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Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae)

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Abstract. *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) is recognized as the vector of citrus leprosis virus that is a significant problem in several South American countries. Citrus leprosis has been reported from Florida in the past but no longer occurs on citrus in North America. The disease was recently reported in Central America, suggesting that *B. phoenicis* constitutes a potential threat to the citrus industries of North America and the Caribbean. Besides *B. phoenicis*, *B. obovatus* Donnadieu, and *B. californicus* (Banks) have been incriminated as vectors of citrus leprosis virus and each species has hundreds of host plants. In this study, *Brevipalpus* mite specimens were collected from different plants, especially citrus, in the States of Florida (USA) and São Paulo (Brazil), and reared on citrus fruit under standard laboratory conditions. Mites were taken from these colonies for DNA extraction and for morphological species identification. One hundred and two Random Amplified Polymorphic DNA (RAPD) markers were scored along with amplification and sequencing of a mitochondrial cytochrome oxidase subunit I gene fragment (374 bp). Variability among the colonies was detected with consistent congruence between both molecular data sets. The mites from the Florida and Brazilian colonies were morphologically identified as belonging to *B. phoenicis*, and comprise a monophyletic group. These colonies could be further diagnosed and subdivided geographically by mitochondrial DNA analysis.

Introduction

The citrus industries within the States of São Paulo State (Brazil) and Florida (USA) are the two largest orange producers in the world (Jackson and Davies 1999). Both citrus producing countries have a series of insects, mites, and plant diseases that affect their industries. Citrus leprosis virus, transmitted by *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae), is a major threat to Brazilian citrus production and millions of dollars are spent annually for chemical control of this mite vector (Rodrigues and Machado 2003). Although

present in the past, citrus leprosis no longer occurs in Florida (Childers et al. 2003a). The disease has been recently reported in Panama and Honduras (Dominguez et al. 2001; J. Zuniga, C.C. Childers and J.C.V. Rodrigues personal communication 2003). Several *Brevipalpus* species were reported in all Florida citrus growing areas (Childers et al. 2003a). However, their potential to transmit citrus leprosis virus remains unproven, and their genetic relationship with South American mite populations belonging to the same species is unknown. Morphological characters used in the identification of *B. phoenicis* perform poorly at delimiting species boundaries (Welbourn et al. 2003). In addition, *B. phoenicis* has been incriminated as the vector of a series of new viruses in crops including: coffee, passion fruit, and various ornamental plants (Kitajima et al. 2003). In order to properly establish their diversity and host ranges and to accurately design control tactics to reduce the spread of citrus leprosis, a clear identification of agronomically important species of pests, especially vectors like certain *Brevipalpus* species, is necessary (Childers et al. 2001a).

The genus *Brevipalpus* Donnadieu, originally described in 1875, is separated into two major groups according to the number of marginal hysterosomal setae (Gonzãlez 1975). The larger group has six pairs of hysterosomal setae and contains 46 species including *B. californicus* (Pritchard and Baker 1958). The other group has five pairs of hysterosomal setae and contains nine species including *B. phoenicis* and *B. obovatus*. The division of This group is further based on the number of sensory rods (solenidia) present on the distal margin of tarsus II (Baker and Tuttle 1987).

Sixteen tenuipalpid species including 10 in the genus *Brevipalpus* occur on citrus worldwide with *B. californicus*, *B. obovatus*, and *B. phoenicis* being the most common species (Childers et al. 2001b). The mites are cosmopolitan and polyphagous with hundreds of host plants identified per species (Childers et al. 2003b). Concern exists about the potential of multiple cryptic species existing within the *B. phoenicis* and *californicus* groups.

Brevipalpus phoenicis feeds mostly on perennial plants and usually co-exists with *B. obovatus* and *B. californicus* as described in azalea by Trindade and Chiavegato (1990). The occurrence of 'hybrids' between these species has been suggested because specimens showing morphological characters from both species have been reported by different researchers (Welbourn et al. 2003).

Molecular markers can be used to verify genetic variability in the field and to distinguish sibling species (Black et al. 1992; Stevens and Wall 1995; Reyes and Ochando 1998; Figueroa et al. 1999; Orui and Mizukubo 1999; Navajas and Fenton 2000). Other uses of molecular markers include identifying the occurrence of parasitism, and (or) endosymbionts (Weeks et al. 2001).

