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Identifying thrips (Insecta: Thysanoptera) using DNA Barcodes

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Abstract

Thrips tabaci Lindeman is an extensively distributed pest insect in many areas that affects plants through direct feeding and at the same time, it makes damage as a vector of different viruses. As a basic first step to control pests is authentic identification, but the inability to determine morphological characters of thrips species makes this process very difficult. For creating an identification key for *T. tabaci*, an economically important species present in Iran, four individuals were selected from four different sites of Mashhad and the vicinity, each as a separate population. The method was based on nucleotide sequencing analysis of the mitochondrial cytochrome C oxidase I (*COI*) gene. Phylogenetic analyses conducted by the neighbor-joining method yielded almost identical phylogenetic reconstructions of trees that separated thrips based on the geographic origin. Molecular data indicate that different thrips species are located in distinct groups. These results show that molecular keys can be a useful method to provide much-needed information on thrips identification for pest management officers and quarantine purposes.

Keywords: *Thrips tabaci*, mitochondrial DNA, barcoding, phylogenetic tree, molecular identification

Introduction

Among the 5800 thrips species described worldwide only 1% are known as pest species with about ten species as vectors of plant viruses (Morris and Mound, 2003). Onion thrips, *Thrips tabaci* Lindeman, is the most harmful species of the Thysanoptera order. It is extremely polyphagous, most damages being reported on bulb plants, tobacco, cabbage, and ornamental plants. The damage is caused either by feeding of larvae or adults. Its role has been proved in the transmission of different viruses such as Tomato Spotted Wilt Virus (TSWV), Eggplant Mottled Dwarf Virus (EMDV), and Iris Yellow Spot Virus (IYSV) to several plants (Babaie and Izadpanah, 2003; Boonham et al., 2002; Gera et al., 1998; Zen et al., 2008**)**. For the first time, *T. tabaci* was reported by Afshar (1938) on tobacco, cotton, cucumber, potato, onion and cabbage in Iran. *Thrips tabaci* is wide-spread in Iran and has been reported from most areas (Alavi et al., 2007) and is the major foliage pest in field cultures. This pest can cause considerable damage due to its feeding behaviour and their rapid reproduction. When conditions are hot and dry, a generation can be completed in only 2-3 weeks (Cranshaw et al., 2005). It is estimated that the yield loss caused by thrips in Iran (onion farms) is more than 50% (Alimousavi et al., 2007). *Thrips tabaci* is a very small insect that shows a high degree of similarity in appearance, particularly in preadult stages, (e.g. larval thrips are often mistaken for Collembola, whereas adults are commonly confused with Staphylinidae beetles (Vierbergen, 1995), which can make them extremely difficult to identify at the species level. On the other hand, in plant consignments, rapid identification is important to prevent the introduction of new pests into non-infested areas. Consequently, the rudiment and accurate recognition of thrips species is important in species-specific control programs, especially for thrips that have determined insecticide resistance (Roehrdanz, 1997).

‘‘DNA barcoding’’ is a method based on DNA sequencing of a standard gene region (Herbert et al., 2003b). It can be helpful in species diagnosis because sequence divergences are usually much lower among individuals of a species than between closely related species (Herbert et al., 2003a).

Recent researches show that it is possible to create credible identification systems established on the analysis of sequence diversity in small fragments of DNA (Tautz et al., 2003) and theoretical aspects (De Salle et al., 2005; Savolainen et al., 2005), methods (Blaxter et al., 2005; Steinke et al., 2005), and applied cases (Chase et al., 2005;

Monaghan et al., 2005) of the DNA barcoding

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are today under quite an intense development. Hebert et al. (2003b) focused this discussion by proposing that a DNA barcoding system for animal life could be based upon sequence diversity in the mitochondrial gene cytochrome C oxidase I (*COI*).

Insect mitochondrial genome (mtDNA) analysis is a powerful tool for the study of population genetics and phylogenetics. In the past few years primer sequences for the PCR amplification of various insect mtDNA genes have been published (Kambhampati and Smith, 1995). For example, congeneric species of moths show an average sequence divergence of 6.5% in *COI*) whereas divergences between conspecific individuals average only 0.25%. Similar values were obtained in birds, with intraspecific divergences at *COI* averaging 0.27%, while congener divergences averaged 7.93% (Herbert et al., 2004).

