T. variablilis* (Quaintance), and *Aleurotrachelus socialis* (Bondar) were studied using scanning electron microscopy to aid in identification of adult whiteflies in cassava and beans in Colombia. Random amplified polymorphic DNA polymerase chain reaction markers proved complementary to the morphological identification of whitefly species and the only rapid method to distinguish individuals in the *Bemisia tabaci* species complex. From each species of whitefly, a region of the mitochondrial 16S rDNA gene was amplified, cloned and the sequence determined. Parsimony and distance analyses were performed and the results were similar to those based on morphology. The distance between the two species of *Trialeurodes* was greater than expected for two species within the same genus. The combination of morphological and molecular traits is useful in understanding the diversity and evolution of these whitefly species.

KEY WORDS Aleyrodidae, whitefly, compound eye, polymerase chain reaction, random amplified polymorphic DNA, 16S rDNA

WHITEFLIES ARE AGRICULTURAL pests in most tropical and subtropical countries. *Bemisia tabaci* (Gennadius), *Bemisia argentifolii* Bellows & Perring, *Bemisia tuberculata* (Bondar), *Trialeurodes vaporariorum* (Westwood), *Trialeurodes variabilis* (Quaintance), *Trialeurodes abutiloneus* (Haldman), *Aleurotrachelus socialis* (Bondar), and *Aleurocanthus woglumi* (Ashby) are considered the important whitefly pests in Colombia and Central America (Caballero 1992). Identification of adults found on *Manihot esculenta* (Crantz) or *Phaseolus vulgaris* (L.) often is necessary in our investigation of whiteflies as vectors of viruses. Conventional taxonomy and identification of whiteflies is based on morphological characters of the pupal stage. The pupa may present difficulties because of variation in setae or shape of pupal cases. In some polyphagous species, the variation is correlated with the host plant and environmental factors (Russell 1948; Mound 1963, 1983; David and Ananthakrishnan 1976; Mohanty and Basu 1986). It is not always possible to find pupae when collecting whiteflies from the field, particularly on young plants. For practical reasons it is desirable to be able to identify whitefly adults.

Few studies have been done on the morphology of adult whiteflies. Hill (1969), Bink-Moenen (1983), and Gill (1990) analyzed adult morphological structures in light microscope studies of whitefly species from temperate regions. Our study adds both morphological and molecular information on three species of economic importance found in tropical America, *B. tuberculata*, *T. variabilis*, and *A. socialis*, as well as the better-known species *B. tabaci*, *B. argentifolii*, and *T. vaporariorum*. The terminology and description of morphological structures of adult whiteflies used in this article are given by Gill (1990). Using a scanning electron microscope (SEM) enables the detection of variation of the sensorial receptors on the antenna and the number of ommatidia connecting the upper and lower compound eyes of adult whiteflies encountered on cassava and beans in Colombia.

Bemisia tabaci B biotype is reported to be a distinct species called *B. argentifolii* (Perring et al. 1992, 1993; Bellows et al. 1994). The molecular data are not convincing because the variation at the mitochondrial 18S rDNA gene is only a single unique nucleotide difference between *B. tabaci* biotype A and *B. argentifolii* (Campbell et al. 1994). Detailed studies (Brown et al. 1995, Frohlich et al. 1999) suggest that *B. tabaci* should be considered a cryptic species complex and that *B. argentifolii*, a member of the complex, is a recent introduction from the Old World to the Americas. In a study based on the ribosomal internal transcribed spacer (ITS 1), the authors reached the same conclusion (DeBarro et al. 2000). *Bemisia argentifolii* is widespread in many countries of Latin America including Colombia (Quintero et al. 1998). In tomatoes, this pest causes hundreds of millions of dollars annually in direct damage and as a vector of whitefly-transmitted viruses (Polston and Anderson 1997). In beans, this pest is associated with an increased incidence of geminiviruses. Although increased host range, silverleaf symptoms, and increased populations of whiteflies are

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Trialeurodes and *Bemisia*. The 3
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studies of distantly related tax
and was proposed for the study
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Materials and M

Whiteflies were collected in
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their identification. The *B. taba*
Rica (CR), Puerto Rico (PR), a
T. vaporariorum from Arizona
previously (Frohlich et al. 199

indicators that the population is *B. argentifolii*, morphology is not reliable to distinguish between whiteflies in the *Bemisia tabaci* complex. Pupal morphological features contain sufficient variation that they are not reliable characteristics to distinguish between *B. tabaci* biotype A and *B. argentifolii* (Rosell et al. 1997).

Molecular detection methods using polymorphisms of PCR (random amplified polymorphic DNA [RAPD]-PCR) products were developed to distinguish native Australian populations of *B. tabaci* and *B. argentifolii* (De Barro and Driver 1997). A RAPD PCR-based test is very convenient because the samples can be preserved in alcohol and relatively large numbers of samples can be processed rapidly.

Both morphological and molecular data are useful in determining taxonomic relationships. Because there is little fossil evidence and significant variability in the key traits in both nymphs and adult whiteflies, molecular data can be important in determining phylogenetic relationships (Campbell et al. 1996). The ITS region of the nuclear 18S rDNA gene was used to study phylogenetic relationships in the Aleyrodidae (Campbell et al. 1994). Molecular analysis of the genus *Bemisia* was made by comparing the mitochondrial 16S rDNA gene and a variable portion of mitochondrial cytochrome oxidase I (COI) gene (Frohlich et al. 1999). These studies demonstrated that different markers have different degrees of variability. The nuclear 18S data were the least variable and the COI data the most variable. Here we analyzed both the relationships between three genera as well as species of *Trialeurodes* and *Bemisia*. The 3′ half of the mitochondrial 16S gene was chosen because it proved useful in studies of distantly related taxa (Simon et al. 1994), and was proposed for the study of whiteflies (Frohlich et al. 1996), and was useful in distinguishing drosophilid genera (DeSalle 1992). We have also tested the utility of RAPD PCR to identify whiteflies in tropical America.