In this study, COI mitochondrial sequences and RAPD markers were used to estimate the genetic variability of *Brevipalpus* mites collected mainly from citrus in Florida in the USA and in São Paulo State in Brazil. We also

addressed the phylogenetic relationships among *Brevipalpus* mites collected from four plant genera other than *Citrus* (*Hibiscus*, *Rhododendron*, *Ligustrum* and *Coffea*) that were successfully reared on citrus fruit. The phylogenetic and geographic diversity of the sampled mitochondrial haplotypes are discussed in the context of species delimitation in order to provide a basis for future studies on the potential of these mites to colonize citrus plants and vector citrus leprosis.

Materials and methods

Mite samples

Table 1 presents the details about the populations that were included in this study with geographical co-ordinates obtained by a GPS III Plus (Garmin, Olathe, KS, USA). Six *Brevipalpus* mite samples were collected from São Paulo (Brazil) (Figure 1). Most of these samples were collected from citrus. In addition mites collected on other plants were also examined, including *Coffea arabica* L., *Hibiscus rosa-sinensis* L., *Ligustrum japonicum* Thunberg, *Rhododendron* sp. and tea (*Camellia sinensis* (L.) Kuntze). We collected mites primarily from Florida and São Paulo State because the two areas together represent the largest citrus producers in the world and both have reported prior or existing *Brevipalpus*-leprosis occurrence. The Florida colonies were maintained in the Entomology and Nematology Department, University of Florida in Gainesville. The Brazilian populations were maintained at the Centro de Citricultura 'Sylvio Moreira', Instituto Agronômico de Campinas, SP in Cordeirópolis. Mites were maintained in each laboratory on immature fruits of *Citrus sinensis* Osbeck at 25 ± 1 °C and $70 \pm 5\%$ relative humidity. Adult female mites (1–20) were taken from a single branch or plant to take advantage of the thelytokous reproduction of mites in this genus. Only colonies able to be established on sweet orange fruits were utilized in this study, in order to provide a 'bottle neck' so that all populations accessed were able to colonize *C. sinensis*. In addition, mites from five locations were collected into 95% ethanol from their original hosts in the field and kept in the freezer at -80 °C until processed. Additionally, the mites from colony #61, collected in August 1998, were originally used during the transmission of citrus leprosis virus of the cytoplasmic type reported by Rodrigues et al. (2000), and the mites from colony #17 came from a single female.

Brevipalpus mites from different geographic regions including Texas (USA) and Rio de Janeiro (Brazil) on citrus, and from Minas Gerais (Brazil) on coffee were collected. Specimens of *Cenopalpus pulcher* (Canestrini and Fanzago) (Tenuipalpidae) from apple collected in Oregon, *Eutetranychus banksi* (McGregor) (Tetranychidae) from sweet orange col-

Table 1. Collection data of mite populations included in this study.

Collection number	Location	Position	Original host	Reared on citrus fruits	Date collected	Species[a]	GenBank Accession No.
	USA						
2	Lake Alfred, FL	N28°06′/W81°42′	Sweet orange 1	Yes[c]	Nov 2001	*B. phoenicis*	AY320007
3	Plant City, FL	N27°58′/W82°04′	Sweet orange	Yes[c]	Feb 2002	*B. phoenicis*	AY320008
4	Montverde, FL	N28°35′/W81°40′	Sweet orange	Yes[c]	Sep 2001	*B. phoenicis*	AY320009
5	Bowling Green, FL	N27°37′/W81°53′	Sweet orange	Yes[c]	Aug 2001	*B. phoenicis*	AY320010
6	Lake Alfred, FL	N28°06′/W81°42′	Sweet orange 2	Yes[c]	Sep 2001	*B. phoenicis*	AY320011
7	Oak Hill, FL	N28°53′/W80°53′	Sweet orange	Yes[c]	Jan 2002	*B. phoenicis*	AY320012
8	Merritt Island, FL	N28°32′/W80°40′	Sweet orange	Yes[c]	Aug 2001	*B. phoenicis*	AY320013
9	Pembroke, FL	N26°01′/W80°20′	*Hibiscus*	Yes[c]	Sep 2001	*B. phoenicis*	AY320014
10	Lake Alfred, FL	N28°06′/W81°42′	*Rhododendron*	Yes[c]	Sep 2001	*B. phoenicis*	AY320015
11	Plant City, FL	N27°56′/W82°06′	Sweet orange	Yes[c]	Feb 2002	*B. phoenicis*	AY320016
12	Eustis, FL	N28°50′/W81°42′	Sweet orange	Yes[c]	Feb 2002	*B. phoenicis*	AY320017
13	Lake Alfred, FL	N28°06′/W81°42′	*Ligustrum*	Yes	Sep 2001	*B. phoenicis*	AY320018
55	Weslaco, TX	–	Pummelo	[b]	Nov 2000	*B. phoenicis*	AY320025
56	Donna, TX	–	Grapefruit	[b]	Nov 2000	*B. phoenicis*	AY320026
	Brazil						
17	Conchal, SP	–	Sweet orange	Yes	Apr 2001	*B. phoenicis*	AY320019
28	Monte Azul Paulista, SP	–	Sweet orange	Yes	Apr 2000	*B. phoenicis*	AY320020
30	Bebedouro, SP	–	Sweet orange	Yes	Apr 2001	*B. phoenicis*	AY320021
32	Teresopolis, RJ	–	Sweet orange	Yes	Oct 2000	*B. phoenicis*	AY320022
39	Araraquara, SP	S21°44′/W48°14′	Sweet orange	[b]	Dec 2002	*B. phoenicis*	AY320023
40	Patrocinio, MG	–	Coffee	[b]	Nov 2002	*B. phoenicis*	AY320024
61	Cordeiropolis, SP	S22°27′/W47°24′	Cleopatra mandarin	Yes	Aug 1998	*B. phoenicis*	AY320027
	Out-group species						
44	Charleston, SC	–	Tea	No	Oct 2002	*B. obovatus*	AY320028
45	Corvalis, OR	–	Apple	[b]	Aug 2002	*Cenopalpus pulcher*	AY320029
1	Lake Alfred, FL	N28°06′/W81°42′	Sweet orange	Yes[c]	May 2002	*Eutetranychus banksi*	AY320030

