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PHYLOGENETIC ANALYSIS OF *TRIALEURODES* SPP. (HEMIPTERA: ALEYRODIDAE) FROM INDIA BASED ON DIFFERENCES IN MITOCHONDRIAL AND NUCLEAR DNA

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Abstract

About 55 species of *Trialeurodes* have been described with only a few viruliferous species like *T. vaporariorum* and *T. abutilonea,* which are able to transmit Criniviruses (Closteroviridae) and *T. ricini*, which is able to transmit *Begomovirus* spp. (Geminiviridae). Quick and accurate identification of whitefly vectors in the early life stages is important from the point of view of understanding the epidemiology of crinivirus associated plant diseases, and in their management and quarantine. Morphological identification is further strengthened by DNA barcoding. In this regard, we examined genetic differences in mitochondrial cytochrome oxidase-I (COI) gene and the internal transcribe spacers (ITS) sequences of ribosomal DNA among various populations of *T. vaporariorum* collected from different geographic locations in the state of Karnataka, Tamil Nadu, and Andhra Pradesh in India. Our studies are the first to analyze the genetic variation of *T. vaporariorum* populations in India, which showed no appreciable differences. This clearly indicated that there are no cryptic species or biotypes in *T. vaporariorum* in India in contrast to the studies of *B. tabaci* in which there is evidence for many biotypes. The phylogenetic analyses comprised of 4 *Trialeurodes* species showed 2 clades. Clade I is comprised of *T. vaporariorum* and *T. abutilonea* which are genetically close. Clade II consists of the remaining 2 species, viz., *T. lauri* and *T. ricini*. Also the current study provided evidence of the suggested emergence of biotypes *T. ricini.*

Key Words: Criniviruses, *Trialeurodes vaporariorum,* DNA barcoding, cryptic species, biotypes

Resumen

Se han descrito cerca de 55 especies de Trialeurodes y de estas hay unas cuantas especies que son reportadas como vectores de Crinivirus (Closteroviridae). T*rialeurodes vaporariorum* y *Bemisia tabaci* son dos vectores importantes del virus de enrollamiento amarillo de las hojas del tomate (TYLCV) y del virus de enanismo amarillo de la papa (PYDV). Una identificación rápida y precisa de los vectores de la mosca blanca en las etapas tempranas de vida es importante desde el punto de vista de la comprensión de la epidemiología de los crinivirus, su manejo y también la cuarentena. Utilizando las técnicas moleculares, la identificación morfológica ha sido reforzada aun mas por el uso de la codificación de barras del ADN utilizando primers universales de códigos de barras. En este estudio se analizaron las diferencias genéticas en el COI y ITS en 14 poblaciones de *T. vaporariorum* recolectadas de diferentes sitios geográficos en Karnataka, India. Nuestros estudios sobre *T. vaporariorum* mostró por primera vez que no hay diferencias apreciables genéticas entre las diferentes poblaciones. Esto indica claramente que no existe especies crípticas o biotipos en *T. vaporariorum* al contrario de las observaciones en *B. tabaci*, donde se han reportado varios biotipos. Un filograma fue construido para cuatro especies de *Trialeurodes* utilizando información disponible del nucleótidos del CO1 en el NCBI-GenBank junto con nuestros datos.

Palabras Clave: Crinivirus, *Trialeurodes vaporariorum*, codificar bandas de ADN, especies crípticas, biotipos

