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Morphometric and Molecular Variation in *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) Populations on Onion and Tobacco in Iran

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ABSTRACT

Onion thrips, *Thrips tabaci* Lindeman, is a broadly distributed pest that attacks a wide range of crops. To investigate the intra-specific morphometric variation and the genetic diversity of the species in Iran, four populations from tobacco plus 18 populations from onion were studied in some 17 provinces of Iran. Morphological analysis, using principal components and canonical discriminant analyses indicated that the populations living on tobacco were significantly different from those living on onion. DNA sequence data for the COI gene was obtained for all the populations including some other 21 population sequences retrieved from the GenBank database. Maximum parsimony analyses revealed the distinct clades of *T. tabaci* on tobacco and on onion with the exception of one population collected from tobacco grown in Golestan Province. The results were identical for maximum likelihood and neighbor-joining analyses. Both molecular and morphometric analyses show heterogenecity of *T. tabaci* populations representing at least two different biotypes on tobacco and on onion.

Keywords: Molecular analysis, Morphometrics, Onion, *Thrips tabaci*, Tobacco.

INTRODUCTION

Onion thrips, *Thrips tabaci* Lindeman, is a major pest of alliaceous crops distributed worldwide (Toda and Murai, 2007). The importance of onion or tobacco thrips, *T. tabaci*, as a major global pest of agricultural and greenhouse crops has become further evident during the past decades (Pourian *et al.,* 2009; Sedaratian *et al.,* 2010; Reitz *et al.,* 2011). The thrips reduces crop production either by direct feeding or as a vector of Tomato Spotted Wilt Virus (TSWV) (Chatzivassiliou, 2002; Cabrera-La Rosa and Kennedy, 2007). *T. tabaci* has attracted special attention as the first identified vector of a tospovirus, TSWV, as well as its ability in transmitting Iris Yellow Spot Virus (IYSV) (Doi *et al.,* 2003; Hsu *et al.,* 2010).

As the eggs are laid within leaf tissue, pupae are hidden in the soil or in leaf litter with larvae and adults getting highly protected within buds and flowers, use of insecticides being usually rendered inefficient (Wu *et al.,* 2013). In addition, the unchecked use of pesticides has accelerated the rapid development of insecticide resistance as regards *T. tabaci* (Robb *et al.,* 1995; Martin *et al.,* 2003). In order to achieve optimal control of onion thrips and reduce the application of pesticides, tremendous effort has been devoted to develop Integrated Pest Management (IPM) based on an accurate identification of *T.*

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tabaci biotypes. Interestingly, the host-plant preference and the potential of the onion thrips as a vector for TSWV and host-plant preferences varies considerably among its populations (Zawirska, 1976). Zawirska (1976) proposed two biotypes for *T. tabaci* in Poland, i.e. the oligophagous "tabaci type" (feeding on tobacco plants; associated with the spread of TSWV) and the polyphagous "communis type" (neither feeding on tobacco nor a vector for TSWV). In the presence of vector biotype (tabaci-type), it is necessary to apply the insecticides quickly to prevent the spread of TSWV, while in the case of non vector biotype (communis type) the use of biological control techniques is favored (Brunner *et al.,* 2004).

In addition, varying susceptibility of *T. tabaci* to different groups of insecticides (Nishimori *et al.,* 2003; Shelton *et al.,* 2003; Murai, 2004; Allen *et al.,* 2005) convinced Toda and Murai (2007) as regards the possible existence of different strains of *T. tabaci* in various parts of the world. Such biotypic variation has been proposed for *Frankliniella occidentalis* (Pergande) (De Kogel *et al.,* 1997) too.

The striking similarity among all the developmental stages of thrips and polymorphism leads to confusion and inaccuracy when trying to make a morphological identification. Unlike the traditional methods of identification, polymorphism and sex or stage of development cannot limit the molecular identification of target species (Asokan *et al.,* 2007). Furthermore, any prevalence of biotypes and biotype associated strain variations in tospoviruses could be elucidated using molecular techniques (Asokan, *et al.,* 2007).

