Comparison of Laboratory Colonies and Field Populations of *Tamarixia radiata*, an Ectoparasitoid of the Asian Citrus Psyllid, Using Internal Transcribed Spacer and Cytochrome Oxidase Subunit I DNA Sequences

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ABSTRACT The genetic diversity of *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) laboratory colonies derived from collections in China, northern Vietnam, Pakistan, and a mixed colony from Taiwan and southern Vietnam was evaluated using the internal transcribed spacer (ITS) region 1, ITS-2, and the 5′ end of the cytochrome oxidase subunit I gene. The strains share the same ITS sequence, consistent with the morphological hypothesis that the collections represent a single species. The COI marker was variable and could distinguish the northern Vietnam and Pakistan colonies from each other and from the other colonies. Comparison of COI sequences from field-collected populations of Puerto Rico, Guadeloupe, and Texas indicates that Florida is not a likely source of the introduction into Puerto Rico but is a likely source of the introduction into Texas.

KEY WORDS biocontrol, citrus greening, pathway analysis, DNA barcode

Tamarixia radiata Waterston (Hymenoptera: Eulophidae) is a species-specific ectoparasitoid of the Asian citrus psyllid *Diaphorina citri* Kuwayama (Meyer and Hoy 2008). In addition to causing direct damage to citrus crops, *D. citri* is a vector of *Candidatus* Liberibacter asiaticus, a bacterium that causes citrus greening also known as "huanglongbing" (HLB) disease (Bové 2006, Meyer and Hoy 2008). Adult females of *T. radiata* host-feed on younger instars of *D. citri* and prefer to oviposit underneath the later instars, particularly the fifth-instar nymph (Chu and Chien 1991). A single female parasitoid can lay up to 300 eggs at 25–30°C (Chu and Chien 1991, Étienne et al. 2001). Through combined behaviors of host feeding and oviposition, a single *T. radiata* is capable of destroying 500 *D. citri* nymphs during her life time (Chien 1995).

Native to Asia, indigenous populations of *T. radiata* have been reported from India (Chien 1995), Pakistan (Waterston 1922), Nepal (Lama et al. 1988), China (Tang 1988), and Vietnam (R. N., personal observation). To reduce the spread of *D. citri* and citrus greening, *T. radiata* from northwestern India was introduced to Reunion in 1978 (Aubert 1987b). This established Reunion population was subsequently introduced to Mauritius (Quilici 1986), Taiwan (Chiu et al. 1988), and Guadeloupe (Étienne et al. 2001).

Additional introductions of *T. radiata* were made to the Philippines (from Reunion; Gavarra et al. 1990), Saudi Arabia (Aubert 1984), East Java, Indonesia (Nurhadi 1987), and Florida (Hoy et al. 1999). The *T. radiata* released in Florida were from a lab colony initiated using collections from an established population in Taiwan (i.e., Reunion derived) and a population from Tien-Giang, in southern Vietnam (Hoy 1998, Hoy et al. 1999). Nonintentional introductions of *T. radiata* also have been reported in Puerto Rico (Pluke et al. 2008), Brazil (Torres et al. 2006), Venezuela (R.N., unpublished), Mexico (D.G.H., unpublished), and Texas (French et al. 2001).

In total, 12,000, 16,800, and 8,000 adults of the mixed origin colony (i.e., Taiwan and south Vietnam) maintained by Florida's Department of Plant Industry (DPI) were released in Florida in 1999, 2000, and 2001, respectively (Skelley and Hoy 2004). *T. radiata* was reported as established and overwintering in southeastern Florida in 1999–2000 (Hoy and Nguyen 2001). Reported rates of parasitism of *D. citri* by *T. radiata* within Florida have been lower, rarely exceeding 20%, compared with rates reported for previous biocontrol programs by using *T. radiata* (Tsai et al. 2002, Michaud 2004, Hall et al. 2008, Qureshi et al. 2009). In Reunion, Guadeloupe, and Puerto Rico, reported parasitism rates were much higher (often exceeding 90%), and *T. radiata* was reported to significantly suppress the psyl-

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lid populations (Étienne and Aubert 1980, Chien and Chu 1996, Étienne et al. 2001, Pluke et al. 2008). Establishment of *T. radiata* and *Tamarixia dryi* Waterston (parasitoid of *Trioza erytreae,* vector of African greening) was credited with virtually arresting the HLB epidemic in Reunion (Aubert 1987a).