Whiteflies (Hemiptera: Aleyrodidae) are important sap sucking insect pests of agricultural and horticultural crops, and can cause crop damage either by direct feeding or by transmission of plant pathogenic viruses. The three most damaging and ubiquitous species are the cotton whitefly or sweet potato whitefly (*Bemisia tabaci* Gennadius), sliver leaf whitefly (*Bemisia argentifolii* [Bellows and Perring]*)* and the green house whitefly (*Trialeurodes vaporariorum* Westwood). Their rapid population growth is due in part to arrhenotoky or arrhenotokous parthenogenetic reproduction of whiteflies in which unfertilized eggs develop into males and fertilized eggs to females. The piercing-sucking feeding behavior of whiteflies can cause considerable crop damage. Currently 55 species in the world are assigned to the genus *Trialeurodes* (Mound & Halsey 1978), which is one of the largest genera in the family Aleyrodidae. The greenhouse or glasshouse whitefly, *Trialeurodes vaporariorum*, was described by Westwood in 1856 as *Aleyrodes vaporariorum*. It is a polyphagous insect, and plants belonging to 82 families serve as its hosts (Mound & Halsey 1978). Feeding by nymphs and adults draws proteins and other nutrients of plant sap, and they excrete surplus sugars as 'honeydew'. At high populations, the copious honeydew produced leads to the growth of sooty mold, which restricts photosynthesis, stunts plants and causes leaf necrosis (Byrne et al. 1990). *T. vaporariorum* can also act as a vector by transmitting viruses to plants, viz., *Beet pseudo yellow virus* (BPYV) (*Closterovirus*) (Duffus et al. 1965.; Tzanetakis et al. 2003, 2004a), *Potato yellow vein virus* (PYDV) (Alba 1950), *Tomato infectious chlorosis virus* (TICV), *Tomato chlorosis virus* (ToCV)(Duffus et al. 1995; Wisler et al. 1998a, 1998b) and *Strawberry pallidosis associated virus* (SPaV) (Tzanetakis et al. 2003). Recent observation on *B. tabaci* states, it's a complex of 24 morphologically indistinguishable species/biotypes and defined by analyzing consensus sequences and 3.5% of mt-COI sequences pairwise genetic divergence. (Paul J. De Barro 2011). Development of biotypes in *B. tabaci* may be due to various causes including insecticide selection pressure and development of resistance, host variation, and geography; all of which may influence vector potential for various viruses (Bird 1957; Mound 1963; Costa and Russell 1975; Bird & Maramorosch 1978). In contrast to the case of *B. tabaci*, fewer studies were carried out with *T. vaporariorum*, which is becoming a serious pest in glasshouse or polyhouse cultivation of agricultural crops. In addition through the characterization of esterase banding patterns, Idriss et al. (1997) reported the existence of 5 different biotypes in *T. ricini* populations.

This study investigates the evolutionary relationship of *Trialeurodes* spp. based on sequence variation of the mitochondrial cytochrome oxidase-I (CO-I) gene, and internal transcribed spacer (ITS) sequences of ribosomal DNA. This is one of the first studies to examine the genetic population structure of *T. vaporariorum* over a broad range.

Materials and Methods

Collection of Whitefly Populations

Adults and nymphs of the whitefly, *T. vaporariorum*, were collected using a hand-held aspirator from 2009 to 2011 on ornamental and vegetables at 22 different locations in South India (Table 1). The specimens were preserved in 70% ethanol for further analysis.

Morphological Identification

Specimens were slide mounted with Canada balsam using the procedure described by Brown (1997). The taxonomic characters were used for identification based on Martin (1987).

DNA Extraction and Polymerase Chain Reaction (PCR)

Trialeurodes vaporariorum were taken individually and a small part of each abdomen was used to extract the genomic DNA using the modified cetyl trimethyl ammonium bromide buffer (CTAB) method (Saghai-Maroof et al. 1984). The rest of the insect body was retained as the specimen voucher and placed in the Division of Biotechnology, IIHR, Bengaluru. Briefly, a part of the abdomen was ground with 1.0 mL of CTAB 2%, 100 mM Tris-HCI (pH 8.0), 1.4 M sodium chloride, 20 mM EDTA, 0.1% of 2-mercaptoethanol (added just prior to use) and suspended in the same buffer. The suspension was incubated at 65 °C for 2 h and then an equal volume of chloroform: isoamylalcohol (24:1) was added. The suspension was centrifuged at 10,000 rpm (Eppendorf 5415 C) for 10 min at 8 °C. The upper aqueous layer was transferred to a fresh micro centrifuge tube taking care not to disturb the middle protein interface. DNA was precipitated by adding an equal volume of ice-cold 95% ethyl alcohol. The precipitated DNA was spun at 10,000 rpm (Eppendorf 5415 C) and the resultant DNA pellet was washed with 70% ethanol and dissolved in 50 µL DNase-, RNase- and Protease-free molecular biology water (5 PRIME Hamburg, Gaithersburg, Maryland, US). Extracted DNA was further purified to be free of RNA contaminants by addition of 10 µL of RNase. The intact genomic DNA was visualized using in a 0.8% agarose gel and quantified using a fluorometer (DyNa quant 200™, Hoefer, www.hoeferinc.com) following standard procedures. Depending upon the concentration, the DNA samples were diluted with sterile water