Few morphometric studies have been conducted on thrips species throughout the world. Raizada (1976) carried out a morphometric analysis on the populations of *Scirtothrips dorsalis* Hood and *S. oligochaetus* (Karny) with reference to their biological and ecological variations, suggesting that considerable differences existed between these two thrips species. Based upon the morphometric analysis of *T. hawaiiensis* (Morgan) species-group, two distinct groups were found in the Orient, Pacific and American regions, corresponding to *T. hawaiiensis* and *T. florum* Schmutz, and an intermediate group, *T. exilicornis* Hood from Africa (Palmer and Wetton, 1987). Kumar *et al.* (2011) conducted a preliminary morphometric analysis of various populations of *Scirtothrips dorsalis* from five different geographical regions including India, Japan, Israel, and St. Vincent and Florida in the United States of America. Comparing fourteen morphometric characteristics of each population indicated that the Japan population was significantly more robust than the other populations.

Resolution of the taxonomic status of *T. tabaci* populations in Iran has been hampered by a lack of genetic studies of populations. The development of various molecular techniques during the last few decades, particularly analysis of mitochondrial DNA (mtDNA), has resulted in significant progress in understanding inter as well as intra-species genetic diversity and speciation issues (Moritz *et al*., 1987; Avise, 1994). These new approaches are particularly useful in such different puzzling groups of insects as thrips, in which a mosaic of various ecological traits prevents the occurrence of conserved morphological features (Brunner *et al*., 2004).

Klein and Gafni (1996) observed molecular variations in Israeli populations of *T. tabaci* using RAPD-PCR method and found significant differences among populations. Applying RAPD-PCR analysis, Jenser *et al*. (2001) observed intraspecific molecular variations between *T. tabaci* populations collected from tobacco and from onion. The amplified DNA-banding patterns of tobacco populations were significantly different from those propagating on onion. Moreover, Brunner *et al.* (2004) conducted molecular phylogenetic analysis in some European populations of *T. tabaci*, as based on sequences of the mitochondrial

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Cytochrome Oxidase subunit I (COI) gene and suggested that variation in host preference is related to the existence of a complex of cryptic (sub) species group in *T. tabaci* populations. To investigate the intra-specific genetic diversity of this species, Toda and Murai (2007) used a 810 bp region of the mtCOI gene and found 17 haplotypes. They observed apparent differences between arrhenotokous and telytokous populations. Furthermore, the phylogenetic tree showed two distinct groups. They identified two haplotypes as problematic ones which could cause a greater deal of damage and exerted a higher level of insecticide resistance. Glover *et al.* (2010) assessed loci for DNA barcoding in the genus *Thrips* Linnaeus and demonstrated that COI provided sufficient variation to be used in DNA barcoding within this genus. Zhang *et al.* (2011) studied a species-specific COI marker for rapid diagnosis of the invasive species of western flower thrips, and found that their diagnostic PCR assay provides a quick, simple and reliable molecular technique for the identification of *F. occidentalis*.

Because of a wide range of host plants, broad geographical distribution as well as a reduced susceptibility of the *T. tabaci* to insecticides; it seems likely that different strains of *T. tabaci* exist on different hosts and localities in Iran. Iranian populations of *T. tabaci* have been poorly studied, and therefore, there is a dire need for a critical study to fill up the gap as regards the analysis of this species. As an example cited, employing the same method fails to control this species in different localities and/or on different host plants. Size differences have also been observed in some populations. So, detailed analyses of morphometric characteristics of different populations colonized on onion and tobacco were carried out. The nucleotide sequences of the COI gene of different populations of *T. tabaci* collected from tobacco and onion in Iran were also analyzed and compared. An analysis of nucleotide sequence data provides an opportunity to test the validity of morphometric differences. Morover, the genetic structure of onion thrips populations was examined and congruence of these findings with morphometric and ecological features were tested.

MATERIALS AND METHODS

Thrips tabaci samples were collected from tobacco and onion crop plants from throughout some 22 sites in Iran (Table 1), immediately stored in 75% ethanol for morphometric analysis and as well in 96% ethanol until DNA extraction to be carried out.

Morphometric Analysis

A total of 631 slide-mounted specimens, including 28-30 individuals from each population, were prepared and examined. For each female, 24 continuous variables were assessed using a compound microscope and an ocular graticule. Measurements used for Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) are illustrated in Figure 1 and listed in the legend. Morphological terminology follows Palmer *et al.'s* (1992).

To reduce the size effects, all variables were divided by the length of the 7th antennal segment. Due to the relatively high number of cases with missing values, replacing missing values with "mean" was not carried out. Therefore, cases with missing values (382 cases) were excluded from the analyses. Analyses were carried out on both transformed and non-transformed data (ratio variables) (Manzari *et al.*, 2002; Polaszek *et al.*, 2004) using the statistical package SPSS 16.

DNA Extraction, Amplification and Sequencing

Species were first identified through observation of morphological traits and only thrips unambiguously identified as *T. tabaci*, were subjected to molecular analysis.