The use of genetic markers, like mtDNA, represents a valuable addition or alternative to classical methods of species identification. The strategy used in the present research is based on nucleotide sequencing analysis of the *COI* gene for the rapid and accurate identification of *T. tabaci*.

Materials and Methods

DNA extraction and COI sequencing

Four populations of *T. tabaci* were collected during 2007-2008 from Mashhad and the vicinity in Iran. DNA for PCR templates was extracted from an adult using DNeasy blood and tissue qiagen kit following the manufacturer's protocol. A double-stranded *COI* template was generated using the universally conserved mtDNA *COI* primers, LCO1490 and HCO2198 (Folmer et al., 1994). PCR reactions were performed with 10 mM dNTPs, 5 U/µl Amplitaq, 25mM $MgCl_2$, 10X PCR buffer, 20mM sense and antisense primers. The PCR thermal regime was 60 s at 94°C, 30 s at 52°C, and 90 s at 72°C with 35 cycles using a Biomethra thermocycler. PCR-amplified products were purified using Bioneer's PCR purification kit. Samples were sequenced from both directions using an ABI 377 sequencer.

Data analysis

Sequences were edited and aligned using BioEdit 7.0.5.2 (Hall, 1999) (figure 1). The nBLAST program (http://www.ncbi.nlm.nih.gov/blast/) was employed to identify similarities between the sequences obtained in this work and previously published data (*Haplothrips* spp., *Thrips palmi*, *T. vulgatissimus*, *T. tabaci*, *Frankliniella occidentalis*) (table 1). A pair wise sequence divergence (the evolutionary distances) was calculated using the Kimura two-parameter distance model with MEGA4 (Kimura, 1980); Sequences were compared to identify intra- and interspecific nucleotide differences (tables 2 and 3). To visualize these patterns of divergence, the neighbor-joining tree (Saitou and Nei, 1987) and minimum evolution trees were constructed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test by 100,000 replicates (Felsenstein, 1985). All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 397 positions in the final alignment. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

Results

DNA was sequenced from four samples of thrips from Mashhad and the vicinity. DNA sequencing resulted in a fragment of the *COI* gene. All samples were successfully amplified. We selected a 413bp segment of DNA for further analysis. Distance estimates (figure 1) were used to generate a neighbor joining (NJ) tree. This tree (figure 2) shows the thrips sequences in six major clades, corresponding to the *Frankliniella occidentalis*, *Haplothrips* spp., *Thrips palmi*, *T. vulgatissimus and T. tabaci* (two clades) species. *COI* sequences of specimens from Mashhad formed two single clades, whereas the sequences of two other species formed two related sister clades, which together formed a larger group. The grouping of the taxa on the tree corresponded to species designation and geographic region, within each major clade and there was segregation based on their origins.

The trees (maximum parsimony analysis and minimum evolution trees not shown) produced by this analysis showed the same overall topology as the NJ tree.

Our result confirm the previous data reported by Crespi et al. (1996) that supports the deep phylogenetic split between *Terebantia suborder* (i.e. *Thrips* spp. and *Frankliniella occidentalis*) and *Tubulifera suborder* (include *Haplothrips* spp.) and corroborates the sister-taxon relationship of these two probably monophyletic suborders. Distance values (table 2) are correlated with geographic distance between specimen collection sites. For example, Palestine territory is the next region to Iran (in this research) and calculated sequence distances between individuals *T. tabaci* from these locations ranged to 0.019. In contrast, the sequence distance between Iran and the United Kingdom specimens, is much higher (0.043).

Table 1. Thrips species used in the study.