Materials and Methods

Whiteflies were collected in June 1997 from cassava and bean fields and were reared in cages in greenhouses. Specific sites in Colombia and host plant are as follows: *B. tabaci*, Palmira, Valle de Cauca, on beans; *B. tuberculata*, Quilcasé, Cauca, on cassava; *T. vaporariorum*, Fusagasugá, Cundinamarca, on beans; *T. variabilis*, Quilcasé, Cauca, on cassava; *A. socialis*, Pescador, Cauca, on cassava. *Bemisia argentifolii* was collected on *Arachis* sp. in Palmira, Valle de Cauca, and was compared with *B. tabaci* biotype A using RAPDs, esterase enzymes, host range, and symptom severity (Quintero et al. 1998). Whitefly pupae from each colony were collected and brought to the laboratory in petri dishes, where the adults were allowed to emerge. Pupae samples were sent to the USDA Agricultural Research Service in Beltsville, MD, to confirm their identification. The *B. tabaci* biotype A from Costa Rica (CR), Puerto Rico (PR), and Israel, as well as the *T. vaporariorum* from Arizona (AZ), were described previously (Frohlich et al. 1999).

To prepare the adult whiteflies for inspection with the SEM, they were collected using an aspirator and stored in 70% ethanol (EtOH). Specimens were immersed in a graded series of EtOH (80, 90, 100%) for 20 min for each step and cleaned with xylene overnight to remove the wax adhering to the surface of the specimens. They were immersed in EtOH:xylene (1:1, vol:vol), transferred to 100% EtOH, and critical-point dried in CO_2 with a Tousimis 780A apparatus (Rockville, MD). The antennae were cut from the heads and viewed in a horizontal position for optimal resolution. Specimens were mounted in holders with a conductive lacquer adhesive. The specimens were coated with 18 nm of gold in a sputter coater and viewed with a SEM (JEOL JSM-820, Tokyo). In total, 13 males and 15 females of *B. tabaci* biotype A, 12 males and 16 females of *B. argentifolii*, and five males and five females of *B. tuberculata*, *T. vaporariorum*, *T. variabilis*, and *A. socialis* were examined. Adults of each species studied were preserved and stored in 70% EtOH. Representative specimens of each whitefly culture were deposited in the insect collection at CIAT, Cali, Colombia.

RAPD PCR Analysis. Total DNA was isolated from individual whiteflies using a method developed for plants (Gilbertson et al. 1991) with volumes of reagents appropriate for the low weight of the whiteflies. The DNA was amplified using the PCR. The primers used were Operon F2 (5′GAGGATCCCT3′) (Operon, Alameda, CA), F12 (5′ACGGTACCAG3′), H9 (5′TGTAGCTGGG3′), and H16 (5′TCTCAGCTGG3′) (De Barro and Driver 1997). The reactions were carried out using *Taq* polymerase and programmable thermal controllers (PTC-100, MJ Research, Waltham, MA). The reaction conditions for the first cycle were 5 min at 94°C, 2 min at 40°C, and 3 min at 72°C. This was followed with 39 cycles of 1 min at 94°C, 1.5 min at 40°C, and 2 min at 72°C. The PCR products were run in agarose gels, stained with ethidium bromide, and visualized using UV light.

PCR, Cloning and Sequence Analysis of a Region of the 16S Mitochondrial DNA. The mitochondrial DNA was amplified using the PCR. The primer 4119 (5′ CGCCTGTTTAACAAAAACAT) was the forward primer and primer 4118 (5′ CCGGTCTGAACTCAGATCACGT 3′) was the reverse primer (Xiong and Kocher 1991). The PCR reaction conditions were 30 cycles of 1 min at 95°C, 50 s at 50°C, and 50 s at 72°C. In the last cycle, the 72°C reaction was for 10 min. The products were purified using the Wizard PCR purification columns (Promega, Madison, WI) and were visualized by agarose gel electrophoresis with ethidium bromide. The PCR products were cloned into the plasmid PCR script amp SK(+) (Stratagene, LA, Jolla, CA). Plasmid DNA was purified using Wizard plasmid purification columns (Promega). Nucleotide sequences were determined using an ABI Prism 377 sequencer (Perkin-Elmer, Foster City, CA) by the dideoxynucleotide chain termination procedure (Sanger et al. 1977) using the ABI dye terminator reaction ready kit. The sequence data were analyzed

using DNAMAN Version 4.13 (Lynnon Biosoft, Vaudreuil, Quebec).

Phylogenetic Analyses. Phylogenetic analyses were done with multiple individuals within populations. DNA sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) by the ClustalW 1.7 program (BCM Search Launcher at the Human Genome Center, Baylor College of Medicine, Houston, TX). Because different tree building algorithms make different evolutionary assumptions, data were evaluated by parsimony, neighbor joining, and maximum-likelihood. All analyses were performed with PAUP, version 4.0b2, for Macintosh (Swofford 1999). For parsimony, the branch-and-bound method was used (characters unordered, equal weight). Bootstrapping was performed with the branch and bound option for 2,000 replicates (stepwise sequence addition, tree-bisection-reconnection [TBR], MulTrees option). For neighbor joining, distances were calculated using the Kimura two-parameter model. Maximum-likelihood trees were constructed with a transition/transversion ratio of 2.0 by heuristic search (100 replicates, random addition sequence, MulTrees, TBR) (Swofford 1999).

Results

Morphology Comparison of Six Whitefly Species. When specimens are stored in 100% EtOH, it is more difficult to remove all the wax adhering to the surface. Although this usually does not prevent the identification of whiteflies, it does lower the quality of the micrographs. The best results were obtained when fresh specimens were processed quickly at least until the overnight treatment in xylene. After that treatment, storage in 70% EtOH did not cause degradation.

The genitalia were viewed to confirm the sex of the individuals examined. Females of all whitefly species examined in this study tended to be larger than males. All whiteflies used for observation of the compound eyes were positioned on their side. The upper compound eye is located below the ocellus, and the lower compound eye is located above and near the clypeus. The number of ommatidia connecting the upper and lower compound eyes was of specific importance. The upper and lower compound eyes of *B. tabaci* (Fig. 1A) and *B. argentifolii* (Fig. 1B) were connected by one ommatidium in both sexes. The upper and lower compound eyes of *B. tuberculata* (Fig. 1C) were connected by two ommatidium in both sexes. The upper and lower compound eyes of *T. vaporariorum* (Fig. 1D) were completely divided in both sexes. Only two species, *T. variabilis* (Fig. 1E, male) and *A. socialis* (Fig 1F, female), had a different number of ommatidia between the sexes. The upper and lower compound eyes of *T. variabilis* and *A. socialis* in males were connected by four ommatidia compared with only three in females. A characteristic, not apparent in the micrographs, is that the eye of *T. variabilis* is not pigmented but the lower compound eye of *A. socialis* is black, and that the upper compound eye is red.