FL, Florida; TX, Texas; SC, South Carolina; and OR, Oregon, USA; SP, São Paulo; RJ, Rio de Janeiro; and MG, Minas Gerais, Brazil.
[a]Species identifications completed based on Baker (1949) and Baker and Tuttle (1987); [b]Mites were directly collected from field plants into 95% ethanol.
[c]Mites included for RAPD analysis.

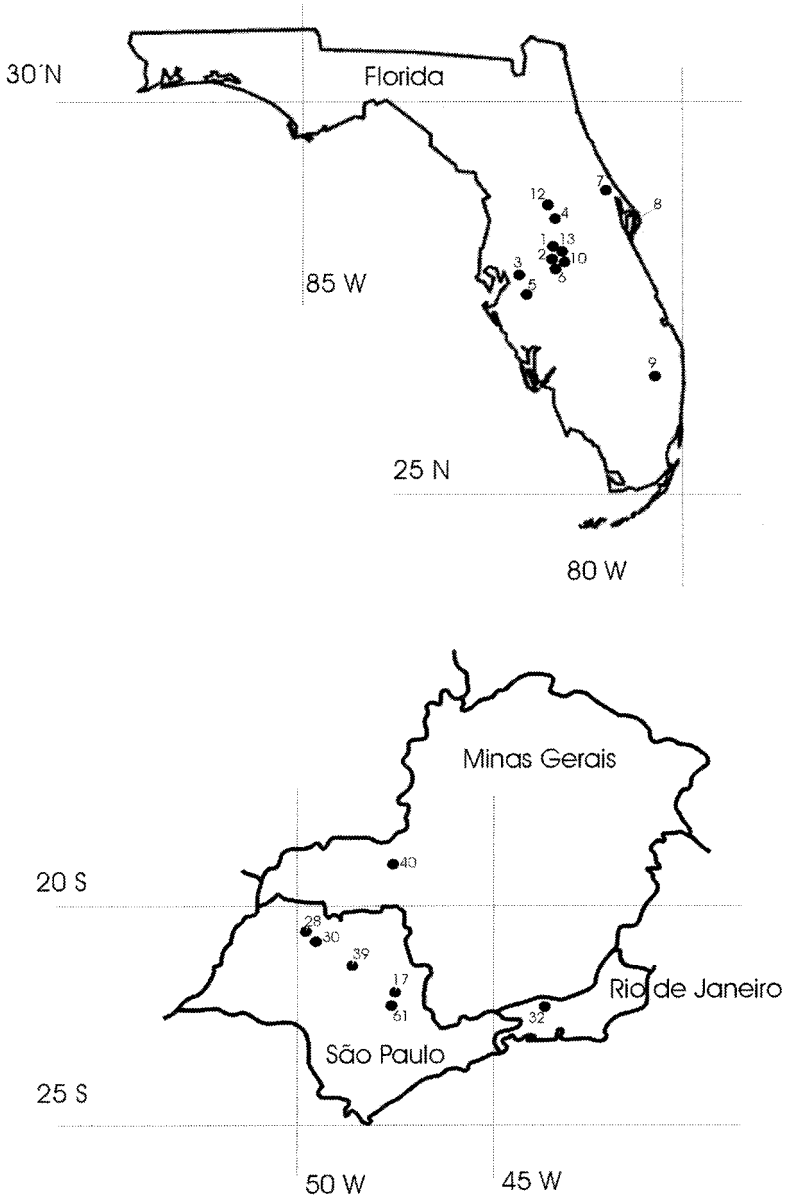

Figure 1. Map of Florida State (USA) and Southeastern States of São Paulo, Minas Gerais and Rio de Janeiro (Brazil) showing sample locations of specimens analyzed in the present study. Numbers of the collecting sites correspond with the numbers in Table 1.