Species	**Geographic Region**	**Specimen/Clone Designation**	**GenBank Accession No.**
Genus *Frankliniella*			
F. occidentalis	South Africa	CSL T166	AM932023
F. occidentalis	Italy	CSL T185	AM932026
F. occidentalis	Kenya	ENTOBAR0588	FN545993
F. occidentalis	UK	ENTOBAR0484	FN545981
Genus *Haplothrips*			
H. cenchricola	Spain	ENTOBAR0545	FN545925
H. distinguendus	UK	ENTOBAR0591	FN545929
H. setiger	Spain	ENTOBAR0695	FN545939
H. statices	UK	ENTOBAR0633	FN545936
H. subtilissimus	UK	ENTOBAR0603	FN545933
Genus *Thrips*			
Th. palmi	India	ENTOBAR0576	FN546147
Th. palmi	Dominican Republica	CSL T122	AM932013
Th. tabaci	Bosnia and Herzegovina	CSL T97	AM932006
Th. tabaci	UK	ENTOBAR0652	FN546169
Th. tabaci	Palestine	ENTOBAR0583	FN546148
Th. tabaci	Japan	IW	AB277235
Th. tabaci	Japan	SM	AB277237
Th. tabaci	Bosnia and Herzegovina	CSL T123	AM932014
Th. tabaci	Japan	ON2	AB277236
Th. tabaci	Bosnia and Herzegovina	ENTOBAR0654	FN546171
Th. tabaci	UK	CSL T223	AM932043
Th. tabaci	Bosnia and Herzegovina	ENTOBAR0419	FN546157
Th. tabaci	Palestine	ENTOBAR0585	FN546150
Th. tabaci	Palestine	ENTOBAR0584	FN546149
Th. tabaci	Iran	FUM11	Current study
Th. tabaci	Iran	FUM 12	Current study
Th. tabaci	Iran	FUM13	Current study
Th. tabaci	Iran	FUM14	Current study
Th .vulgatissimus	UK	ENTOBAR0629	FN546059
Th. Vulgatissimus	UK	ENTOBAR0363	FN546068

Table 2. Pairwise Kimura 2-parameter distances between groups of *T. tabaci* (±SE).

	Bosnia	**UK**	**Palestine**	**Japan**	**Iran**	
Bosnia		0.006	0.009	0.003	0.009	
UK	0.03		0.007	0.007	0.008	
Palestine	0.041	0.039		0.01	0.005	
Japan	0.004	0.033	0.043		0.009	
Iran	0.04	0.043	0.019	0.04		

Table 3. Mean distances between groups based on different species of thrips by Kimura 2-parameter distance (± SE).

	F. occidentalis	***Haplothrips spp.***	***T. palmi***	***T. vulgatissimus***	***T. tabaci***
F. occidentalis		0.034	0.026	0.024	0.026
***Haplothrips* spp.**	0.369		0.033	0.034	0.033
T. palmi	0.236	0.373		0.022	0.024
T. vulgatissimus	0.206	0.379	0.198		0.022
T. tabaci	0.237	0.379	0.221	0.197	