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Table 1. Population code, sampling locations, host plant and accession numbers of *T. Tabaci* populations.

Population code	Locality (City-province)	Host plant	Accession numbers
TO1	Shirhesar - Khoasan Razavi	*Allium cepa*	HM600786
TO2	Ashkhaneh - Khorasan Shomali	*A.cepa*	HM600787
TO3	Galikash - Golestan	*A.cepa*	HM600788
TO4	Sari - Mazandaran	*A.cepa*	HM600789
TO5	Rasht - Gilan	*A.cepa*	HM600790
TO6	Azarshahr - East Azarbaijan	*A.cepa*	HM600791
TO7	Oshnavieh -West Azarbaijan	*A.cepa*	HM600792
TO8	Tarome Sofla - Ghazvin	*A.cepa*	HM600793
TO9	Zanjan - Zanjan	*A.cepa*	HM600794
TO10	Harsin - Kermanshah	*A.cepa*	HM600795
TO11	Marivan - Kordestan	*A.cepa*	HM600796
TO12	Boroojerd - Lorestan	*A.cepa*	HM600797
TO13	Khomein - Markazi	*A.cepa*	HM600798
TO14	Flavarjan - Esfahan	*A.cepa*	HM600799
TO15	Shiraz - Fars	*A.cepa*	HM600800
TO16	Ahvaz - Khoozestan	*A.cepa*	HM600801
TO17	Dezful - Khoozestan	*A.cepa*	HM600802
TO18	Borazjan - Booshehr	*A.cepa*	HM600803
TT1	Aliabad - Golestan	*Nicotiana tobacum*	HM600804
TT2	Darab Kola - Mazandaran	*N. tobacum*	HM600805
TT3	Somee Sara - Gilan	*N. tobacum*	HM600806
TT4	Rasht - Gilan	*N. tobacum*	HM600807

Total genomic DNA was extracted from each single female adult thrips using DNeasy Tissue and Blood Kit (Qiagen) by grinding and then incubating at 56ºC overnight in proteinase K, with elution in 30 µL of distilled water. 25 µL PCR reactions were then carried out in a GeneAmp PRIMUS thermal cycler using 2 µL DNA extract, 2.5 µL *Taq* buffer (1.5 mM $MgCl_2$), 0.25 µL (1.5 U) *Taq* polymerase (Roch), 0.5 µL (0.2 mM) dNTPs and 1 µL (0.4 µM) of each primer.

The Cytochrom Oxidase subunit I (COI) region of mitochondrial DNA was amplified using the following primers (Simon *et al.,* 1994):

C1-J-1751: 5´-GGA TCA CCT GAT ATA GCA TTC CC-3´

C1-N-2191: 5´-CCC GGT AAA ATT AAA ATA TAA ACT TC-3´

PCR products were then sequenced with the same primers using Big Dye terminators. PCR conditions were as follows: 40 cycles of 94ºC denaturation (45 seconds), 50ºC annealing (45 seconds), and 72ºC extension (1 minutes) with an initial denaturation for 5 minutes and a final extension of 7 minutes.

Phylogenetic Analysis

The sequences obtained, together with 21 additional ones, two as the outgroups and 19 as the ingroups, retrieved from GenBank (from Inoue and Sakurai (2007); accession numbers AB277231, AB277226 and Brunner *et al*., (2004); accession numbers AY196831-AY196849) were aligned by eye. Maximum Likelihood (ML), Maximum Parsimony (MP) and Neighbor-Joining (NJ) analyses were carried out using software PAUP*4.0b10 (Swofford, 2002) to assess phylogenetic relationships. Heuristic searches were carried out with 10,000 random additions followed by branch swapping, using Tree-Bisection-Reconnection (TBR), and holding a single tree. Maximum parsimony analysis was carried out using all the equal weights. A bootstrap analysis was performed to test for

