



Genetic variability in *Thrips tabaci* (Insecta: Thysanoptera) living on vegetables in Serbia

Cvrković Tatjana, Jović J., Mitrović M., Krstić O.,
and Toševski I.



Institute for Plant Protection and Environment, Serbia



- ✓The onion thrips (*Thrips tabaci* Lindeman) belongs to the family Thripidae (Thysanoptera)
- ✓Widely distributed throughout the world
- ✓Highly polyphagous - causes damages on tobacco, alliaceus crops, cabbage, and ornamental plants
- ✓The damage - caused by feeding or by transmitting *tomato spotted wilt virus* (TSWV) and *Iris yellow spot virus* (IYSV)





- ✓ TSWV and IYSV - tospoviruses with a wide host range that are capable of causing serious epidemics and crop losses
- ✓ The effectiveness of *T. tabaci* as a vector of TSWV and host-plant preference can vary dramatically among populations
- ✓ *T. tabaci* consists of two biotypes (Zawirska, 1976)
- ✓ 'Tabaci type' - associated with tobacco plants; vector of TSWV
- ✓ 'Communis type' - different host plants (not tobacco); couldn't transmit TSWV
- ✓ leek associated populations transmitted efficiently IYSV on onion (Chatzivassiliou et al., 2002).



- ✓ Morphological identification in adult and nymphal stages, is limited by polymorphism and the high degree of similarity
- ✓ Molecular identification, is not hampered by the above factors and can easily be followed
- ✓ Genetic markers, like mtDNA - a valuable addition or alternative to classical methods of species identification
- ✓ This study investigates the genetic variability of *T. tabaci* populations on different vegetable crops in Serbia, based on sequence variation of the mitochondrial cytochrome oxidase I (COI) gene.



MATERIALS AND METHODS



Insect collection

Vegetables from 4 sites in Serbia:

- a) open field and greenhouse production area around village Donji Vrtogoš (near town Vranje, South Serbia)
- b) open field and greenhouse production area located around village Slanci (about 10 km east from Belgrade)
- c) main greenhouse and open field production area located near town Ub (Central Serbia)
- d) open field bean production area in Zemun

Sample code	Locality	Lat/Long	Host plant	Collection date	Accession No.
S8	Vranje	N42 29.192 E21 49.190	cucumber	01-Sept-2010	JX275861
S13	Zemun	N44 51.32 E20 22.625	bean	06-Aug-2010	JX275862
S29	Slanci	N44 48.899 E20 34.436	leek	26-July-2011	JX275863
S58	Ub	N 44 48 298 E 20 34 228	pepper	27-July-2011.	JX275864
S60	Ub	N 44 28 322 E 20 01 278	onion	27 -July-2011.	JX275865



DNA extraction, PCR amplification and sequencing

- ✓DNA was extracted from a single specimen
- ✓Mitochondrial COI gene
- ✓Amplification for the barcode region of the COI gene was performed using LCO1490 and HCO2198 primers (658bp)



Phylogenetic analysis

- ✓Nine COI sequence data on *T. tabaci* submitted by Brunner et al. (2004) were obtained from the NCBI database and used for phylogenetic analysis employing the sequence data obtained in this study
- ✓The complete mitochondrion sequence data of *T. palmi* (AF378690) and *T. angusticeps* (AF378679) (Brunner et al, 2002) provided from GenBank served as an outgroups
- ✓Phylogenetic analysis - maximum-likelihood (ML) and neighbor-joining method
- ✓Five hundred bootstrap replicates were performed to assess branch support in the resulting tree topology



RESULTS



Phylogenetic analysis

- ✓ Mitochondrial COI gene of *T. tabaci* individuals collected from five host plants were successfully amplified and sequenced (acc. num. JX275861 to JX275865)
- ✓ Selected sequences were trimmed to 471 bp, the length of the shortest fragment which corresponds to all sequences of *T. tabaci*, *T. palmi* and *T. angusticeps*