The differences in size between the sexes did not significantly affect the modal length of the sensorial cone or its position on the antenna. In all whiteflies studied, the sensorial area on the third antennal segment was apical. All species in both sexes had two primary sensoria near the sensorial cone on the third antennal segment. The second sensoria are not always visible (Fig. 2) because the antennal segment was selected for the optimum view of the sensorial cone. In the third antennal segment of *B. tabaci* and *B. argentifolii*, the first primary sensorium was subapical, and the second primary sensorium was apical. They were separated by one ring with respect to each other. The sensorial cone of *B. tabaci* averaged 7.2 μm in length compared with 8.2 μm for *B. argentifolii* and did not reach the first primary sensorium. The position of the sensorial cone with respect to the primary sensoria and the shape of the cones were important in differentiating the species. There was a 12% difference in the average length of the sensorial cone between *B. tabaci* biotype A and *B. argentifolii*. Although this difference was statistically significant (analysis not shown), the range of the length of the senorial cones overlapped. In the third antennal segment of *B. tuberculata*, the first primary sensoria were apical and subapical, and were located on separate rings. The sensorial cone was farther from the first primary sensorium than in *B. tabaci* or *B. argentifolii*, and the sensorial cone was larger (10.2 μm long). In the third antennal segment of *T. vaporariorum*, the sensorial cone arose on the same ring near the first primary sensorium. In *B. tabaci* the sensorial cone was on a separate antennal ring. In *T. vaporariorum* the sensorial cone did not extend beyond the second primary sensorium and was longer than in *B. tabaci* (10.4 μm long). In the third antennal segment of *T. variabilis*, the primary sensoria were both on the same ring. The sensorial cone lay between the primary sensorium and was farther from the second primary sensorium than in *T. vaporariorum*. The length of the sensorial cone was 11.1 μm. In the third antennal segment of *A. socialis*, the sensorial cone was the longest of the species studied (19.9 μm) and reached the second primary sensorium. The central peg on the primary sensoria was elongated and was surrounded by a flowerlike ring of erect spinules.

RAPD PCR Analysis of Whiteflies. Of the oligonucleotide primers that were tested for RAPD PCR analysis, the primers H9 and H16 synthesized fewer PCR products and were useful in distinguishing between the whiteflies tested in this study. Using the primer H9 (Fig 3) for *B. argentifolii* and *T. vaporariorum*, there were prominent PCR products at ≈600 and 800 bp that can sometimes make distinguishing the two species difficult. There was a PCR product in *B. argentifolii* at ≈950 bp, and *T. vaporariorum* had a PCR product at ≈500 bp that was important for distinguishing between these two species. There were PCR products of ≈350, 550, and 600 bp that were similar in *B. tabaci* biotype A and *B. tuberculata*. These species could be identified by a 250 bp PCR product in *B. tuberculata*, and an ≈900 bp product in *B. tabaci* A biotype. The H9

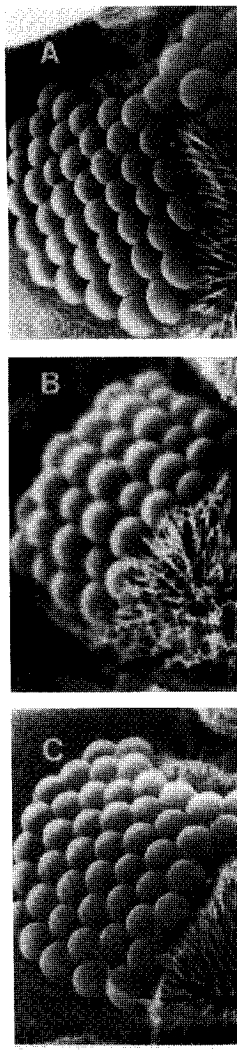

Fig. 1. Compound eyes of adult
A on beans, (B) *Bemisia argentifolii*
T. variabilis on cassava, (F) *Aleurot*

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were PCR products of similar siz
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type A.

The primer H16 was useful to
whitefly species (Fig. 3). Altho
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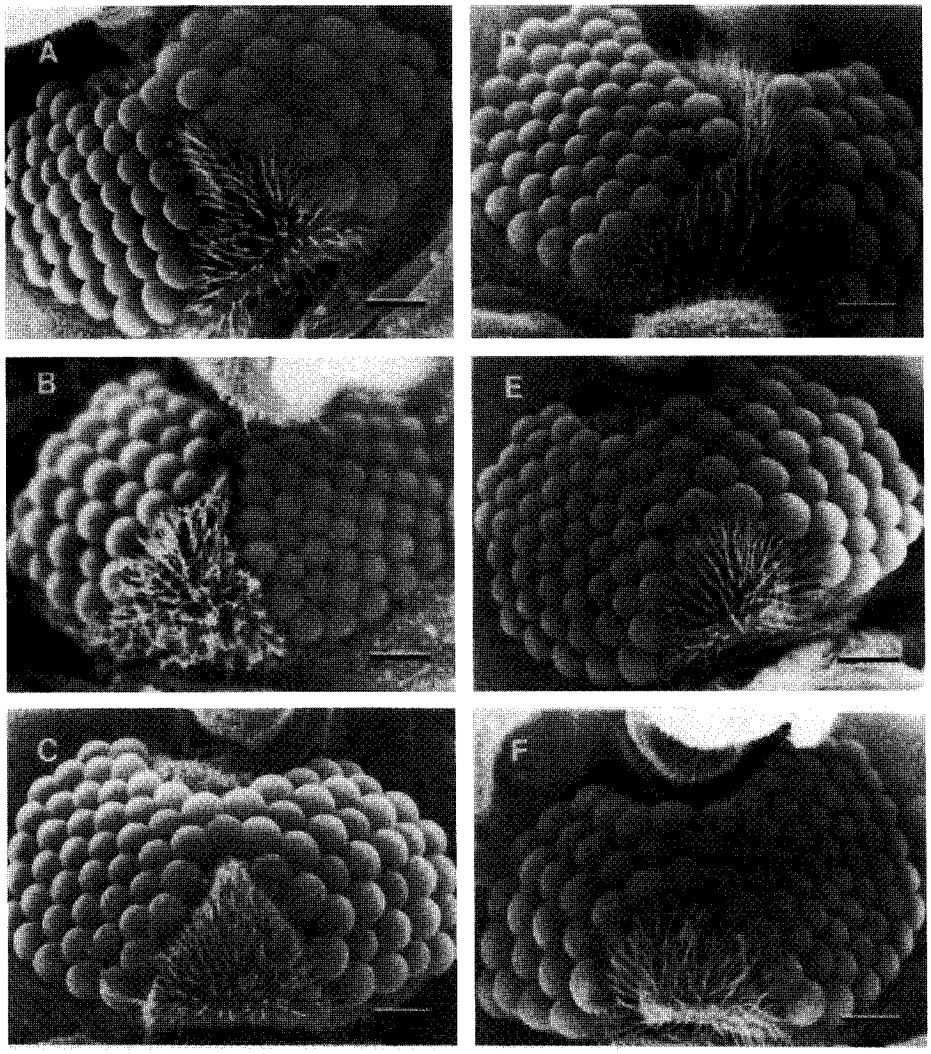