lected in Florida, and *B. obovatus* from South Carolina (tea) were also included in this study to serve as out-group taxa and polarize in-group character states.

Morphological analysis

Brevipalpus larvae, protonymphs, deutonymphs, and adults from each colony were slide-mounted in Hoyer's mounting medium (Krantz 1978) and cured for 2 weeks at 45 °C in an oven for species identifications and future morphological comparisons. Species identifications were completed from 20 slides of each sample using keys of Baker (1949) and Baker and Tuttle (1987). A differential interference contrast and phase contrast microscope (Leica DMR, Olympus, BH) was used to identify the mite specimens. Voucher specimens were deposited in the USDA-ARS Systematic Entomology Laboratory in Beltsville, Maryland and Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

DNA extraction

Genomic DNA from each mite sample was extracted using the CTAB protocol described by Navajas et al. (1998). The resulting DNA pellet was resuspended in 20 μl double-distilled deionized and autoclaved water, and then 2 μl of the DNA solution was used for PCR. Twenty adult *Brevipalpus* females per isofemale line or field collected colony, and one adult *E. banksi* female, were used for DNA extraction. The same DNA extraction protocol was adopted for RAPD and Mitochondrial DNA procedures.

Mitochondrial DNA

The DNA fragment encoding the mitochondrial cytochrome oxidase subunit I (Mit-COI) gene was amplified using primers 5′-TGATTTTTTGGTCACCCAGAAG-3′ and 5′-TACAGCTCCTATAGATAAAAC-3′ (Navajas et al. 1996). PCR reactions were carried out in a final 50 μl volume, using 2 μl of the DNA solution as template, 1.4 mM of each dNTP, 1.4 μM (10-mer) of each primer, and 1 U/μl of *Taq polymerase* (Promega). The temperature profile was 4 min at 92 °C, 35 cycles of 1 min at 92 °C, 1 min at 50 °C, and 1 min at 72 °C, followed by a final extension of 10 min at 72 °C, using a GeneAmp 2400 thermal cycler (PerkinElmer, Inc., Wellesley, MA). The amplified 400 bp fragment was purified using the MinEluteTM PCR Purification Kit (Qiagen Operon, Alameda, CA) and sequenced directly, in both directions, using the same two amplification primers. A. PEC 9600 automated DNA thermalcycler was used in fluorescent cycle sequencing reactions (PerkinElmer-Cetus, Foster City, CA).

RAPD analysis

For a general analysis of the mite genome, randomly amplified polymorphic DNA (RAPD) profiles were compared. The commercially available primers:

A01, A02, A04, A07, A10, A11, A12, A13, A14, A16, and A20 (Operon Technologies, Alameda, CA), were used for the screening. DNA from mite colonies from Florida, numbers 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 (Table 1), were PCR amplified. PCR reactions were carried out in a final 25 μl volume, using 2 μl of the DNA solution as template, 1.4 mM of each dNTP, 1.4 μM (10-mer) of each primer, and 1 U/μl of *Taq polymerase* (Promega). The temperature profile was 4 min at 92 °C, 45 cycles of 1 min at 92 °C, 1 min at 36 °C, and 2 min at 72 °C, followed by a final extension of 10 min at 72 °C, using a PTC-100 programmable thermal cycler (MJ Research, Watertown, MA). PCR products were separated by electrophoresis in a 1.5% agarose gel and observed under UV light after ethidium bromide staining.

Phylogenetic and phenetic analysis

Data from COI sequences and RAPD banding patterns were compared independently. For RAPD, all included colonies were visually compared for presence or absence of bands of the same size, and scored as 1 or 0, respectively. Phenetic analysis of RAPD markers representing the 11 mite colonies culminated in a phenogram constructed from the calculated distance relations, using the Unweighted Pair Group Method with Arithmetic Mean Method (UPGMA; Sneath and Sokal 1973).