TABLE 1. DETAILS OF SPECIMENS FOR WHICH *CO-I* AND *ITS-1* MARKERS WERE SEQUENCED.

Species	Voucher number	Host plant	Site of collection	GenBank accessions		
				COX-1	mtCO1	ITS
T. vaporariorum	ORPRP-01	*Gerbera jamesonii*	Tubgere	JQ339309	JQ339323	JQ339337
T. vaporariorum	ORPRP -02	*Rosa spp*	Arallu mallige	JQ339310	JQ339324	JQ339338
T. vaporariorum	ORPRP -03	*Gerbera jamesonii*	Byrasandra	JQ339311	JQ339325	JQ339339
T. vaporariorum	ORPRP -04	*Gerbera jamesonii*	IIHR	JQ339312	JQ339326	JQ339340
T. vaporariorum	ORPRP -05	*Gerbera jamesonii*	Moodal Palya	JQ339313	JQ339327	JQ339341
T. vaporariorum	ORPRP -06	*Cucurbita moschata*	Malur	JQ339314	JQ339328	JQ339342
T. vaporariorum	ORPRP -07	*Gerbera jamesonii*	Malur	JQ339315	JQ339329	JQ339343
T. vaporariorum	ORPRP -08	*Rosa spp*	Saragapalli	JQ339316	JQ339330	JQ339344
T. vaporariorum	ORPRP -09	*Rosa spp*	Kodiyala	JQ339317	JQ339331	JQ339345
T. vaporariorum	ORPRP -10	*Rosa spp*	Kurabatti	JQ339318	JQ339332	JQ339346
T. vaporariorum	ORPRP -11	*Gerbera jamesonii*	Srinivaspura	JQ339319	JQ339333	JQ339347
T. vaporariorum	ORPRP -12	*Solanum lycopersicum*	Solur	JQ339320	JQ339334	JQ339348
T. vaporariorum	ORPRP -13	*Capsicum annum*	Solur	JQ339321	JQ339335	JQ339349
T. vaporariorum	ORPRP -14	*Rosa spp*	Solur	JQ339322	JQ339336	JQ339350
T. vaporariorum	ORPRP -15	*Hibiscus spp*	Yarcaud	JX841200	JX841208	JX841216
T. vaporariorum	ORPRP -16	*Ipomea spp*	Yarcaud	JX841201	JX841209	JX841217
T. vaporariorum	ORPRP -17	*Gerbera jamesonii*	Dharmapuri	JX841202	JX841210	JX841218
T. vaporariorum	ORPRP -18	*Rosa spp*	Dharmapuri	JX841203	JX841211	JX841219
T. vaporariorum	ORPRP -19	*Solanum lycopersicum*	Guntur	JX841204	JX841212	JX841220
T. vaporariorum	ORPRP -20	*Gerbera jamesonii*	Guntur	JX841205	JX841213	JX841221
T. vaporariorum	ORPRP -21	*Nicotiana tabacum*	Bengaluru	JX841206	JX841214	JX841222
T. vaporariorum	ORPRP -22	*Phaseolus vulgaris*	Bengaluru	JX841207	JX841215	JX841223

Table 2. Primers employed in the current study.