To further study *T. radiata* parasitism rates and augment the currently established populations within Florida, additional quarantined lab strains of *T. radiata* are being maintained at Florida's DPI. These strains were derived from collections made in southern China, Pakistan, and northern Vietnam. A comparison of behavioral (handling time and plant host preferences) and physiological (responses to temperature and humidity) characteristics of these strains with the original lab strain in Florida should yield useful information on how to improve the efficacy of the biocontrol program through augmentation within Florida or introductions of *T. radiata* strains to other regions where *D. citri* is present.

There were no significant morphological differences observed among these four strains of *T. radiata* (Greg Evans, personal communication), and the development of molecular markers useful to distinguish among them would enhance any future studies of their behavior and physiology. Molecular analysis of DNA can provide a useful option for the development of such identifying markers (Gariepy et al. 2007). To date, there are no published molecular genetic analyses of *T. radiata* that either report diversity estimates of the species or compares diversity among geographic populations. The identification of strain-specific genetic markers could facilitate future study of strain-specific behaviors that affect parasitism rates in pre- and postrelease experiments (Taylor and Szalanski 1999, Gariepy et al. 2007).

In this study, we use DNA sequences from the cytochrome oxidase subunit I (COI), internal transcribed spacer (ITS) region 1, and ITS-2 loci to compare the genetic diversity of the four *T. radiata* lab strains initiated at DPI and field-collected populations from Florida, Texas, Puerto Rico, and Guadeloupe. Despite morphological evidence that these collections represent a single species, it is possible that *T. radiata,* as currently recognized, comprises distinct evolutionary lineages or even cryptic species that can only be detected using genetic analysis (Avise 2004). Evidence of cryptic species within *T. radiata* would impact future decisions to release the new strains. The three loci selected for analysis of *T. radiata* in our study have been used extensively as markers to study phylogenetics, taxonomic identification, and population structure of insect species (Hillis and Dixon 1991, Ji et al. 2003, Avise 2004, Ratnasingham and Hebert 2007, Coleman 2009) and should be useful in the detection of cryptic species. In addition, genetic evidence of potential reproductive isolation among the collections also could be used to evaluate the probable source of nonintentional *T. radiata* introductions (Barr 2009).

Materials and Methods

Insect Collections. *T. radiata* were reared from parasitized nymphs of *D. citri.* In Florida, the parasite was reared in a cage made up by a Plexiglas cylinder 36 cm in height and 12 cm in diameter. The top of the cylinder was sealed by a piece of nylon organdy (60 mesh). Four holes were cut on the wall of the cylinder and also sealed by a piece of nylon organdy for aeration. A pot of orange jasmine, *Murraya paniculata* (L.) Jack, infested with *D. citri* of third and fourth nymphal stages was covered with the cage. The cage was housed in a greenhouse of the DPI quarantine laboratory at a temperature of 26.5°C and 40–70% RH. The life cycle of parasite from egg to adult was 11–12 d. Before analysis, insects were collected as adults and killed in 95% ethanol at 4°C. Vouchers of the lab colonies were deposited in the Florida State Collections of Arthropods (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL), and vouchers of the field collections were deposited in the USDA–ARS insect collections at the Subtropical Insects Research Unit in Fort Pierce, FL. Unless stated otherwise, adult *T. radiata* specimens were identified by Dr. Gregory A. Evans (USDA–APHIS–PPQ/Systematic Entomology Laboratory, Beltsville, MD).

Florida Department of Agriculture and Consumer Services–DPI Laboratory Strain Derived from Taiwan and Vietnam Collections (DPI-TV). Collections from the DPI-TV lab strain were made at the Gainesville Lab on 23 October 2006 and 13 November 2007 and at the Mission Lab in Texas on 16 June 2008. The Mission lab colony was started on 7 May 2008 from the DPI-TV strain.