The Mit-COI sequence data were processed using BioEdit software (Hall 1999) and aligned using Clustal W (Thompson et al. 1994). The Mit-COI data matrix is publicly available at TreeBase (http://www.treebase.org; accession number SN1599–4891). As several of the taxa had identical Mit-COI sequences, duplicate sequences were excluded from the analyses.

A Neighbor Joining analysis of the Mit-COI data was done in MEGA version 2.1 (Kumar et al. 2001), and assumed the Kimura 2-parameter model of sequence evolution (Kimura 1980) with gamma distributed rate heterogeneity (inferred empirically). For maximum likelihood analyses, the model of sequence evolution was selected using the ratio tests implemented in ModelTest (Posada and Crandall 1998). Accordingly, the Tamura and Nei (1993) model was chosen with an expected transition/transversion ratio of 0.68, pyrimidine/purine transition ratio of 1.95 and predicted nucleotide frequencies of pi(A) = 27.2%, pi(C) = 11.3%, pi(G) = 13.7%, pi(T) = 47.8% (all estimated from the empirical data). Maximum likelihood analyses were carried out using PUZZLE 4.0.2 (Strimmer and von Haeseler 1996) that allowed for a gamma distributed rate heterogeneity of 0.3 to be distributed among eight categories. The aligned sequences did not show significant departure from homogeneous nucleotide use (5% chi-square test, ns).

Maximum Parsimony analysis was carried out in PAUP* ver. 4.0b10 (Swofford 2002). All characters in the matrix were treated as unordered, and polarized via out-group comparison with *Eutetranychus banksi*. Of the 374 characters in the matrix, 255 were constant and 79 were phylogenetically

informative. Forty variable characters were phylogenetically uninformative due to symplesiomorphic or autapomorphic polarities. Starting trees for the heuristic search strategy were obtained by stepwise addition and proceeded via TBR branch swapping. Bootstrap support for nodes was carried out under a similar full heuristic search strategy but with 1000 replicates. Character state analyses were explored and visualized in MacClade 4.06 (Maddison and Maddison 2002).

Results and discussion

Morphological identifications completed by contrast phase microscopy identified all samples as *B. phoenicis*, with exception of one collected from tea, that was identified as *B. obovatus* (Table 1).

Of the 374 bp fragment of the Mit-COI used to compare the phylogenetic relationships among the 24 mite samples examined in this study, a shared fragment (329 bp) of a Mit-COI sequence of *Cenopalpus pulcher* (European population), strain 'Montpellier' (GenBank accession number X80873) showed 90% similarity to the *C. pulcher* population included in this study from an Oregon (USA) apple orchard. *C. pulcher* is a pest mite of pome and stone fruits and is widely distributed in Europe, the Middle East, Central Asia, and North Africa and recently found in the Corvallis area of Oregon (USA) (Bajwa et al. 2001). The apparent low similarity reported here could be an indication that this strain from Oregon was not introduced from Europe. This statement should be confirmed by analysis of additional specimens from Europe, Asia and also a more detailed set of samples from Oregon. The Unweighted Pair-Group Average (UPGMA) (not shown) and Neighbor Joining (NJ) algorithms force the resolution of all nodes for unique (non-redundant) taxa and produced identical tree topologies (Figure 2) The maximum parsimony analysis yielded five equally parsimonious trees, the combinable components consensus of which is concordant with the quartet puzzling maximum likelihood solution, including an unresolved polytomy for the three main lineages of *B. phoenicis* (Figure 3). Bootstrap support for the major nodes on the NJ and UPGMA trees were virtually identical to the indices of the parsimony bootstrap analysis. However, the UPGMA and NJ trees form a cluster of the Brazilian haplotypes together and they are apart from the USA haplotypes. This differentiation, along with careful analysis of the DNA sequences themselves (Figure 3) indicates that there may be Brazilian and North American mitochondrial variants that are diagnosable within *B. phoenicis*. Improved sampling between these two regions will clarify whether these populations are truly structured geographically.