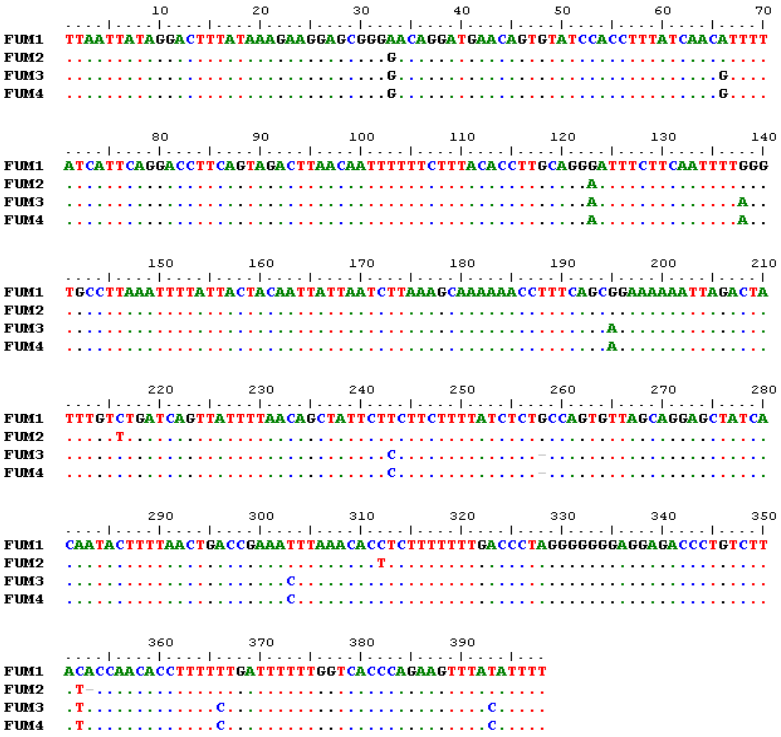

Figure 1. Aligned sequences from the mitochondrial cytochrome C oxidase I (*COI*) gene of four populations of *Thrips tabaci* species from Iran. Dots indicate nucleotides that are identical throughout the compared sequences, R indicates G/A nucleotides and dashes indicate insertions/deletions.

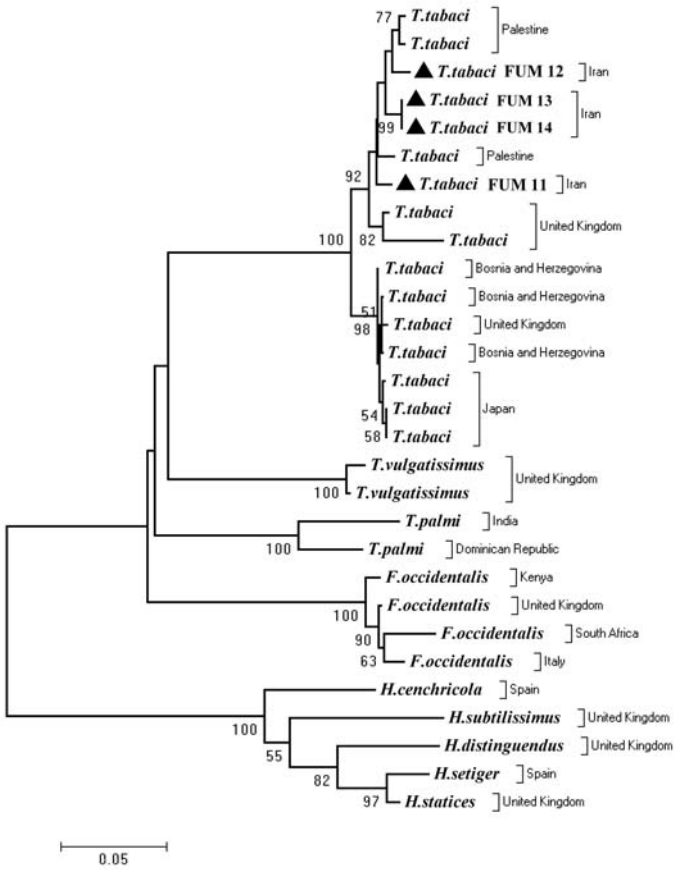

Figure 2. Unrooted neighbor-joining tree (with 100,000 replicate) constructed with the Kimura two-parameter distance calculation based on mtDNA *COI* sequence data. Taxa are labeled with the collection site. Bootstrap support ≥ 50% is indicated at branches.

Discussion

Recently, the *COI* gene has been used for identification purposes in projects known as species barcoding. The idea behind barcoding is to sequence the *COI* gene of as many different species as possible and then use the *COI* sequence to identify unknown specimens by comparing their COI sequence the catalogued or named species (Blaxter, 2004; Hebert et al., 2003a, b; Tautz et al., 2003; Van Driesche et al., 2008). Whereas geographic isolation and genetic drift contribute to pronounced intraspecific phylogeographic structure, gene flow retards the genetic divergence of populations (Avise et al., 1987). The latter may be massive enough to reverse adaptive differentiation, unless the integrity of populations is maintained by reproductive solation (Brunner et al., 2004).