Region	Gene Location	Primer Name	Sequence	Reference
Mitochondria	COX-1	LCO1490 HCO2198	5'-GGTCAACAAATCATAAAGATATTGG-3' 5'- TAAACTTCAGGGTGACCAAAAAATCA-3'	Folmer et al. 1994
Mitochondria	mtCOI	C1-J-2195 L2-N-3014	5'-TTGATTTTTTGGTCATCCAGAAGT-3' 5'-TCCAATGCACTAATCTGCCATATTA -3'	Simon et al. 1994
Nuclear DNA	ITS-1	TW81 5.8R	5'- GTTTCCGTAGGTGAACCTGC -3' *5'*- ATCCGCGAGCCGAGTGATCC -*3'*	Brust et al. 1998 De Barro et al. 2000

to get a working solution of 20-25 ng/µL. A portion of the total DNA was preserved in glycerol (10%) at -80 ˚C for future reference.

The polymerase chain reaction (PCR) was carried out in a thermal cycler (AB-Applied Biosystems, Veriti 96 wells) with the following cycles; 94 °C for 4 min as initial denaturation followed by 35 cycles of 30 s at 94 °C, for 45 s at 47 °C, 54 °C, and 52 °C for COX-I, mtCO-I and ITS-1 respectively, for 45 s at 72 °C and for 20 min at 72 °C as the final extension. The primers as mentioned in Table 2 were specific to mitochondrial DNA; and nuclear DNA fragments were used in the present study. PCR was performed in 25-µL total reaction volume containing 20 picomoles of each primer, 10 mM Tris HCl (pH -8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase (Fermentas GmBH, St. Leon-Rot, Germany). The amplified products were resolved in 1.5% agarose gel, stained with ethidium bromide (10µg/mL) and visualized in a gel documentation system (UVP)

Molecular Cloning and Sequencing

The PCR amplified fragments were eluted using Nucleospin® Extract II according to the manufacturer's protocol *(*Macherey-Nagel, Duren, Germany) and ligated into the general purpose-cloning vector, InsT/Aclone (Fermentas GmBH, St. Leon-Rot, Germany) according to the manufacturer's protocol. The transformed cells were spread on LB agar plates containing X-gal (300 ug/mL), IPTG (120 ug/mL) and ampicillin (100 ug/mL). The plates were then incubated at 37 °C overnight to screen blue and white colonies; and all the white colonies (colonies harboring the insert) were maintained on LBA containing ampicillin (100 mg/ml), incubated at 37 °C overnight and stored at 4 °C until further use. Plasmids were prepared from the overnight culture of the positive colonies cultured in LB broth (enzymatic casein- 10 g, yeast extract-5 g, NaCl-5 g in 1000 mL of water, pH 7.0) using GeneJET™ Plasmid Miniprep Kit (Fermentas, Germany) according to manufacturer's protocol. Cloning was confirmed by colony PCR and recombinant plasmid comparison with colony without insert. Sequencing was carried out in an automated sequencer (ABI Prism 310; Applied Biosystems, USA) using M13 universal primers both in forward and reverse directions. Homology search was carried out using BLAST (http://www.ncbi.nlm.nih.gov), and the differences in nucleotide sequences of *T. vaporariorum* were determined using the sequence alignment editor 'BioEdit'.

Phylogenetic Analysis

All the sequences generated in the present study, corresponding to COX-I, mtCO-I and ITS-1 was aligned using BioEdit. 4. 0 program using Clustal W. The alignment was further analyzed using MEGA.4.0 (Kumar et al. 1993). Maximum- Parsimony (MP) and Neighbour-Joining (NJ) trees were constructed using the Kimura-2-parameter (K2P) distance model (Kimura 1980; Saitou & Nei 1987). All the corresponding sequences for different mitochondrial and ribosomal markers of *T. vaporariorum* were deposited in the NCBI-GenBank.

Results and Disussion

Molecular diversity studies in various insects of importance in agriculture pave the way in identifying new species (Ball & Armstrong 2006), biotypes (Perring 2001), cryptic species (Hebert et al. 2004) and haplotypes (Toda & Murai 2007), which are difficult to identify through morphological

Table 3. Maximum composite likelihood estimate of the pattern of nucleotide substitution from different populations of *Trialeurodes vaporariorum.*

	A	T	C	G
A	—	*6.38*	*5.35*	**12.28**
T	*6.45*	—	**11.83**	*5.51*
C	*6.45*	**14.12**	—	*5.51*
G	**14.4**	*6.38*	*5.35*	—

Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold, and those of transversional substitutions are shown in italics.