Fig. 1. Compound eyes of adult female whiteflies found on cassava and beans in Colombia. (A) *Bemisia tabaci* biotype A on beans, (B) *Bemisia argentifolii* on beans, (C) *B. tuberculata* on cassava, (D) *Trialeurodes vaporariorum* on beans, (E) *T. variabilis* on cassava, (F) *Aleurotrachelus socialis* on cassava. Bar = 10 μm.

primer was most useful in distinguishing between *B. tabaci* biotype A and *B. argentifolii.* At ≈600 bp there were PCR products of similar size in both biotypes, but the biotype A had several unique PCR products including doublet bands at 300–350 bp. The *B. argentifolii* had PCR products at ≈600, 700, and 900 bp compared with one product of ≈850 bp in *B. tabaci* biotype A.

The primer H16 was useful to distinguish among the whitefly species (Fig. 3). Although there were some common bands in the 500–1000 bp range for both *B. tabaci* biotype A and *B. argentifolii,* there were three products amplified from *B. argentifolii* of ≈350, 450, and 550 bp that were consistently present. *Trialeurodes vaporariorum* was distinguished by having only three prominent PCR products and an ≈700 bp product that is not present in *B. argentifolii.*

When the reactions were run using the primers F2 and F12, there were a large number of PCR products (data not shown). Although the pattern of the PCR products can be used to distinguish the whitefly species, the results often were more difficult to interpret because of the large numbers of PCR products. Therefore F2 and F12 were less useful than H9 and H16 to distinguish between the whiteflies in this study.

Mitochondrial 16S Gene Comparisons. After alignment, 450 characters were used in a parsimony analysis (unordered, equal weight) of which 233 were constant, 51 were variable and uninformative, and 166 were both variable and informative. The branch-and-bound search yielded eight parsimonious trees of equal length (=377) (trees not shown). A bootstrap analysis produced the 50% majority-rule consensus tree shown in Fig 4. The retention index of the tree was

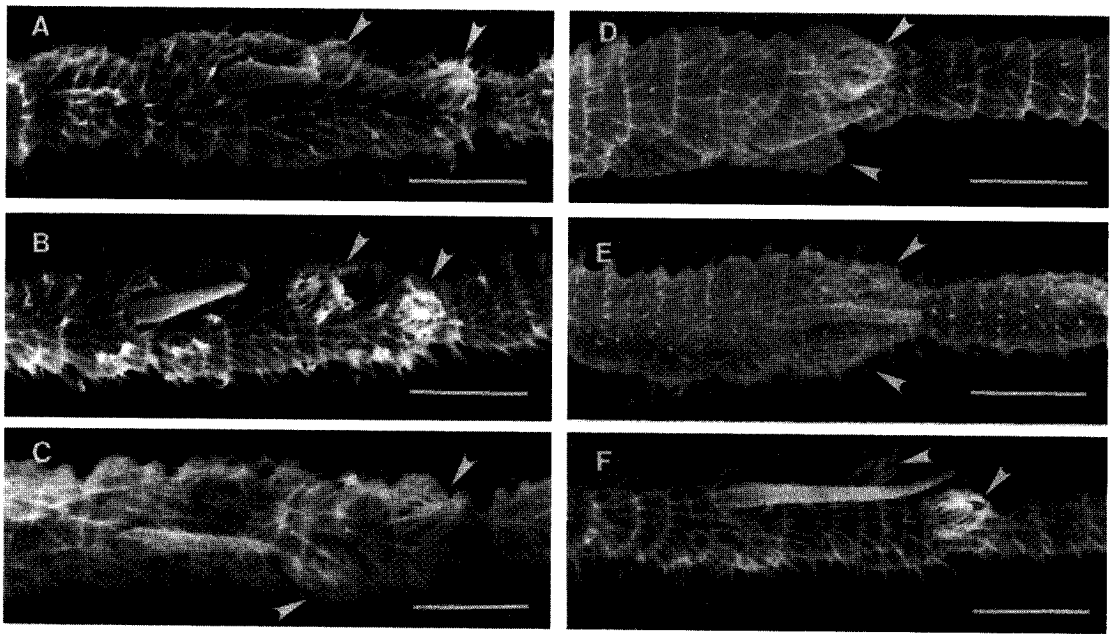


Fig. 2. Third antennal segment of adult male whiteflies found on cassava and beans in Colombia. Arrows point to the primary sensoria. (A) *Bemisia tabaci* biotype A on beans, (B) *Bemisia argentifolii* on beans, (C) *B. tuberculata* on cassava, (D) *Trialeurodes vaporariorum* on beans, (E) *T. variabilis* on cassava, (F) *Aleurotrachelus socialis* on cassava. Bar = 10 μm.

0.82 and the consistency index was 0.86. Tree reconstruction by both neighbor joining and maximum-likelihood produced relationships between the terminal taxa that were exactly the same as those produced by parsimony (trees not shown). With respect to the *Bemisia tabaci* species complex, two robust clades are strongly supported. The first consists of *B. argentifolii* individuals from Colombia Sucre, Beans, Yuca, and a *B. tabaci* sequence from Israel. Values from the distance table (Table 1) indicate that there is no difference between the *B. tabaci* Israeli sequence and *B. argentifolii* yuca. The second clade consists of New World sequences from *B. tabaci* biotype A from Costa Rica, Puerto Rico, Arizona, and Colombia.