DPI Lab Strain Derived from Northern Vietnam Collection (DPI-NV). Strain started from collection of parasitized nymphs made by R.N. in Bac-Ninh Province (21° 11′40.76″ N, 106° 6′21.45″ E) on 21 October 2007. Adult specimens were collected on 29 November 2007 and 24 December 2007.

DPI Lab Strain Derived from a Southern China Collection (DPI-CH). Strain started from collections of parasitized nymphs made by P. Stansly and J. Qureshi from *Citrus* spp. and *M. paniculata* in Nansheng (22° 50′0″ N, 112° 03′51″ E; 105-m elevation), Weizheng, (23° 34′35″ N, 112° 42′34″ E; 31-m elevation), and Guangzhou (23° 09′31″ N, 113° 21′08″ E, 32-m elevation), Guangdong Province, China on 6–9 November 2008. Specimens were collected from second generation lab colony on 9 December 2008.

DPI Lab Strain Derived from Pakistan (DPI-PK). Strain started from a collection made by A. Rehman from citrus in Punjab, Pakistan (30° 09′15.79″ N, 71° 26′51.32″ E) on 29 August 2008. The adult specimens were collected from first generation of lab colony on 17 September 2008.

Field Collections of *Tamarixia* from Florida Groves Where Lab Strain DPI-TV Was Released. Eight citrus groves (locations) across six counties of Florida were sampled for *D. citri* nymphs parasitized by *T. radiata:* Stapely Grove (sweet orange; 29 November 2006, Bre-

vard County; 27° 59′2.94″ N, 80° 50′48.06″ W), Vero Beach Grove (sweet orange; 13 March 2007, Indian River County; 27° 39′3.6″ N, 80° 28′19.02″ W), Williamson Grove (sweet orange; 13 November 2006, Okeechobee County; 27° 17′58.65″ N, 80° 46′32.46″ W), Fort Pierce Grove (sweet orange; 14 December 2004, Saint Lucie County; 27° 24′39″ N, 80° 24′26.4″ W), Shadow Lakes Grove (sweet orange; 26 September 2006, Martin County; 27° 12′10.38″ N, 80° 21′16.26″ W), Sexton Grove (sweet orange; 3 August 2006, Saint Lucie County; 27° 32′53.4″ N, 80°25′19.2″ W), Callery Grove (grapefruit; 15 May 2006, Palm Beach County; 27° 24′45.36″ N, 80° 23′49.98″ W), and USDA Grove (sweet orange; 24 May 2006, Saint Lucie County; 27° 26′2.82″ N, 80°25′37.26″ W).

At each site, fourth- or fifth-instar *D. citri* nymphs were collected from trees. Flush shoots (as defined by Hall and Albrigo 2007) containing nymphs were placed in a ziplock bag, labeled, and transported in an insulated cooler to the laboratory where actual numbers of fourth- and fifth-instar nymphs were identified and counted by using a stereoscopic microscope. Nymphs were kept in ventilated containers held at ambient temperatures in a laboratory for at least 2 wk to rear adults of *T. radiata.* Adult specimens were identified by D.G.H.

Field Collections of *Tamarixia* from Edinburg, TX. Adults from the Rio Grande Valley (Hidalgo County) of southern Texas were collected from a citrus tree in a USDA shade house on Moore Air Base (26° 23′41.64″ N, 98° 20′5.71″ W, 57-m elevation) on 16 June 2008 and a Citrus tree at the intersection of 3809 Mon Mack and Shadowood in the City of Edinburg (26° 18′35.96″ N, 98° 12′5.62″ W, 28-m elevation) on 26 June 2008.

Field Collections of *Tamarixia* from Puerto Rico. Collections of parasitized nymphs were made by R. Pluke from San Juan (18° 24′15″ N, 66° 02′42″ W, 24-m elevation) and Isabela (18° 27′46″ N, 67° 03′07″ W, 134-m elevation), Puerto Rico in September 2005.