The Brazilian and North American variants, except accession #7 from Florida, all showed local silent substitutions. Accession number #7 was collected from a 100-year-old citrus orchard located in an isolated Atlantic coastal area near Oak Hill in Volusia County, Florida. The last reported cases of citrus

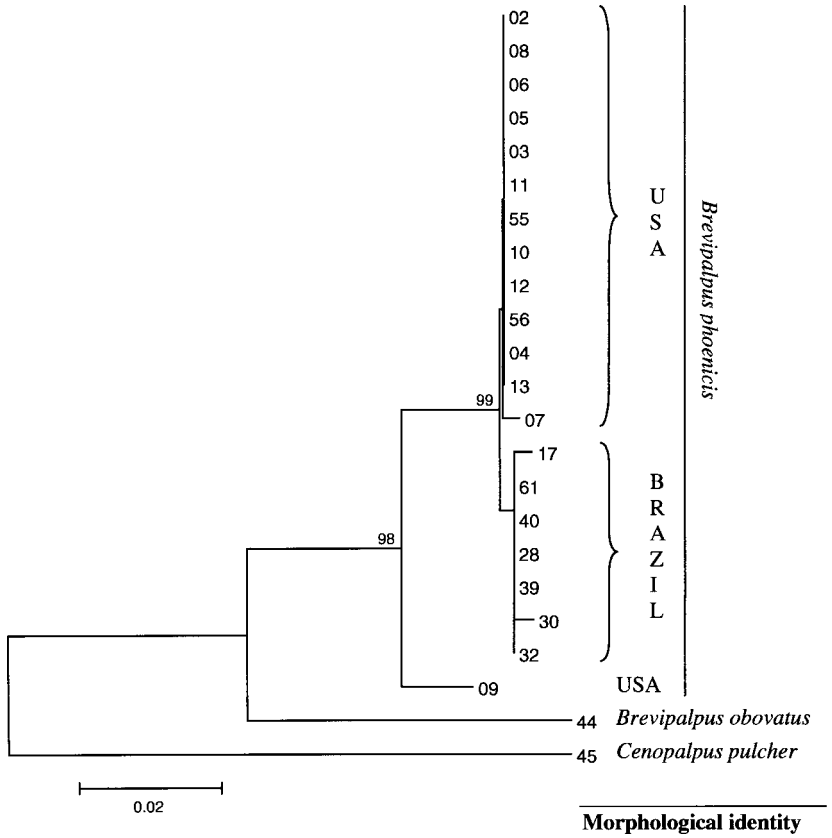

Figure 2. Phylogenetic relationships among *Brevipalpus* samples based on mitochondrial COI fragment (374 bp) (see Table 1 for details). Numbers at nodes refer to bootstrap values (1000 replicates) greater than 75%. The Neighbor Joining (NJ) method was used based on distances calculated using Kimura's two-parameter model with Gamma distributed rate heterogeneity. The Tenuipalpid *Cenopalpus pulcher* was used as an out-group species in the tree.

leprosis within Florida came from this area (Rodrigues et al. 2003). Upon exclusion of the silent base substitutions, the two Brazilian and North American groups cluster with minor internal differences. It is uncertain if the Florida populations are as efficient in vectoring citrus leprosis as the Brazilian or Central American mite populations, where citrus leprosis currently exists.

Sample #9, collected from *Hibiscus* in South Florida was phylogenetically distinct, from all of the others. *B. phoenicis* samples examined having evolving autapomorphic states at two bases (positions 136 and 291; Figure 3). Although those mites were morphologically identified as *B. phoenicis* they did not share any of the synapomorphic base substitutions uniting the other 20 colonies identified as *B. phoenicis* (Table 2). Since it was collected from *Hibiscus*, there is the possibility that it represents a new introduction into Florida by ornamental