Our analyses clearly indicate that genetic differentiation is significant among populations of *T. tabaci* collected from different locations and then mtDNA sequences could be used in many studies to determine the origin of an invasive species. An example is the study by Havill et al. (2006) to determine the origin of the hemlock woolly adelgide, *Adelges tsugae* Annand (Homoptera: Adelgidae), which has invaded eastern North America. *COIt* has a great ability to help identify the invasive species (Scheffer et al., 2006) and natural enemies (Greenstone et al. 2005). Perdikis et al. (2003) used mitochondrial DNA sequences to distinguish between two closely related predatory hemipterans encountered in field studies.

Different markers are useful for inferring phylogeny of this insect group. For example Inoue and Sakurai (2007) used partial sequences of *COI*, *28S* ribosomal, and *EF-1α* for determining the phylogenetic relationships between the species of thrips and the vector competence of thrips for tospoviruses.

This study investigated the utility of *COI* for identifying thrips species. As demonstrated in this work, there is a relationship between phylogeny and origin evolution of thrips species. This can reveal that climate changes have important effects on diversification of species of thrips. Variation and polymorphism is common between species, nevertheless, it is often ignored by taxonomists. Molecular studies have the potential for detection of genetic polymorphism within species, and such information will be useful in identification of important species, study of population genetic, ecology, vector transmission, insecticide resistance, biological control and quarantine.

In conclusion, *COI* appears to be a good candidate marker to be used in DNA barcoding projects and can be particularly suitable in combination with the sequencing of additional genes or when biological and morphological characteristics are also studied to supplement *COI* data.

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References

1- Afshar J., (1938) Pests of summer crops, vegetables, industrial plants, and pastures in Iran and their control. 124 pp. General Office of Agriculture, Tehran.

2- Alavi J., zur Strassen R. and Bagherani N. (2007) Thrips (Thysanoptera) species associated with wheat and barley in Golestan province, Iran. Journal of Entomological Society of Iran 27(1): 1-28.

3- Alimousavi S., Hassandokht M. and Moharramipor S. (2007) Evaluation of Iranian onion germplasms for resistance to *Thrips*. International journal of Agriculture and Biology 9: 455–8.

4- Avise J. C., Arnold J., Ball M. R., Bermingham E., Lamb T. and Neigel J. E. (1987) Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematic. Annual Review of Ecology and Systematics 18: 489–522.

5- Babaie G. and Izadpanah K. (2003) Vector transmission of eggplant mottled dwarf virus in Iran. Journal of Phytopathology 151:679–682.

6- Blaxter M. L. (2004) The promise of a DNA taxonomy. Philosophical. Transactions of the Royal Society of London, Series B, Biological. Sciences 359:669–679.

7- Blaxter M., Mann J., Chapman T., Thomas F., Whitton C., Floyd R., Eyualem A. and Abebe E. (2005) Defining operational taxonomic units using DNA barcode data. Philosophical. Transactions of the Royal Society of London, Series B, Biological. Sciences 360: 1935–1943.

8- Boonham N., Smith P., Walsh K., Tame J. and Morris J. (2002) The detection of tomato spotted wilt virus (TSWV) in individual thrips using real time fluorescent RT-PCR (TaqMan). Journal of Virological Methoes 101:37-48.

9- Brunner P. C., Chatzivassiliou E., Katis N., and Frey J. (2004) Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. Heredity 93:364–370.

10- Chase M. W., Salamin N., Wilkinson M., Dunwell J.

M., Kesanakurthi R. P., Haidar N. N. and Savolainen V. (2005) Land plants and DNA barcodes: short-term and long-term goals. Philosophical. Transactions of the Royal Society of London, Series B, Biological. Science 360: 1889–1895.

11- Cranshaw W., Grafius V., T-X Liu, Nault B. and Riley D. (2005) Chapter 11. Onions in Vegetable Insect Management. ed. R. Foster and B. Flood. Meister Publishing Co. Willoughby, Ohio, 264 pp.

12- Crespi B., Carmean D., Vawter L. and Von Dohlen C. (1996) Molecular phylogenetics of Thysanoptera. Systematic Entomology 21: 79-87.

13- De Salle R., Egan M. G. and Siddall M. (2005) The unholy trinity: taxonomy,species delimitation and DNA barcoding. Philosophical. Transactions of the Royal Society of London, Series B, Biological. Sciences 360:1905–1916.