Field Collection of *Tamarixia* from Guadeloupe. Collections made by J. Etienne at Petit Bourg (16° 03′46″ N, 61° 40′49″ W, 230-m elevation) on 25 January 2005.

DNA Isolation. DNA was isolated from specimens using 1) a phenol-chloroform extraction protocol, 2) a modified Wizard Genomic DNA protocol (Promega, Madison, WI), or 3) the DNeasy extraction method for animal tissue (QIAGEN, Valencia, CA).

Using the Phenol-Chloroform protocol a specimen was placed into a 1.5-ml tube with 300 μl of extraction buffer (0.1 M Tris-HCl, pH 8.0, 0.05 M EDTA, 0.5 M NaCl, and 1% *N*-lauroylsarcosine), homogenized with a sterile pestle, and incubated for 1 h at 55°C. After incubation, 300 μl of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the tube, and the tube was vortexed and centrifuged at 12,000 × *g* for 10 min at 4°C. The resulting supernatant was transferred to a new tube containing 100% ethanol at 4°C, mixed, and centrifuged at 14,000 × *g* for 10 min at 4°C. The supernatant was discarded and the pellet washed with 70% ethanol. The dried DNA pellet was rehydrated in 15 μl of sterile water and stored at −20°C.

Specimens extracted using the modified Wizard Genomic DNA purification protocol were transferred to tubes containing 500 μl of cell lysis buffer and crushed with a sterile pestle. This tissue buffer solution was mixed with 300 μl of nuclei lysis solution and then incubated with 5 μl of proteinase-K (20 mg/ml) at 37°C for 3 h. The sample was treated with 3 μl of RNase for 30 min at 37°C and then mixed with 100 μl of protein precipitation solution. The samples were incubated at 4°C for 5 min and then centrifuged at 16,000 × *g* for 5 min. The supernatant (containing DNA) was transferred to a tube containing 300 μl of isopropanol, mixed, and centrifuged at 16,000 × *g* for 5 min at room temperature. The supernatant was discarded, and the DNA pellet washed with 600 μl of 70% ethanol at room temperature in a centrifuge at 16,000 × *g* for 1 min. The ethanol was discarded and the pellet air-dried. The isolated DNA was rehydrated by incubating the pellet at 65°C for 1 h in 15 μl of DNA rehydration solution. The resulting extractions were stored at 4°C.

Specimens extracted using the DNeasy method were crushed in 180 μl of ATL lysis buffer by using a sterile pestle and then incubated overnight at 56°C with 20 μl of proteinase K. The DNA was isolated following the manufacturer's protocol and eluted in 100 μl of EB buffer and stored at 4°C.

Polymerase Chain Reaction (PCR) and Sequence Analysis. Overlapping fragments of the cytochrome oxidase subunit 1 (COI) gene were amplified using the LCO-1490 (5-GGTCAACAAATCATAAAGATATTGG) and HCO-2198 (5-TAAACTTCAGGGTGACCAAAAATCA) primer pair combination (Folmer et al. 1994) or the C1-J-1718 (5-GGAGGATTTGGAAATTGATTAGTTCC) and C1-N-2191 (5-CCCGGTAAAATTAAAATATAAACTTC) primer pair combination (Simon et al. 1994). Reactions were performed in total volumes of 50 μl by using 1 μl of DNA template and Ex *Taq* polymerase (Hot Start Version, Takara Bio USA, Madison, WI) at the manufacturer's recommended reagent concentrations: 1× buffer, 0.2 mM each dNTP, 0.2 μM each primer, and 0.025 U/μl *Taq.* The COI fragments were amplified under the following conditions: 94°C × 3 min., followed by 39 cycles of (94°C × 20 s., 50°C × 20 s., 72°C × 30 s.), and an extension step of 72°C × 5 min. PCR products were visualized on agarose gels to confirm amplification of samples and nonamplification of negative controls (water).