The relationship between the three genera, *Bemisia, Trialeurodes,* and *Aleurotrachelus,* was not clear. Considering that *T. vaporariorum* and *T. variabilis* are in the same genus, the mean distances were much greater as compared with the mean distance between the whiteflies in the genus *Bemisia.*

Discussion

The structure, size, and position of the sensorial cone with respect to the primary sensoria on the third antennal segment differed among the six species of whiteflies studied. Although sensorial cones are found on segments III-VII, the third segment is the longest and shows the most sensoria. There are also other modifications of specific importance found on adult whiteflies, such as the male genitalia, the position of the combs and brushes on the legs, and the ventral wax plates. In some genera, such as *Paraleyrodes, Tetraleurodes,* and *Dialeurodes,* the antennae are distinct between the sexes, but for the genera studied here few differences were noted between the sexes. The position of the sensorial cone with respect to the primary sensoria was the only morphological trait that was statistically different for *B. tabaci* biotype A and *B. argentifolii.* Because of the overlap in range and the difficulty in measuring this trait accurately (the antennae must be cut off and laid flat on the viewing platform), it is not useful for studies monitoring the range of these species. The characteristic may be important because few differences have been noted between these species. Additional studies using populations collected from different host plants and geographical locations are needed to determine if the differences found in this study remain consistent over the range of *B. argentifolii* and *B. tabaci* biotype A.

In the case of *B. tabaci* and *B. argentifolii,* it is proposed that they be considered a species complex, and morphological features do not reliably distinguish between them. The oligonucleotide primers found useful in RAPD analysis to separate the native Australian *B. tabaci* from *B. argentifolii* were tested first on known whiteflies from colonies and then on populations of whiteflies from 10 countries in South and Central America. Because the host plants for *B. tabaci* biotype A (not found on cassava) and *B. tuberculata* (principal host cassava) are mutually exclusive, the similarities in the RAPD banding pattern using primer H9 did not cause confusion in identifying these whiteflies. Using H9 to distinguish between *B. argentifolii* and *T. vaporariorum* led to some ambiguous results, and they do have many common host plants. A similar result occurred between *T. vaporariorum* and *B. tabaci* biotype A using the primer H16. Therefore, the results

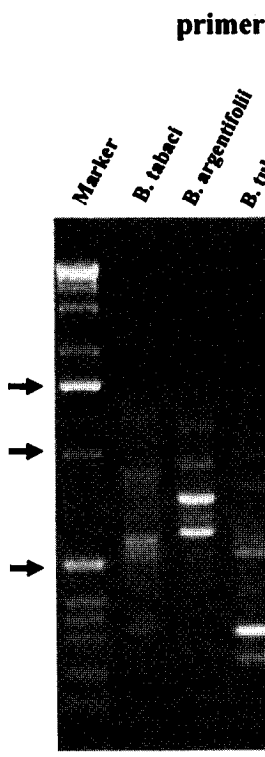


Fig. 3. RAPD PCR products fro
tuberculata. Lane 4: *T. vaporariorum.*
and 1600 bp (1 kb DNA ladder, BRI

are most reliable if both the H9
used in RAPD analysis of indivi
Based on mt18S sequence dat
differences, it was suggested th
belong in a different genus with
rodini than *T. vaporariorum* and

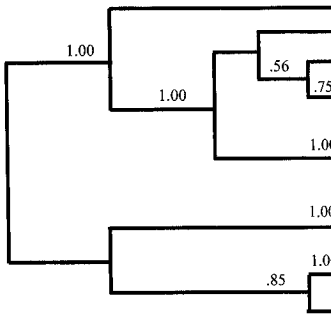
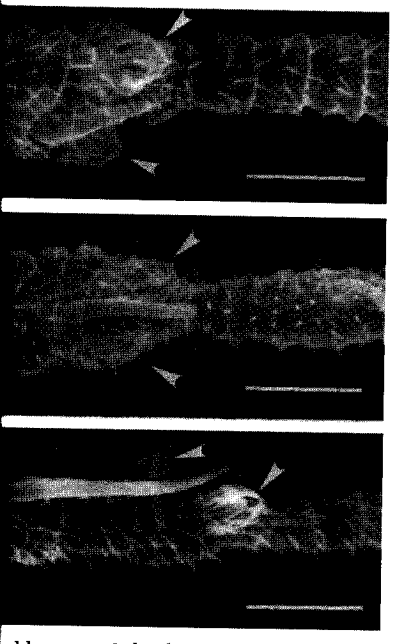


Fig. 4. Cladogram shows the re
whiteflies in this study. The cladogr
parsimonious tree inferred from the
a region of the mitochondrial 16S g
branches indicate the level of statist
responding node from 2,000 bootstra
sion 4.0b2 for Macintosh, Swofford



nd beans in Colombia. Arrows point to the
lii on beans, (C) *B. tuberculata* on cassava,
otrachelus socialis on cassava. Bar = 10 μm.

s, but for the genera studied here few
re noted between the sexes. The posi-
orial cone with respect to the primary
he only morphological trait that was
ferent for *B. tabaci* biotype A and *B.*
ause of the overlap in range and the
easuring this trait accurately (the an-
e cut off and laid flat on the viewing
not useful for studies monitoring the
species. The characteristic may be im-
e few differences have been noted be-
ecies. Additional studies using popula-
l from different host plants and
cations are needed to determine if the
nd in this study remain consistent over
argentifolii and *B. tabaci* biotype A.
of *B. tabaci* and *B. argentifolii*, it is
hey be considered a species complex,
ical features do not reliably distinguish
The oligonucleotide primers found
analysis to separate the native Aus-
from *B. argentifolii* were tested first on
es from colonies and then on popula-
lies from 10 countries in South and
a. Because the host plants for *B. tabaci*
found on cassava) and *B. tuberculata*
cassava) are mutually exclusive, the
e RAPD banding pattern using primer
e confusion in identifying these white-
to distinguish between *B. argentifolii*
orum led to some ambiguous results,
e many common host plants. A similar
etween *T. vaporariorum* and *B. tabaci*
the primer H16. Therefore, the results