14- Felsenstein J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

15- Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

16- Gera A., Cohen J., Salomon R. and Raccah B. (1998) Iris yellow spot spovirus detected in onion (*Allium cepa*) in Israel. Plant Disease 82:127.

17- Greenstone M. H., Rowley D. L., Heimbach U., Lundgren J. G., Pfannenstiel R. S. and Rehner S.A. (2005) Barcoding generalist predators by polymerase chain reaction: carabids and spiders. Molecular Ecology14: 3247–66.

18- Hall T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/. NT. Nucleic Acids Symposium Series 41:95-98.

19- Havill N. P., Montgomery M. E., Yu G., Shiyake S. and Caccone A. (2006) Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Annals of the Entomological Society of America 99:195–203.

20- Herbert P., Cywinska A., Ball S. and deWaard J. (2003a) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B Biological Sciences 270:313–321.

21- Hebert P., Ratnasingha S. and deWaard J. R. (2003b) Barcoding animal life: cytochrome C oxidase subunit 1 divergences among closely related species .Proceedngs of the Royal Society of London B (Supplement): online 15.05.03.

22- Hebert P., Penton E. H., Burns J. M., Janzen D. and Hallwachs W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences 101:14812-14817.

23- Inoue T. and Sakurai T. (2007) The phylogeny of thrips (Thysanoptera: Thripidae) based on partial sequenences of cytocrome oxidase I, 28S ribosomal DNA and elongation factor-1α and the association with vector competence of tospoviruses. Applied Entomology and Zoolog 42:71-81.

24- Kambhampati S. and Smith P. T. (1995) PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Molecular Biology 4: 233-236.

25- Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

26- Monaghan M. T., Balke M., Gregory T. R. and Vogler A. P. (2005) DNAbased species delineation in tropical beetles using mitochondrial and nuclear markers. Philosophical Transactions of the Royal Society of London, Series B, Biological. Sciences 360:1925–1933.

27- Morris D. C., and Mound L. A. (2003) The morphological background to Thysanoptera phylogeny. Entomologische Abhandlungen 61:151-153.

28- Perdikis D. C., Margaritopoulos J. T. and Stamatis C. (2003) Discrimination of the closely related biocontrol agents *Macrolophus melanotoma* (Hemiptera: Miridae) and *M. pygmaeus* using mitochondrial DNA analysis. Bulletin of Entomological Research 93:507–14.

29- Roehrdanz R. L. (1997) Identification of tobacco budworm and corn earworm (Lepidoptera: Noctuidae) during early developmental stages by polymerase chain reaction and restriction fragment length polymorphism. Annals of the Entomological Society of America 90:329–332.

30- Savolainen V., Cowan R. S., Vogler A. P., Roderick G. K. and Lane R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. Philosophical. Transactions of the Royal Society of London, Series B, Biological. Sciences 1805–1811.

31- Saitou N. and Nei M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

32- Scheffer S. J., Lewis M. L. and Joshi R. C. (2006) DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines. Annals of the Entomological Society of America 99:204–10.

33- Steinke D., Vences M., Salzburger W., Meyer A. (2005) TaxI: a software tool for DNA barcoding using distance methods, Philosophical. Transactions of the Royal Society of London, Series B, Biological. Sciences 360:1975–1980.

34- Tamura K., Dudley J., Nei M. and Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

35- Tautz D., Alexander P., Minelli A., Thomas R. H. and Vogler A.P. (2003) A plea for DNA taxonomy. Trends in Ecology and Evolution 18: 70–74.

36- Van Driesche R. G., Hoddle M. and Center T. (2008) Control of Pests and Weeds by Natural Enemies. Blackwell, London, PP. 437.

37- Vierbergen G. (1995) International movment, detection and quarantine of Thysanoptera pest, Thrips Biology and Management, Plenum Press, New York, PP. 119-132.

38- Zen S., Okuda M., Fuji S. and Iwanami T. (2008) The Seasonal occurrence of viruliferous *Thrips tabaci* and the incidence of iris yellow spot virus disease on *Lisianthus*. Journal of Plant Pathology 90:511-515.