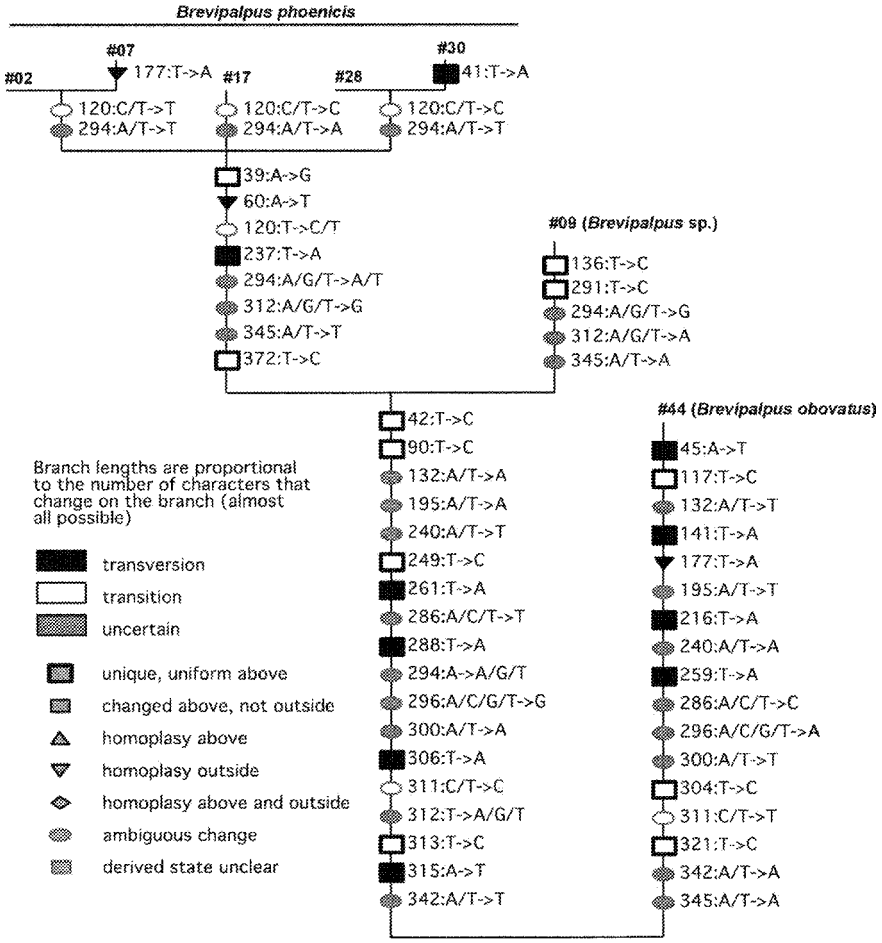

Figure 3. Character map of 374 bp of mitochondrial cytochrome oxidase subunit I gene for *Brevipalpus* taxa. Characters were mapped on the best estimate of phylogenetic relationships via maximum likelihood and parsimony analyses (congruent MP and ML trees; see text for details). Colonies #02, #07 and #09 were collected in Florida; #28 and #30 in Brazil (see Table 1 for details).

importations from other regions. Recently, *Brevipalpus* mites were detected, in ornamental plants arriving at the Miami Air Cargo Facilities from Central America (Rodrigues and Childers unpublished data). More intensive sampling efforts and molecular and morphological character analyses are needed to determine whether this lineage is representative of a new species or simply a variant population of *B. phoenicis*. The colonies collected from *Citrus, Rhododendron*, and *Ligustrum* collected from the Citrus Research and Education Center (CREC), in Lake Alfred, Florida showed the same sequences. No differentiation was observed between samples #2 and #6 collected from the same citrus orchard in Lake Alfred on different sampling dates. Historically, CREC

Table 2. DNA distance matrix[a] among different phylogenetic levels assessed by the analysis of the 374 bp fragment of the mitochondrial Cytochrome Oxidase I gene (COI).

A	B	C	Number	Species	02	17	09	44	45	01
Tetranychoidea	Tenuipalpidae	*Brevipalpus*	02	*Brevipalpus phoenicis* – USA	0					
			17	*Brevipalpus phoenicis* – Brazil	0.0054					
			09	*Brevipalpus* sp. – USA	0.0245	0.0273	0			
			44	*Brevipalpus obovatus*	0.0899	0.0897	0.0802	0		
			45	*Cenopalpus pulcher*	0.1537	0.1574	0.1543	0.1661	0	
			01	*Eutetranychus banksi*[b]	0.2751	0.2798	0.2751	0.2480	0.2896	0

[a]The model of nucleotide substitution used was the maximum likelihood phylogeny calculated using the DNAML program (Felsenstein 1995); A, Superfamily; B, Family; C, Genus.
[b]Tetranychidae.

was the location where transmission assays with citrus leprosis were conducted in Florida during 1949 and reported by Knorr (1968). *Brevipalpus phoenicis* collected from coffee in Minas Gerais State and from citrus in Rio de Janeiro State did not show patterns of nucleotide differentiation when compared with the Brazilian citrus colonies. The estimate of genetic distance by the Kimura 2-parameter model between group averages of the Brazilian and Florida samples was 0.0057 and was reduced to 0.0037 when sample #9 was excluded from the analysis. The estimate was even lower within the group average of the USA samples (from 0.0039 to 0.0004), when the population #9 was excluded.

Despite a small number of samples from plant species other than citrus, it is possible to speculate about the occurrence of host-range expansion, alternate hosts, and gene flow between *B. phoenicis* colonizing citrus and others plants. For example, samples from ornamentals plants, i.e. *Ligustrum* and *Rhododendron*, did not show detectable differences in their mitochondrial haplotype, or any restriction in their ability to establish colonies on citrus fruits. The occurrence of genetic isolation associated with the host plant effect could not be accurately assessed by this study because of the 'bottle neck' strategy adopted during the rearing process of the colonies. However, we noticed that many colonies were not able to establish on citrus fruit when the mites were collected from non-citrus plants. Those samples were not included in this study, which focused solely on mite diversity in citrus.