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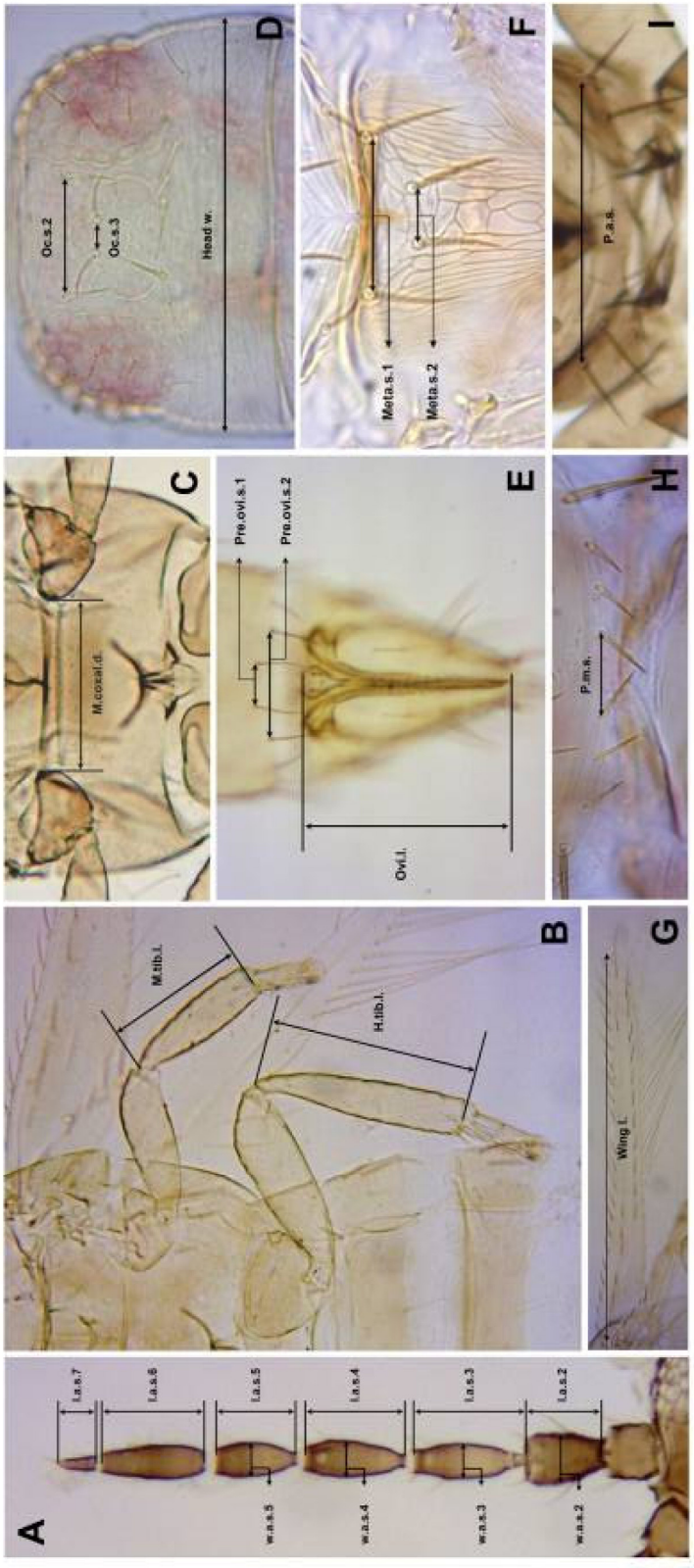

Figure 1. (A) l.a.s. and w.a.s., length and width of antennal segments, respectively; (B) M.tib.l and H.tib.l, length of mid and hind tibiae, respectively; (C) M.coxal.d., distance between mid coxae; (D) Oc.s.2 and Oc.s.3, distance between second and third ocellar setae, respectively, Head w., head width; (E) Pre.ovi.s.1 and Pre.ovi.s.2, distance between posteromarginal setae S1 and S2 of 7th abdominal sternite, respectively, Ovi.l., ovipositor length; (F) Meta.s.1 and Meta.s.2, distance between lateral and median metanotal setae; (G.) Wing l., wing length; (H) P.m.s., distance between median posteromarginal setae; and (I) P.a.s., distance between posteroangular setae.