The ITS-1 and ITS-2 PCR fragments were generated using the ITS1-F/18sF (5-GTGAACCTGCGGAAGGA) and 5.8sR (5-GTTCATGTGTCCTGCAGTTCACA) primer pair and the 5.8sF (5-TGTGAACTGCAGGACACATGAAC) and 28sR (5-ATGCTTAAATTTAGGGGGTA) primer pair (Porter and Collins 1991), respectively. Amplifications of the two ITS fragments were performed in 50-μl reactions using 1–2 μl of DNA as template and an Ampli *Taq* polymerase protocol (Applied Biosystems; 1× buffer, 0.1 mM dNTP, 0.2 μM each primer, 0.03 U/μl *Taq*), the Platinum PCR SuperMix (0.2 μM each primer) (Invitrogen, Carlsbad, CA), or the Ex *Taq* protocol as reported previously for PCR of COI. The ITS-1 fragment was

amplified under the following conditions: 94°C × 3 min., followed by 35 cycles of (94°C × 30 s., 55°C × 30 s., 72°C × 50 s.), and an extension step of 72°C × 7 min. The ITS-2 fragment was amplified using the same program file as ITS-1 except the annealing temperature was set to 50°C.

PCR products were visualized on 1% agarose gels to confirm amplification of samples and nonamplification of negative controls (water). These products were then purified using QIAquick purification columns (QIAGEN). Sequencing reactions were performed in both directions using the DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA) on a Beckman-Coulter CEQ8000 sequencer or using the BigDye terminator version 3.1 chemistry on a 3730 xl DNA analyzer (Applied Biosystems, Foster City, CA). Sequences were edited using Sequencher version 4.8 (Gene Codes, Ann Arbor, MI), aligned by hand using the MEGA4 alignment editor (Tamura et al. 2007), and submitted to GenBank (accessions GQ401922-GQ401993, ITS-1; GQ401745–GQ401811, ITS-2; and GQ401812–GQ401921, FJ152417–FJ152427, COI).

The number and location of variable sites and genetic distances (p-) of the aligned ITS-1, ITS-2, and COI gene fragments were calculated using MEGA4 (Tamura et al. 2007). Haplotypes were identified by comparing pairwise distances and the topological placements of sequences on a neighbor-joining tree (p-distance) using MEGA4. Phylogenetic structure of unique haplotypes was estimated using the parsimony network analysis implemented in the program TCS version 1.21 (Clement et al. 2000), with a 95% connection limit.

Results

Based on agarose gel separation, all the tested specimens produced PCR amplicons of equal size for ITS-1 (≈550 bp), ITS-2 (≈550 bp), and COI (≈700 bp, by using the Folmer et al. [1994] primer pair; ≈470 bp using the Simon et al. [1994] primer pair). From these amplicons, bidirectional DNA sequences were generated for 72, 67, and 121 specimens for the ITS-1, ITS-2, and COI, respectively.

The ITS-1 and ITS-2 sequences include *T. radiata* specimens collected from each of the four lab strains currently maintained at the DPI lab (i.e., TV, CH, PK, and NV) and field collections from Florida, Puerto Rico, and Guadeloupe. Most of the edited, bidirectional ITS-1 sequences (97%) ranged in size from 400 to 465 bp. The amplified ITS-1 fragment includes parts of the 18S ad 5.8S rRNA genes but because the 18SF primer is located near the beginning of the ITS-1 locus it is not included in the sequence data. A portion of the 5.8S gene is included in the reported sequence data at the 3′ end. Comparison of our alignment with the 18S and 5.8S sequences of another hymenopteran sequence also in the family Eulophidae (*Diglyphus isaea*, AY948084) indicates that the length of the *T. radiata* ITS-1 locus is expected to be 436 bp. There are no variable sites within the alignment of *T. radiata* ITS-1 sequences.

Most of the edited, bidirectional ITS-2 sequences (98%) ranged in size from 430 to 465 bp. The amplified ITS-2 fragment includes parts of the 5.8S ad 28S rRNA genes but, because the 28SR primer is located near the end of the ITS-2 locus, it is not included in most of the reported sequence data. The 5.8S gene is included in the reported sequence data at the 5′ end. A comparison with the 5.8S and 28S sequences of another hymenopteran sequence (*Trichomalopsis dubois*, U02961) in the closely related family Pteromalidae indicates that the length of the *T. radiata* ITS-2 locus is expected to be 411 bp. There are no variable sites within the alignment of *T. radiata* ITS-2 sequences.