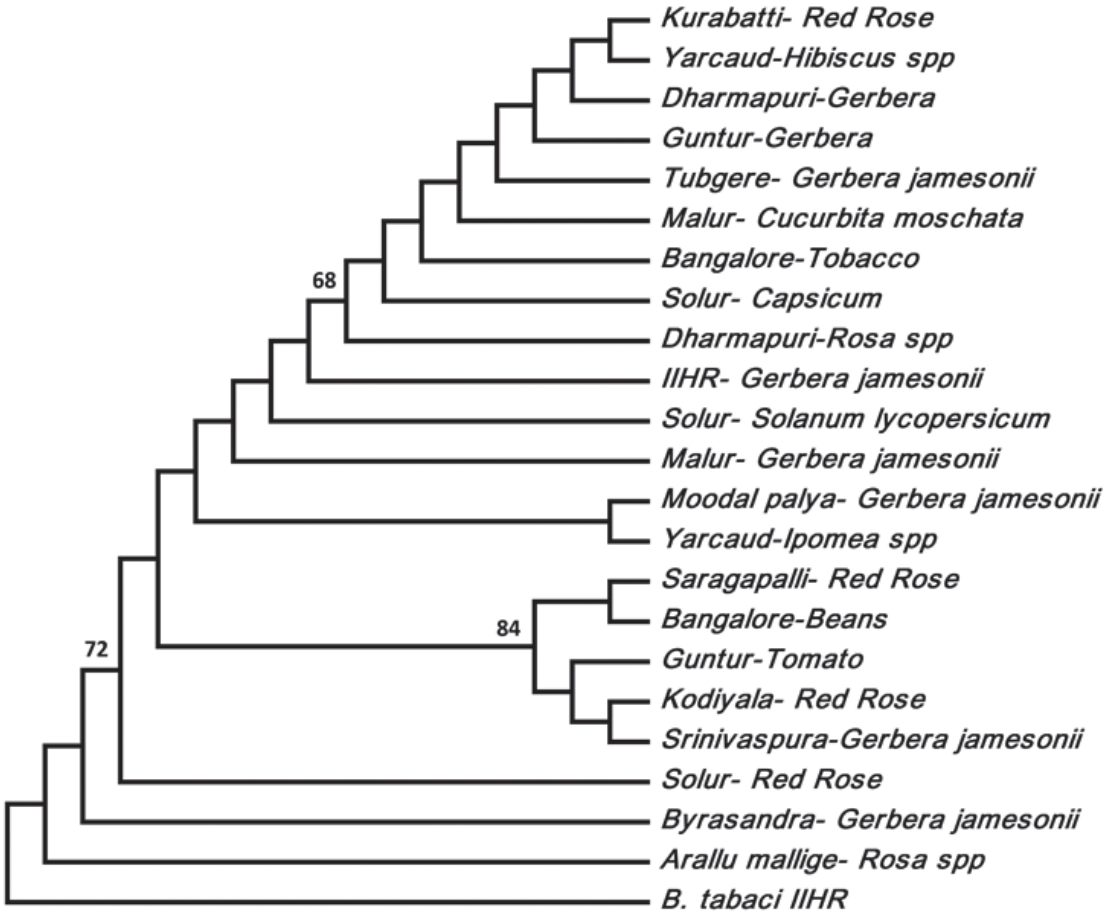

Fig. 1. Maximum Parsimony tree using COI and ITS sequences with bootstrap support (1000 replicates) showing phylogram for *Trialeurodes vaporariorum* collected from different hosts and different geographical locations in South India. *Bemisia tabaci* was used as an out group in the analysis. (MEGA. 4.0)