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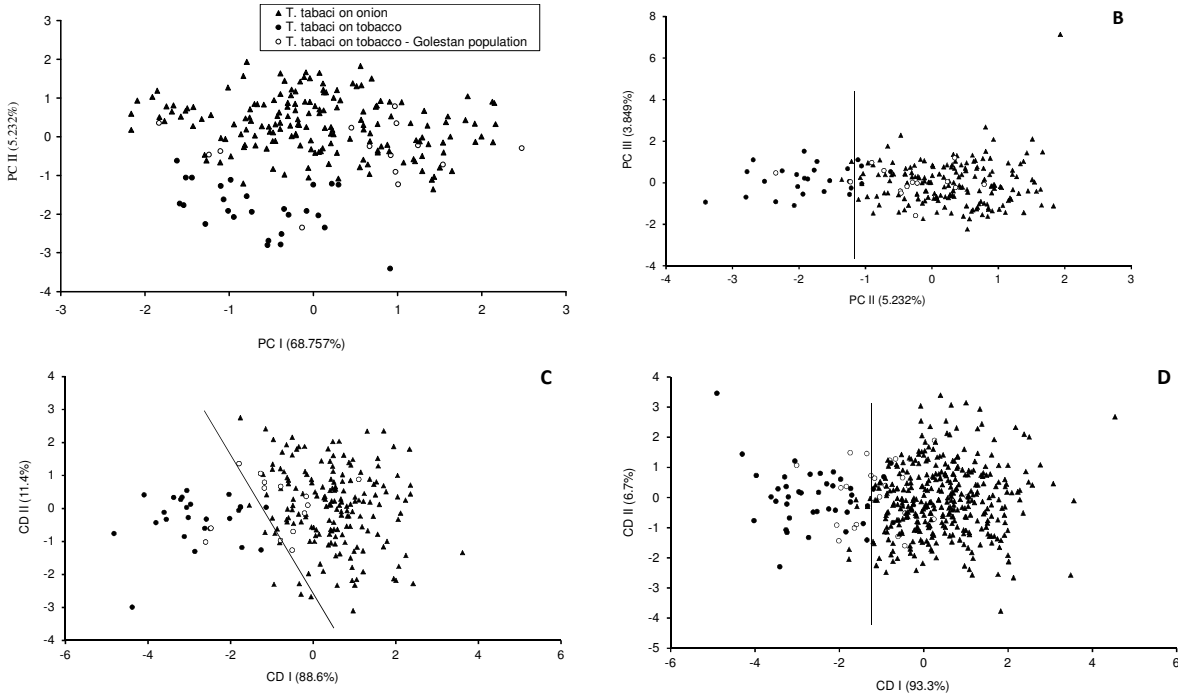

Figure 2. (A) Scatter diagram with respect to first and second principal components; (B) Scatter diagram with respect to components II and III; (C) Scatter diagram with respect to first and second canonical discriminant function using the "entering independent variables together" method, and (D) Scatter diagram with respect to first and second canonical discriminant function using the stepwise method.

statistical significance of the trees generated with 1,000 pseudoreplicates and under stepwise addition option for MP. GenBank sequences from *T. palmi* (AB277231) and *T. hawaiiensis* (AB277226) used as outgroups. The Jackknife support for each alignment was found using 1,000 pseudoreplicates holding only one tree.

RESULTS

Morphometric Analysis

Principal Component Analysis

Using nontransformed data, five components were extracted as based on 23-variables and biplots of all the pairwise combinations of those examined. The extracted communality values of variables (the proportion of variance accounted for, by the factors for each variable) were observed as high (0.556-0.964). Eigenvalues and weights (component score coefficients) for five principal components are presented in Table 2. Analysis of log-transformed data gave virtually the same results (not presented).

Figure 2-A shows a scatter diagram with respect to the first and second principal components obtained using ratio variables. It can be seen as two separate groups with some overlap. Thrips collected from tobacco (group 2) are evidently distinct from those collected from onion (group 1). The overlap is mainly related to the Golestan population, collected from tobacco. Thrips individuals were projected on the first two Principal Components (PCI and PC-II), accounting for 73.989 of the overall variance. l.a.s4/l.a.s7, l.a.s6/l.a.s7, Ovi.l./l.a.s7, Wing L./l.a.s7,

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Table 2. Eigenvalues and weights for five principal components extracted as based on ratio variables.[a]