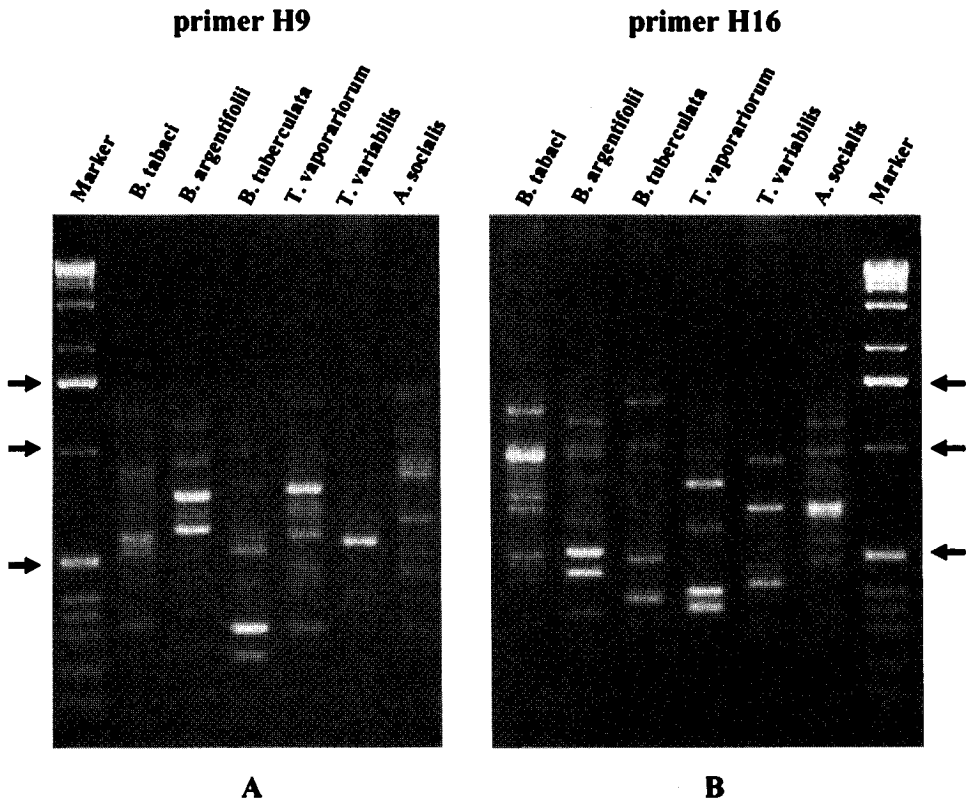


Fig. 3. RAPD PCR products from individual whiteflies. Lane 1: *B. tabaci* biotype A. Lane 2: *B. argentifolii*. Lane 3: *B. tuberculata*. Lane 4: *T. vaporariorum*. Lane 5: *T. variabilis*. Lande 6: *A. socialis*. M: Markers indicated by arrows are ≈500, 1000, and 1600 bp (1 kb DNA ladder, BRL). Oligonucleotide primers were Operon H9 (3A) and H16 (3B).

are most reliable if both the H9 and H16 primers are used in RAPD analysis of individual whiteflies.

Based on mt18S sequence data and morphological differences, it was suggested that *T. intermedia* may belong in a different genus within the tribe Trialeurodini than *T. vaporariorum* and *T. packardi* (Campbell et al. 1994). The relatively large mean distance of the mt16S sequence data between *T. vaporariorum* and *T. variabilis* may also reflect intergeneric differences. The eyes of these two species were very different. In *T. vaporariorum* the compound eye is split and in *T. variabilis* the sections of the compound eye are connected with three (females) or four (males) ommatidia. Detailed comparative molecular and morphological studies of the species in the tribe Trialeurodini are needed to clarify the taxonomic relationships. The mt16S gene sequences of the Arizona and Colombia populations of *T. vaporariorum* were >99% identical. This is similar to the identity found between populations of *B. argentifolii*, which have recently spread throughout the Americas, and less than the variation found in *B. tabaci* biotype A. Because *T. vaporariorum* is thought to be indigenous to the Americas (Vet et al. 1980), one would expect the variation to be greater. More populations of *T. vaporariorum* need to be analyzed to determine the range of diversity within this species.

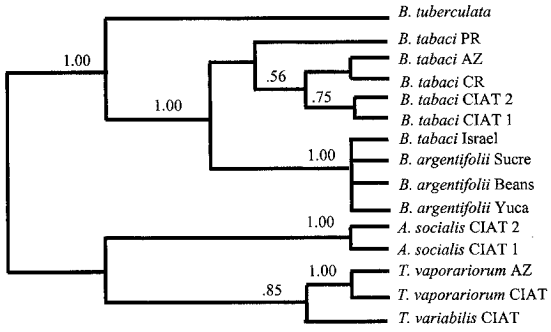


Fig. 4. Cladogram shows the relationship between the whiteflies in this study. The cladogram is based on the most parsimonious tree inferred from the analysis of 450 bases of a region of the mitochondrial 16S gene. Numbers above the branches indicate the level of statistical support for the corresponding node from 2,000 bootstrap replicates (PAUP version 4.0b2 for Macintosh, Swofford 1999).

Mitochondrial DNA is maternally inherited and has a rapid rate of evolutionary change relative to the nuclear genome. These properties make mtDNA suitable for systematic studies among closely related taxa. As expected, the molecular analyses of the sequence

Table 1. Mean distances for a 3′ region of mitochondrial 16S ribosomal gene in 15 individual whiteflies representing different species and populations

	Whitefly	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*B. tabaci* B Sucre	0.00	0.01	0.01	0.01	0.10	0.10	0.09	0.10	0.10	0.22	0.35	0.35	0.37	0.37	0.36
2	*B. tabaci* B CIAT cass		0.00	0.00	0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
3	*B. tabaci* B CIAT bn			0.00	0.00	0.09	0.10	0.09	0.09	0.09	0.21	0.34	0.35	0.37	0.36	0.35
4	*B. tabaci* Israel				0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
5	*B. tabaci* A CIAT 1					0.00	0.01	0.01	0.02	0.00	0.22	0.34	0.35	0.36	0.35	0.38
6	*B. tabaci* A CR						0.00	0.01	0.02	0.01	0.23	0.35	0.36	0.37	0.36	0.39
7	*B. tabaci* A AZ							0.00	0.02	0.01	0.22	0.34	0.36	0.37	0.36	0.38
8	*B. tabaci* A PR								0.00	0.02	0.22	0.35	0.36	0.36	0.36	0.38
9	*B. tabaci* CT 2									0.00	0.22	0.34	0.35	0.36	0.36	0.38
10	*B. tuberculata* CIAT										0.00	0.28	0.28	0.37	0.36	0.38
11	*A. socialis* Mon											0.00	0.07	0.40	0.39	0.43
12	*A. socialis* CIAT												0.00	0.41	0.40	0.41
13	*T. vaporariorum* CIAT													0.00	0.00	0.39
14	*T. vaporariorum* AZ														0.00	0.38
15	*T. variabilis* CIAT															0.00