characters. Insect pests with piercing-sucking mouthparts are of major concern both agricultural and horticultural crops because many of them possess immense vectoring capacity and they are developing insecticide resistance (Denholm *et al.,* 1996, 1998). One of the invasive species, *B. tabaci* is reported as 'species complex' with the existence of 24 biotypes (Paul J. De Barro et al 2011) and also is an important vector for many begomo viruses. Attempts to define these biotypes were made by using esterase banding patterns and a range of biological characters (Bedford et al. 1994). Subsequently many biotypes have been defined primarily by differences in both or either mitochondrial COI or ribosomal ITS1 DNA sequences with little or no meaningful biological data to support their uniqueness (Boykin et al. 2007). In India, another whitefly species, *T. vaporariorum*, also has been gaining importance to rival *B. tabaci* in developing insecticide resistance, especially in polyhouses on ornamentals, vegetables and rarely in open fields. Considering their pest potential, we judged it to be necessary to analyze the molecular diversity in *Trialeurodes* species viz. *T. vaporariorum, T. ricini, T. lauri* and *T. abutilonea*. Recently, Idriss et al. (2009) proved the existence of 5 different biotypes in *T. ricini* using esterase banding patterns, and proved that these biotypes have varying efficiencies in transmitting *Tomato yellow leaf curl virus* (TYLCV).

COX-I, mtCOI and ITS-1 sequencing yielded 658bp, 825bp and 525bp nucleotide sequences, respectively, and a total of 22 specimens for *T. vaporariorum* collected on various hosts from different geographical regions in Karnataka, Tamil Nadu and Andhra Pradesh (Table. 1). All of these sequences have been deposited in the NCBI-Genbank database under the accession numbers JQ339309 to JQ339350 (Table. 1).

CO-I and ITS-1 sequences were aligned using Clustal X (Version 1.83, 2003; Thompson et

al. 1997) with default settings. The Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution for *T. vaporariorum* sequences was performed using MEGA 4.0 (Tamura et al. 2007). The reliability of the clustering pattern in the trees was determined by the bootstrap test, with 1000 replications. Each entry showed the probability of substitution from one base (row) to another base (column) instantaneously. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics (Table 3). The *T. vaporariorum* nucleotide frequencies are 0.272 (A), 0.269 (T), 0.226 (C) and 0.232 (G). The base composition of the COX-I gene fragment was biased toward Adenine (A) and Thymine (T), which together constituted 54.1% of the total. The overall transition (ti)/transversion (tv) bias of *T. vaporariorum* nucleotide sequence is R = 1.02, where R= $[A*G*k_1+T*C*k_2]/[(A+G)*(T+C)]$. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated from the datasets (complete deletion option). All calculations were conducted in MEGA 4.0 (Tamura et al. 2007). Summary statistics for the different substitution changes are shown in Table. 3. Maximum Parsimony (MP) and maximum likelihood (ML) analysis were performed with PAUP*4.0b10 (Swofford 1998), using the heuristic search procedure with 1000 random additions of sequences and 10 trees held at each pseudo-replicate, and the tree bisection reconnection (TBR) branch swapping method; all characters were treated as unordered and equally weighted for the MP analysis.

In India, *T. vaporariorum* is gaining importance as a major direct pest on crops in polyhouses. Therefore, specimens were collected from different geographical locations in Karnataka, Tamil Nadu and Andhra Pradesh and the corresponding sequences for CO-I region and ITS-1 revealed that there are very little sequence variations in the nucleotides. The phylogram suggested that *T. vaporariorum* is a single species (Fig. 1), which contrast with corresponding observations of the *B. tabaci* species complex (Boykin et al. 2007) and *Thrips tabaci* (Brunner et al. 2004). The present study clearly indicated that there are neither biotype nor host associated genetic differences in *T. vaporariorum*. For the phylogenetic analysis various *Trialeurodes spp.* sequences of the mt-CO1 gene were acquired from NCBI- GenBank (Table 4) and were used along with our data generated in the present study. Four different *Trialeurodes* species viz. *T. vaporariorum, T. ricini, T. lauri, and T. abutilonea* were analyzed and the mt-COI sequences revealed that, there are 2 major clades; i.e., clade I consists of *T. vaporariorum* and *T. abutilonea* and clade II consists of *T. ricini* and *T. lauri* (Fig. 2). Another interesting observation resulted from our molecular data was the strong support for the evidence of biotypes emer-

TABLE 4. DETAILS OF MTCO-I SEQUENCES FOR *TRIALEURODES SPP* ACQUIRED FROM NCBI-GENBANK FOR PHYLOGENETIC ANALYSIS.