Variable	Component				
	1	2	3	4	5
Eigenvalues	15.814	1.203	0.885	0.704	0.654
	component score coefficients				
l.a.s.2/l.a.s.7	0.825	-0.214	0.027	0.050	-0.168
l.a.s.3/l.a.s.7	0.870	0.177	-0.159	0.027	-0.169
l.a.s.4/l.a.s.7	0.930	-0.022	-0.075	0.012	-0.134
l.a.s.5/l.a.s.7	0.906	-0.068	-0.019	0.006	-0.101
l.a.s.6/l.a.s.7	0.922	-0.119	-0.040	-0.082	-0.047
w.a.s.2/l.a.s.7	0.840	-0.390	0.064	0.000	-0.038
w.a.s.3/l.a.s.7	0.839	-0.335	0.195	-0.041	0.042
w.a.s.4/l.a.s.7	0.842	-0.412	0.079	0.073	-0.029
w.a.s.5/l.a.s.7	0.838	-0.379	0.085	0.096	-0.023
Oc.s2/l.a.s.7	0.870	0.106	-0.037	0.129	-0.008
Oc.s3/l.a.s.7	0.590	0.266	-0.186	0.673	-0.032
Head w./l.a.s.7	0.727	0.020	-0.015	0.161	-0.030
P.m.s./l.a.s.7	0.614	-0.130	-0.218	0.087	0.718
P.a.s./l.a.s.7	0.886	0.066	-0.047	-0.158	0.080
Meta.s.1/l.a.s.7	0.826	0.279	0.172	-0.133	0.083
Meta.s.2/l.a.s.7	0.465	0.265	0.797	0.154	0.109
M.tib.l./l.a.s.7	0.863	0.318	-0.033	-0.127	-0.040
H.tib.l./l.a.s.7	0.899	0.292	-0.102	-0.081	-0.056
Pre.ovi.s.1/l.a.s.7	0.750	0.129	-0.068	-0.214	0.047
Pre.ovi.s.2/l.a.s.7	0.850	0.123	-0.002	-0.172	0.053
Ovi.l./l.a.s.7	0.913	-0.121	-0.045	-0.007	-0.055
Wing l./l.a.s.7	0.913	0.107	-0.154	-0.089	-0.018
M.coxal.d/l.a.s.7	0.908	0.183	0.060	-0.040	0.082

[a] See Figure 1 for abbreviations

M.coxal.d./l.a.s7 and l.a.s5/l.a.s7 exerted the highest influences on PC-I, and while Meta s2/l.a.s7, Oc.s3/l.a.s7 and P.m.s./l.a.s7 presented the lowest contribution (Table 2). Other variables exhibited approximately equal contributions. Along PC-II, Head w./l.a.s7 and l.a.s4/l.a.s7 had the least influence, while w.a.s4/l.a.s7, w.a.s2/l.a.s7 and w.a.s5/l.a.s7 the greatest (Table 2).

Considering the separation based on other components (components II and III) the discrimination of these two groups was not affected by size (Figure 2-B).

Canonical Discriminant Analysis

Analysis was conducted within group sizes because of the differences in the number of individuals per group. Separation between populations collected from onion and those collected from tobacco was relatively highly marked using canonical discriminant analysis of variables. Based on ratio variables, thrips individuals were projected on the first and second canonical discriminant function (CD-I and CD-II)

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using the "Entering Independent Variables Together" method, which together accounted for 100% of original variance (Figure 2-C). In this method, all the predictor variables are simultaneously analysed and are given equal priority. In other words, there is no prediction or assumption for the existence of any special relationship between each predictor variable and group membership (Polaszek *et al.*, 2004).

Table 3 shows standardized canonical discriminant function coefficients and functions at group centroids (group means are the centroids). Based on this table, the first discriminant function is largely a measure of M.coxal.d./l.a.s7 and w.a.s4/l.a.s7 that separate thrips collected from tobacco from those collected from onion. Along CD-II, H.tib.l./l.a.s7 stands out as important. In total, 81.1% of the original grouped cases were correctly classified. Using stepwise method gave almost the same results of the "entering independents together" method, but 79.4% of the originally grouped cases were correctly classified (Figure 2-D).

Table 3. Standardized canonical discriminant function coefficients calculated from the "entering independents together" method.[a]

Variable	Function	
	1	2
l.a.s.2/l.a.s.7	-0.247	-0.068
l.a.s.3/l.a.s.7	0.190	-0.397
l.a.s.4/l.a.s.7	0.082	-0.440
l.a.s.5/l.a.s.7	-0.324	-0.585
l.a.s.6/l.a.s.7	-0.319	-0.626
w.a.s.2/l.a.s.7	-0.454	0.432
w.a.s.3/l.a.s.7	0.014	0.331
w.a.s.4/l.a.s.7	-0.699	-0.097
w.a.s.5/l.a.s.7	-0.351	0.239
Oc.s2/l.a.s.7	0.022	-0.091
Oc.s3/l.a.s.7	0.267	-0.066
Head w./l.a.s.7	0.188	0.197
P.m.s./l.a.s.7	-0.033	0.269
P.a.s./l.a.s.7	-0.137	0.421
Meta s.1/l.a.s.7	0.453	-0.161
Meta s.2/l.a.s.7	-0.075	-0.208
M.tib.l./l.a.s.7	0.536	0.349
H.tib.l./l.a.s.7	-0.411	0.841
Pre.ovi.s.1/l.a.s.7	0.295	-0.395
Pre.ovi.s.2/l.a.s7	0.353	-0.243
Ovi.l./l.a.s.7	0.042	0.204
Wing l./l.a.s.7	-0.046	0.616
M.coxal.d./l.a.s7	0.922	0.020

[a] See Figure 1 for abbreviations.