Genetic divergences were calculated by PAUP, version 4.0b2, for Macintosh (Swofford 1999). Mean distances were calculated as

$$d_m(i,j) = \frac{d(i,j)}{\sum_{\kappa \in S} w_k}$$

where S = the set of characters not excluded, w_k = weight of character k, x_{ik} and x_{ij} = the states of character k in taxa i and j, and diff (x_{ik}, x_{jk}) = the change cost from x_{ik}, to x_{jk}. AZ, Arizona; PR, Puerto Rico; CR, Costa Rica.

data of the 3′ region of the mt16S gene placed all the whiteflies of the same genera in related clades.

There is considerable molecular information available for the 16S gene of different world wide populations of the *B. tabaci* species complex. Given the rapid spread of *B. argentifolii*, the lack of diversity between populations in Costa Rica, Arizona, the north coast of Colombia, and in inter-Andean regions of Colombia was expected. The individuals of Colombian *B. argentifolii* were at least 98.2% identical with the *B. tabaci* of Israel, Yemen and Sudan (Frohlich *et* al. 1999). There is only slightly more diversity within the few characterized populations of *B. tabaci* biotype A. When the Colombian population was compared with the reported sequences from individuals in the biotype A of Arizona, Costa Rica, and Puerto Rico, the identity was 97% or higher (Frohlich et al. 1999). Although the data are still very limited, it appears that the 3′ region of the mt16S gene shows the New World population of *B. tabaci* to be more homogeneous than studies that used esterases as the determinate of variability (Wool et al. 1989, 1994). Since the expression of esterases can be induced by environmental factors such as applications of insecticides, the maternally inherited genetic marker appears to be a better indicator of diversity between populations of whiteflies. Other markers such as the mitochondrial cytochrome oxidase subunit I gene (Frohlich et al. 1999) and the nuclear ribosomal ITS regions (DeBarro et al. 2000) may be even more useful in understanding diversity within the *Bemisia* complex.

Within the *Bemisia* complex, the relationship of *B. tuberculata* is clear because it has distinct morphological characteristics both in the pupa and the adults. The mt16S gene data indicate that *B. tuberculata* is evolutionarily more distant than are *B. tabaci* biotype A and *B. argentifolii*.

The combination of morphological differences in the adults and the mitochondrial DNA molecular data should be useful in understanding the evolution and taxonomy of the six whitefly species examined in this study. These results extend the morphological and molecular comparison between *B. tabaci* biotype A and *B. argentifolii*, and report on a molecular method to distinguish between these species. Although *B. tuberculata*, *A. socialis*, and *T. variabilis* have narrow host ranges, these little studied species are of economic importance on cassava in the American tropics and deserve greater attention as part of the whitefly complex causing increased crop losses.

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References Cited

Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae) infesting North American agriculture. Ann. Entomol. Soc. Am. 87: 195–206.

Bink-Moenen, R. M. 1983. Revision of the African whiteflies (Aleyrodidae), mainly based on a collection from Tchad. Entomologische Vereninging, Amsterdam.

Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweetpotato/silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? An 511–534.

Caballero, R. 1992. Whiteflies (Ho from Central America and Col mounted pupal and field keys characteristics, hosts, distributio economic importance. M.Sc. thes sity, Manhattan, KS.

Campbell, B. C., J. D. Steffen-Campb Evolutionary origin of whitefli ryncha: Aleyrodidae) inferred quences. Insect Mol. Biol 3: 73–

Campbell, B. C., J. D. Steffen-Campb Origin and radiation of whitefli phylogenetic assessment, pp. 29 R. T. Mayer [eds.], *Bemisia* 19 damage control, and manageme UK.

David, B. V., and T. N. Ananthakrish lated variation in *Trialeurodes ra tabaci* (Gennadius) (Homopte Sci. 45: 223–225.

De Barro, P. J., and F. Driver. 199 distinguish the B biotype from o *tabaci* (Gennadius) (Hemiptera Entomol. 36: 149–152.

DeBarro, P. J., F. Driver, J.W.H. T 2000. Phylogenetic relationship *Bemisia tabaci* (Gennadius) usin Phylogenet. Evol. 16: 29–36.

DeSalle, R. 1992. The phylogeneti the family Drosophilidae ded quences. Mol. Phylogenet. Evol

Frohlich, D. R., J. K. Brown, I. Be 1996. Mitochondrial 16S riboso lar marker in *Bemisia*, and imp variability, pp. 143–145. *In* D. C [eds.], *Bemisia* 1995: taxonomy, and management. Intercept, An

Frohlich, D. R., I. Torres-Jerez, Markham, and J. K. Brown. 1 analysis of the *Bemisia tabaci* s mitochondrial DNA markers. M

Gilbertson, R. L., M. R. Rojas, D Maxwell. 1991. Use of the asym reaction and DNA sequencing t ability of bean golden mosaic g ican Republic. J. Gen. Virol. 72

Gill, R. J. 1990. The morphology o D. Gerling [ed.], Whiteflies: th and management. Intercept, An

Hill, B. G. 1969. A morphological species of whitefly, *Trialeurode* and *Bemisia tabaci* (Genn.) (H which occur on tobacco in the t 1: 127–146.

Mohanty, A. K., and A. N. Basu. 19 and seasonal factors on intrasp morphological of the whitefl (Genn.) (Homoptera: Aleyrodi 19–26.