			GenBank accessions
Species	Host plant	Country	mtCO1
T. vaporariorum.	Unknown	China	JF512474
T. vaporariorum.	Unknown	China	JF693934
T. vaporariorum.	Unknown	Unknown	JF693935
T. ricini	*Ricinus communis*	Egypt	AM179430
T. ricini	*Unknown leaves*	Gambia	AM179428
T. ricini	*Ricinus communis*	Spain	AM179426
T. ricini	*Ricinus communis*	Spain	AM179424
T. ricini	*Ricinus communis*	Spain	AM179422
T. ricini	*Ricinus communis*	Spain	AM179447
T. ricini	*Ricinus communis*	Egypt	AM179431
T. ricini	*Unknown leaves*	Nigeria	AM179429
T. ricini	*Ricinus communis*	Spain	AM179427
T. ricini	*Ricinus communis*	Spain	AM179425
T. ricini	*Ricinus communis*	Spain	AM179423
T. ricini	*Ricinus communis*	Egypt	AM179430
T. ricini	*Unknown leaves*	Gambia	AM179428
T. lauri	*Laurus nubilis*	Croatia	AM179440
T. lauri	*Arbutus andrachne*	Israel	AM179438
T. lauri	*Arbutus unedo*	Turkey	AM179436
T. lauri	*Arbutus unedo*	Turkey	AM179434
T. lauri	*Arbutus unedo*	Turkey	AM179432
T. lauri	*Laurus nubilis*	Croatia	AM179439
T. lauri	*Arbutus andrachne*	Israel	AM179437
T. lauri	*Arbutus unedo*	Turkey	AM179435
T. lauri	*Arbutus unedo*	Turkey	AM179433