Initial analyses of COI were performed using the Folmer et al. (1994) primers and generated DNA sequences between 520 and 571 bp of which only seven sites were variable in an alignment. No gaps were present in the protein encoding gene sequences. Sequencing of these fragments with the LCO primer produced lower quality data than did the HCO primer; consequently, the C1-J-1718 primer was used as an internal sequencing primer to generate complete bidirectional data. Based on the locations of the variable sites and the efficiency of the C1-J-1718 primer, subsequent analyses were performed using the C1-J-1718 and C1-N-2191 primer pair for both amplification and sequencing.

The COI alignment of 412 bp generated from 121 specimens (Table 1) includes six variable sites (Table 2) and no gaps. All six variable sites are synonymous substitutions located at the first or third coding positions. Only two of the sites are parsimony informative; the other four are singletons.

The arrangement of nucleotide substitutions at the six variable sites generates six unique haplotypes within the data set. These haplotypes are coded alphanumerically as Hap-1 thru Hap-6 in our study (Table 2) and range in genetic diversity from 0.2 to 1.2% divergence. A parsimony network of the six haplotypes is reported in Fig. 1 to demonstrate the possible evolutionary history of the haplotypes and the observed mutations separating these haplotypes. In the network each haplotype is represented by a large circle, mutational differences are represented by lines, and "hypothetical" intermediate haplotypes are represented by small circles.

The COI data set includes specimens from the four DPI lab strains, a lab strain in Texas derived from the DPI-TV strain, and field collections from Texas, Florida, Puerto Rico, and Guadeloupe (Table 2). Three of the haplotypes are restricted to lab strains (Hap-4, northern Vietnam and Hap-5, Pakistan) or geographic field sites (Hap-6, Puerto Rico and Guadeloupe). The other three haplotypes are found within the DPI-TV strain and/or collection sites within Florida that are expected to be derived from the DPI-TV strain. Based on our samples, the strain from southern China (DPI-CH) is fixed for Hap-1 and the field-collected population collected from southern Texas is fixed for Hap-2.

Table 1. COI data sorted by collection source: sample sizes (*N*) and haplotype counts indicated

Tamarixia pop	Total tested *N*	Count based on haplotypes					
		Hap-1	Hap-2	Hap-3	Hap-4	Hap-5	Hap-6
Gainesville, FL (Lab strain DPI-TV)	24	22	2				
Mission, TX (Lab strain DPI-TV)	5	5					
Stapely Grove, FL	4	4					
Vero Beach Grove, FL	4	4					
Williamson Grove, FL	1	1					
Fort Pierce, FL	1	1					
Shadow Lakes Grove, FL	4	2	1	1			
Sexton Grove, FL	4	4					
Callery Judge Grove, FL	4	3	1				
USDA Grove, FL	2		2				
Edinburg, TX	7		7				
San Juan, PR	7						7
Isabela, PR	4						4
Guadeloupe	5						5
N. Vietnam (Lab strain DPI-NV)	20				20		
S. China (Lab strain DPI-CH)	10	10					
Pakistan (Lab strain DPI-PK)	15					15	
Total	121	56	13	1	20	15	16

Discussion

In this study, we have tested three genetic markers for variability among lab strains of *T. radiata.* The ITS-1 and ITS-2 loci exhibited no variability among the tested specimens. In general, the ITS loci are useful diagnostic markers because they experience high levels of divergence between species but low level of divergence within a species (Hillis and Dixon 1991, Taylor and Szalanski 1999, Ji et al. 2003). The lack of divergence in the *Tamarixia* data set supports the morphological hypothesis that these different lab strains and collections represent a single species.