Mound, L. A. 1963. Host-correla *tabaci* (Genn.) (Homoptera A tomol. Soc. Lond. 38: 171–180.

dual whiteflies representing different species

10	11	12	13	14	15
0.22	0.35	0.35	0.37	0.37	0.36
0.21	0.34	0.34	0.36	0.36	0.35
0.21	0.34	0.35	0.37	0.36	0.35
0.21	0.34	0.34	0.36	0.36	0.35
0.22	0.34	0.35	0.36	0.35	0.38
0.23	0.35	0.36	0.37	0.36	0.39
0.22	0.34	0.36	0.37	0.36	0.38
0.22	0.35	0.36	0.36	0.36	0.38
0.22	0.34	0.35	0.36	0.36	0.38
0.00	0.28	0.28	0.37	0.36	0.38
	0.00	0.07	0.40	0.39	0.43
		0.00	0.41	0.40	0.41
			0.00	0.00	0.39
				0.00	0.38
					0.00

). Mean distances were calculated as

es of character k in taxa i and j, and diff (x_{ik},

n of morphological differences in nitochondrial DNA molecular data understanding the evolution and whitefly species examined in this ts extend the morphological and ison between *B. tabaci* biotype A and report on a molecular method een these species. Although *B. tu-*, and *T. variabilis* have narrow host studied species are of economic ava in the American tropics and ention as part of the whitefly com-sed crop losses.

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ferences Cited

I. Perring, R. J. Gill, and D. H. Head-tion of a species of *Bemisia* (Ho-dae) infesting North American agri-mol. Soc. Am. 87: 195–206.
83. Revision of the African whiteflies nly based on a collection from Tchad. reninging, Amsterdam.
ohlich, and R. C. Rosell. 1995. The eaf whiteflies: biotypes of *Bemisia*

tabaci or a species complex? Annu. Rev. Entomol. 40: 511–534.

Caballero, R. 1992. Whiteflies (Homoptera: Aleyrodidae) from Central America and Colombia including slide-mounted pupal and field keys for identification, field characteristics, hosts, distribution, natural enemies, and economic importance. M.Sc. thesis. Kansas State University, Manhattan, KS.

Campbell, B. C., J. D. Steffen-Campbell, and R. J. Gill. 1994. Evolutionary origin of whiteflies (Hemiptera: Sternorryncha: Aleyrodidae) inferred from 18S rDNA sequences. Insect Mol. Biol 3: 73–89.

Campbell, B. C., J. D. Steffen-Campbell, and R. J. Gill. 1996. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment, pp. 29–51. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia* 1995: taxonomy, biology, damage control, and management. Intercept, Andover, UK.

David, B. V., and T. N. Ananthakrishnan. 1976. Host correlated variation in *Trialeurodes rara* (Singh) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Curr. Sci. 45: 223–225.

De Barro, P. J., and F. Driver. 1997. Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Aust. J. Entomol. 36: 149–152.

DeBarro, P. J., F. Driver, J.W.H. Trueman, and J. Curran. 2000. Phylogenetic relationship of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. Molec. Phylogenet. Evol. 16: 29–36.

DeSalle, R. 1992. The phylogenetic relationships of flies of the family Drosophilidae deduced from mtDNA sequences. Mol. Phylogenet. Evol. 1: 31–40.

Frohlich, D. R., J. K. Brown, I. Bedford, and P. Markham. 1996. Mitochondrial 16S ribosomal subunit as a molecular marker in *Bemisia*, and implications for population variability, pp. 143–145. *In* D. Gerling, and R. T. Mayer [eds.], *Bemisia* 1995: taxonomy, biology, damage control, and management. Intercept, Andover, UK.

Frohlich, D. R., I. Torres-Jerez, I. D. Bedford, P. G. Markham, and J. K. Brown. 1999. A phylogeographic analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol. Ecol. 8: 1683–1691.

Gilbertson, R. L., M. R. Rojas, D. R. Russell, and D. P. Maxwell. 1991. Use of the asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of bean golden mosaic geminivirus in the Dominican Republic. J. Gen. Virol. 72: 2843–2848.

Gill, R. J. 1990. The morphology of whiteflies, pp. 13–46. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status, and management. Intercept, Andover, UK.

Hill, B. G. 1969. A morphological comparison between two species of whitefly, *Trialeurodes vaporariorum* (Westw.) and *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) which occur on tobacco in the transvaal. Phytophylactica 1: 127–146.

Mohanty, A. K., and A. N. Basu. 1986. Effect of host plants and seasonal factors on intraspecific variations in pupa morphological of the whitefly vector, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). J. Entomol. Res. 10: 19–26.

Mound, L. A. 1963. Host-correlated variation in *Bemisia tabaci* (Genn.) (Homoptera Aleyrodidae). Proc. R. Entomol. Soc. Lond. 38: 171–180.

Mound, L. A. 1983. Biology and identity of whitefly vectors of plant pathogens, pp. 305–313. *In* R. T. Plumb and J. M. Thresh [eds.], Plant virus epidemiology. The spread and control of insect-borne viruses. Blackwell, Oxford, UK.

Perring, T. M., A. Cooper, and D. J. Kazmer. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. J. Econ. Entomol. 85: 1278–1284.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows. 1993. Identification of a whitefly species by genomic and behavioral studies. Science 259: 74–77.

Polston, J. E., and P. K. Anderson. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis. 81: 1358–1369.

Quintero, C., C. Cardona, D. Ramírez, and N. Jiménez. 1998. Primer registro del biotipo B de *Bemisia tabaci* (Homoptera: Aleyrodidae) en Colombia. Rev. Colomb. Entomol. 24: 23–28.

Rosell, R. C., I. D. Bedford, D. R. Frohlich, R. J. Gill, J. K. Brown, and P. G. Markham. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 90: 575–589.

Russell, L. M. 1948. The North American species of whiteflies of the genus *Trialeurodes*. U.S. Dep. Agric. Misc. Publ. 635: 1–85.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Nat. Acad. Sci. USA 74: 5463–5467.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Swofford, D. L. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, MA.

Thompson, J. D., D. G. Higgins, and T. L. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acid Res. 22: 4673–4680.

Vet, L.E.M., J. C. van Lenteren, and J. Woets. 1980. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Z. Angew. Entomol. 90: 26–51.

Wool, D., D. Gerling, B. Nolt, L. M. Constantino, A. C. Bellotti, and F. J. Morales. 1989. The use of electrophoresis for identification of adult whiteflies (Homoptera: Aleyrodidae) in Israel and Colombia. J. Appl. Entomol. 107: 344–350.

Wool, L., L. Calvert, L. M. Constantino, A. C. Bellotti, and D. Gerling. 1994. Differentiation of *Bemisia tabaci* (Genn.) populations in Colombia. J. Appl. Entomol. 117: 122:134.

Xiong, B., and T. D. Kocher. 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34: 306–311.

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