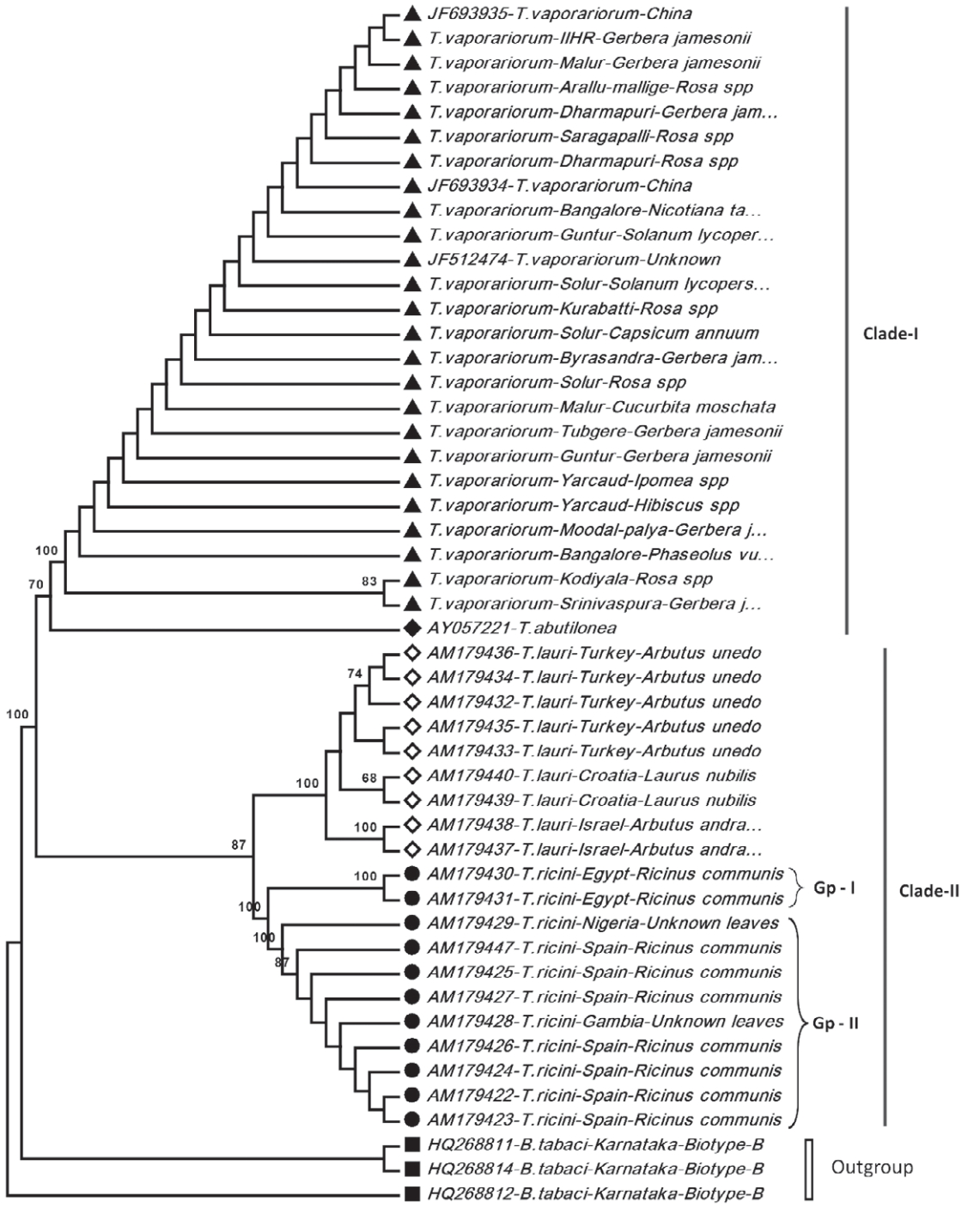

Fig. 2. Maximum Parsimony tree using COI sequences with bootstrap support (1000 replicates) showing two major clades for *Trialeurodes* spp., viz., *T. vaporariorum, T. abutilonea, T. lauri and T. ricini*. Clade I consists of *T. ricini* (●) and *T. lauri* (◇), and Clade II consists of *T. vaporariorum* (▲) and *T. abutilonea* (◆). *Bemisia tabaci* (■) was used as an out group in the analysis. Gp-I and Gp-II are the two new groups of *T. ricini* cryptic species/ biotypes, according to Hebert's barcoding gap of 10X interspecific-to-intraspecific distances.

gence as in earlier case of *T. ricini* by Idriss et al, 2009. The phylogenetic analysis showed that two populations from Egypt, collected on *Ricinus communis* formed the clade I and the rest of the populations collected on various geographical locations viz. Spain, Gambia and Nigeria formed the Clade II. According to Hebert et al, (2004), sequence thresholds of 10X, the mean intraspecific mtCOI variation for group under study as an approximate method of screening for potentially different (cryptic) species or biotypes. Using MEGA.5.0, intraspecific and interspecific distances were calculated for both group-I and group-II observed in the MP tree for *T. ricini.* The intraspecific distance ranges for group-I and group-II of *T. ricini* 0.00-0.35. The interspecific distances for group-I and group-II of *T. ricini* is 6.25%. Thus our studies showed that *T. ricini* is not a single polyphagous species but on contrary, by the standards of genetic and ecological differentiation in other species groups and also based on Hebert's barcoding gap of 10X interspecific-to-intraspecific distances, the recognition of a distinct biotypes may be considered. On the other hand *T. lauri* also formed two clades with the variations in the nucleotide sequences, however unable to satisfy the sequence thresholds of 10X rule.

Our study indisputably proved that *T. vaporariorum* is a single species, and unlike *B. tabaci* species complex. Also the phylogram showed that *T. vaporariorum* is more closely related to *T. abutilonea* than to *T. ricini* and *T. lauri*, which formed the second clade in the phylogram (Fig. 2). This is a much needed starting point with reference to the molecular identification of the greenhouse whitefly, *T. vaporariorum*, and to further attempts to determine if biotypes or cryptic species occur in this globally distributed species.

Endnote

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