The COI marker was variable in our study and supports evidence for reproductive isolation among lab strains. Three of the lab strains exhibited no genetic diversity (0% within China, Pakistan, and northern Vietnam). This is consistent with expectations for a bottlenecked population caused by small samples sizes during the collection and rearing stages. However, population structure or overall low diversity within the source countries could also explain these low values.

The original Florida lab strain (DPI-TV) maintained at the Florida DPI Lab and Texas Mission Lab exhibited two haplotypes (Hap-1 and Hap-2). The relatively greater diversity within this strain may reflect that it was initiated from multiple sources. As expected, the field-collected populations within Florida exhibited both Hap-1 and Hap-2. But a rare, third haplotype (i.e., Hap-3) was documented at one Florida collection site (i.e., Shadow Lake) and not in the DPI-TV lab strain. If rare in the original lab strain, then this haplotype could have been lost from the lab strain due to drift after the parasitoids were released in Florida or it could still be present at a low frequency within the lab strain. The sample sizes in our study are too low to distinguish these possibilities.

Based on our collections, the COI marker is useful for distinguishing among the China (DPI-CH), northern Vietnam (DPI-NV), and Pakistan (DPI-PK) strains because they are fixed for different haplotypes (Hap-1, Hap-4, and Hap-5, respectively). However, the DPI-CH strain cannot be distinguished from the original DPI-TV strain.

The field-collected population from Guadeloupe can be distinguished from all the DPI lab strains. It is interesting that Guadeloupe exhibits a haplotype (Hap-6) distinct from the original DPI-TV strain because the Taiwan population (used to start the DPI-TV strain) was initiated from a collection made in Reunion and the population in Guadeloupe was imported from Reunion during 1999. Perhaps Reunion exhibits a greater level of diversity than the populations included in our study or the variation present in the DPI-TV strain is derived from the southern Vietnam collection. Further study is required to explore these possibilities.

Table 2. Character states and positions of variable nucleotide sites in COI gene for the six haplotypes

Haplotype	Nucleotide position					
	43	67	339	393	399	402
Hap-1	C	T	C	A	T	T
Hap-2	T	T	C	A	G	T
Hap-3	C	T	C	A	T	C
Hap-4	T	T	T	A	G	T
Hap-5	T	T	C	A	A	T
Hap-6	T	C	C	G	A	T

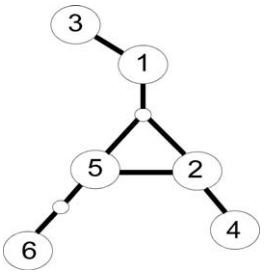

Fig. 1. Parsimony network of the six haplotypes (*Hap*I through Hap-6) reported for *T. radiata* tested in the study.

Fig. 2. Map of known world wide distribution of *T. radiata* (presence indicated by dots on countries).

Regarding nonintentional introductions, the samples from Puerto Rico share the Guadeloupe haplotype. When *T. radiata* was discovered in Puerto Rico during 2002, Florida was considered a possible source for the introduction. Our data does not support this pathway. The field-collected populations of parasitoids in Texas, however, are consistent with the genetic profile of the wasps released in Florida.

An evaluation of the most likely pathways of *T. radiata* movement into Texas (or other regions) will require further analyses including collections from Latin America and Asia (Fig. 2). Although the COI gene region reported in our study will provide useful information to the study of *T. radiata* population genetic structure, additional gene regions will be required to provide greater resolution and confidence in pathway analyses.

The region of the COI gene analyzed in our study has recently been proposed as a diagnostic marker for species level identification of animals by using a method called DNA barcoding (Hebert et al. 2003a). Although application of this marker for species description is problematic (DeSalle et al. 2005, DeSalle 2006), it is interesting to note that the greatest level of divergence within our data set (1.2% between Hap-3 and Hap-6) is within the expected the levels of intraspecific divergence based on empirical studies of other species (Hebert et al. 2003b, Meyer and Paulay 2005, Meier et al. 2006). In conclusion, both the nuclear and mitochondrial markers that we used to study *T. radiata* diversity indicate relatively low levels of divergence among lab strains and suggest no evidence of cryptic species within the taxon.

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