It is worth mentioning that in all analyses; the overlap is largely related to the Golestan population collected from tobacco. Disregarding this overlap, morphometric analyses indicate that thrips collected from tobacco are mostly distinct from those collected from onion. No single measurement or ratio provided a definite separation of the two groups. Excluding some of the variables form analyses, gave nearly the same results (not presented).

Phylogenetic Analysis of Molecular Data

The 422bp region of the mitochondrial COI gene was successfully amplified. A total of 73 variable characters were parsimony-informative while 51 variable characters were parsimony-uninformative. The sequences have been deposited in GenBank with Accession numbers HM600786- HM600807 (Table 1). The MP tree (Length=187, RI= 0.9314, CI= 0.8021) is shown in Figure 3, with bootstrap and Jackknife values indicated. The MP tree revealed two major subdivisions that arrayed *T. tabaci* populations according to host plants (Figure 3). Those groups are called 'clade A' and 'clade B'. Clade 'B' consists of two subgroups: B_1 and B_2. Clade "B" was exclusively composed of haplotypes from thrips individuals collected from onion, with the exception of one haplotype collected from tobacco. The clade "A"

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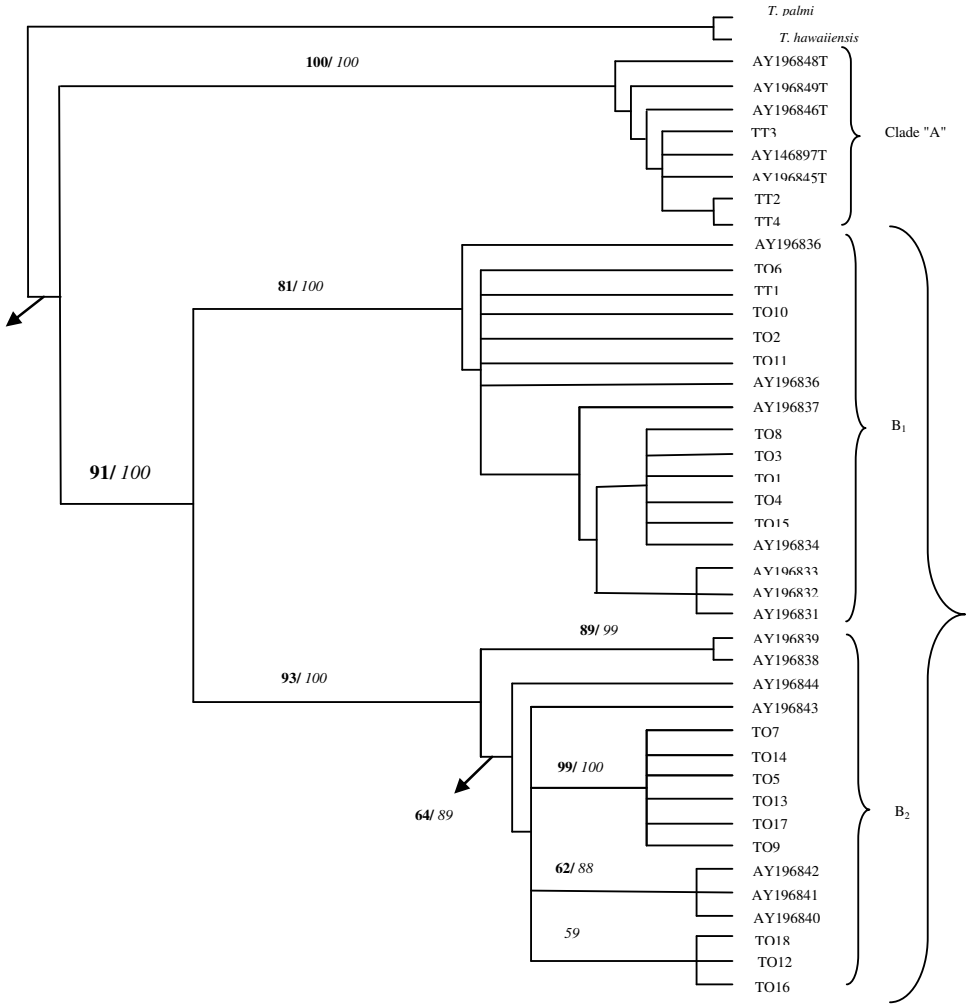

Figure 3. Tree topology using COI sequence data derived from Maximum parsimony (bold and italic figures indicate bootstrap and jackknife values, respectively). See Table 1 for population codes.

contained haplotypes collected merely from tobacco. The maximum likelihood tree (not presented) gave the same clustering as in the MP tree. The Neighbor-Joining tree (NJ) was also the similitude of as MP and ML trees (not presented). Its main stems showed the same pattern as MP and ML trees with high bootstrap values.