Figure 3 summarizes the relationships among *Brevipalpus* taxa included in this study. Nucleotide state changes are mapped on the best estimate of phylogenetic relationships among the sampled taxa. For purposes of clarity, states for two of the out-group taxa (also included in the analysis) are not shown. The orders of nucleotide state transformations are not reconstructed. Thus, apomorphic characters with multiple states are considered ambiguous even though parsimony mapping would suggest that they are autapomorphies (i.e. characters 120 and 294). Still, this conservative approach suggests that several diagnostic, autapomorphic characters unite the clade comprised of samples #2, #7, #17, #28 and #30. This distribution of characters is consistent with the hypotheses that the lineage is evolving independently of its sister taxon, and is behaving like a species (Frost and Kluge 1994; Ghiselin 1997). Although some of the taxa have evolved diagnostic differences (i.e. #7 and #30), their sister taxa can only be defined based on privative evidence (Adams 2001). Such a distribution of character states is not sufficient to infer lineage independence or that these sister taxa represent different species (Adams 1998, 2001; Frost and Kluge 1994; but see Wheeler and Platnick 2000). Instead, the pattern of nascent evolution among the lineages reflects a reticulate pattern of evolution consistent with tokogenetic relationships among entities sharing a common historical origin and fate (a single species; Wiley and Mayden 2000). The #9 taxon appears to have evolved at least two autapomorphies. Further study is required, including mites collected on *Hibiscus* from several geographically disjunct populations, to determine if its status as a separate species is warranted.

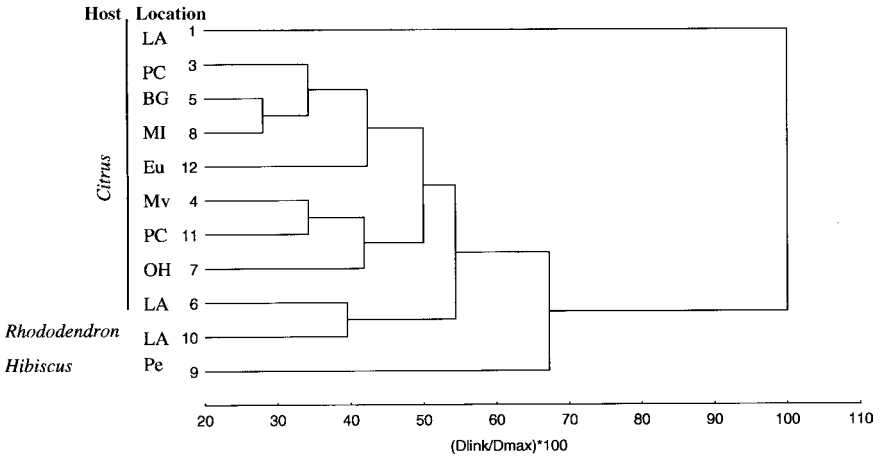

Figure 4. Phenogram based on Unweighted pair-group average (UPGMA) Euclidian distance cluster analysis from 102 RAPD markers for 10 *B. phoenicis* colonies collected in Florida and the *Eutetranychus banksi* (#1). LA, Lake Alfred; PL, Plant City; BG, Bowling Green; MI, Merritt Island; Eu, Eustis; Mv, Montverde; OH, Oak Hill; and Pe, Pembroke.

Since only freshly collected mites provided highly reproducible results using RAPDs we confined this analysis to 11 samples collected in Florida, USA. Consequently, RAPD analysis was conducted with ten *Brevipalpus* mite colonies and one of *Eutetranychus banksi*. *Eutetranychus banksi* belongs to the same mite superfamily, but it is genetically distant from tenuipalpid mites. It was included as a control in the RAPD study because it shows a very consistent, yet comparable banding pattern. The RAPD results agreed with the COI analysis with colony #9 being placed in a distinct group isolated from the cluster formed by the other colonies (Figure 4). High band polymorphisms were observed within the main cluster including the other *B. phoenicis* samples. Similar results were observed previously between *Ligustrum* and citrus colonies *of B. phoenicis* from Brazil (Rodrigues et al. 1996). Because of the lack of specificity of the RAPD primers, it is possible that one or more endosymbiotic organisms or other alien DNA were simultaneously generated during RAPD amplification. Therefore, these results must be validated by another technique. However, in agreement with our RAPD results, Weeks et al. (2000) showed large differences in four samples of mites from citrus by AFLP (amplified fragment length analysis) and expressed doubts about the uniqueness of parthenogenetic reproduction of this mite.

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