DISCUSSION

The study's analyses indicate that genetic differentiation is significant among the populations of *T. tabaci* on different host plants. The thrips' populations on tobacco were clearly distinct from those collected from onion. This may support the existence of different biotypes/forms of the pest on different host plants in Iran, as also suggested in previous studies (Zawirska, 1976; Chatzivassiliou, 2002). Molecular evidence presented in this study strongly suggests that *T. tabaci* forms three distinct, well-supported lineages in consistence with a tobacco group and with two onion groups (Figure 3).

Brunner *et al.* (2004), proposed three distinct major lineages (T, L1 and L2) in *T.*

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tabaci using molecular techniques. They found that *T* is the tobacco-associated lineage, while *L1* and *L2* are the leek-associated lineages with *T*, as well as others, and sibling species with two leek haplotypes (L1 and L2) indicative of host-race. The haplotypes, AY196845-49, belong to the tobacco type (T) while, haplotypes AY196838-44 being members of *L1* plus AY196831-37 members of *L2*. The group "B" in the present study (consisting of two sub groups) corresponds to lineages *L1*, *L2* while the group "A" corresponding to lineages *T,* as previously found by Zawirska (1976). The trees with high bootstrap values confirmed the monophyly of subgroups B_1 and B_2 with the group 'A' as their sister group. This result supports the phylogenetic relationship proposed by Brunner *et al.* (2004).

The results of both molecular and morphometric analyses are suggestive of the same scenario, showing that tobacco populations, with the exception of the Golestan population, form a distinct clade from onion populations. It is speculated that the exception of the Golestan population may have been due to the original development of this population on some other host-plant [other than tobacco (e.g. onion)] or their accidental occurrence on tobacco; for example, short-rang dispersal by either flight or wind. This idea is in agreement with the results of the present paper's biological study on these populations (Fekrat *et al.,* 2009).

Finally, it becomes evident that an accurate identification of *T. tabaci* biotypes is of paramount importance in improving the efficiency in properly handling of the chemical control strategies.

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تنوع مولکولی و مورفومتریکی جمعیت‌های مختلف تریپس پیاز، *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) روی پیاز و توتون در ایران

ل. فکرت، ش. منظری، و پ. شیشه بر

چکیده

تریپس پیاز، *Thrips tabaci* Lindeman، آفتی با پراکنش وسیع است که طیف وسیعی از محصولات زراعی را مورد حمله قرار می‌دهد. به منظور بررسی تنوع مورفومتریکی و ژنتیکی این آفت در ایران، ۴ جمعیت از روی توتون و ۱۸ جمعیت از روی پیاز از هفده استان کشور جمع‌آوری گردید. آنالیزهای مورفولوژیکی با استفاده از روش‌های تجزیه به مولفه‌های اصلی و تجزیه تشخیص کانونیکی، آشکارا جمعیت‌های جمع‌آوری شده از روی توتون را از جمعیت‌های جمع‌آوری شده از روی پیاز تفکیک نمودند. داده‌های تعیین توالی DNA ژن میتوکندریایی COI برای تمامی جمعیت‌ها به همراه توالی‌های مربوط به ۲۱ جمعیت که از بانک ژن استحصال شده بودند، مورد بررسی قرار گرفتند. آنالیزهای بیشینه پارسیمونی جمعیت‌های جمع‌آوری شده از روی توتون را به استثنای جمعیت مربوط به استان گلستان، به خوبی از جمعیت‌های جمع شده از روی پیاز تفکیک نمود. نتایج آنالیزهای بیشینه احتمال و neighbor-joining نیز مشابه بودند. آنالیزهای مولکولی و مورفومتریکی موید هتروژن بودن جمعیت‌های تریپس پیاز بوده و مشخص کردند که این آفت روی پیاز و توتون، حداقل دارای دو بیوتیپ مختلف می‌باشد.

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