Phylogenetic analysis

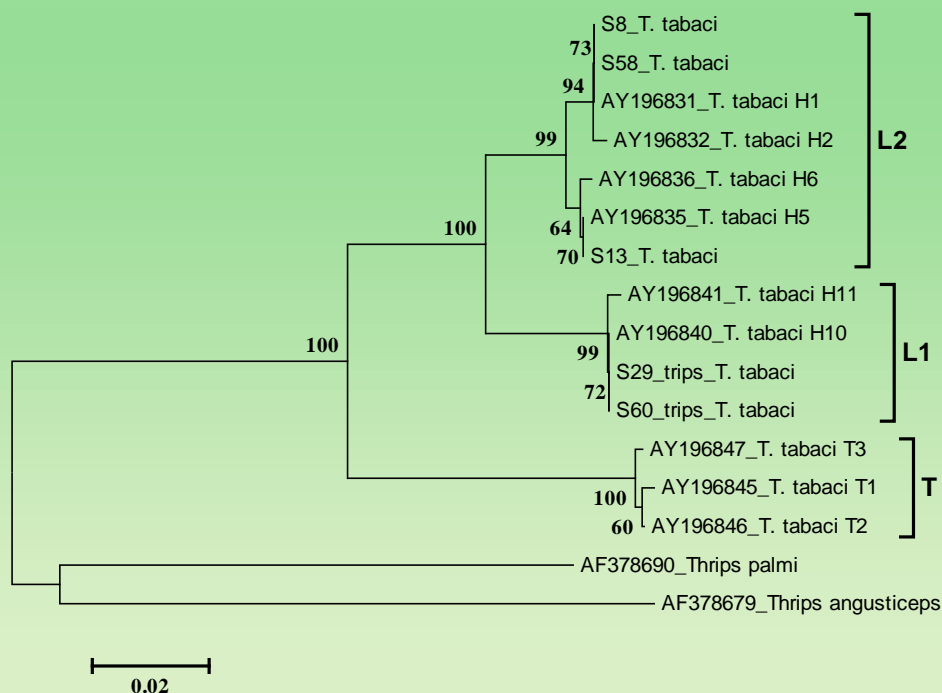
✓The COI sequences showed polymorphism at 18 nucleotide positions (0.4%) among the five Serbian samples. All variations were in the form of silent, single base-pair substitutions, resulting in no amino-acid replacement

Sequence code	Nucleotide position																	
	78	87	99	114	117	189	246	288	294	307	309	321	354	363	381	384	390	399
S8	A	G	A	A	G	A	G	C	T	T	G	G	C	C	A	A	G	T
S58
S13	G	A	.	.	.	A
S29	.	A	G	G	A	G	A	T	C	C	.	A	T	T	G	G	A	C
S60	.	A	G	G	A	G	A	T	C	C	.	A	T	T	G	G	A	C



Phylogenetic analysis

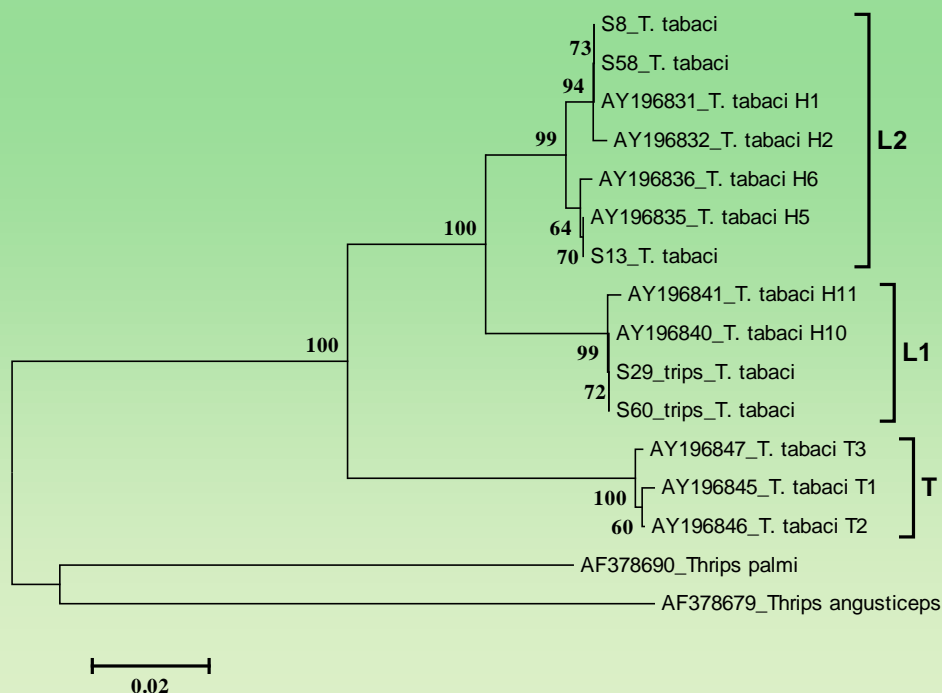
✓ Phylogenetic trees were estimated using the neighbor-joining and maximum likelihood method. For both analyses, trees showed the same pattern of phylogenetic clustering with main stems supported by high bootstrap values, and due to that we showed only the NJ tree





Phylogenetic analysis

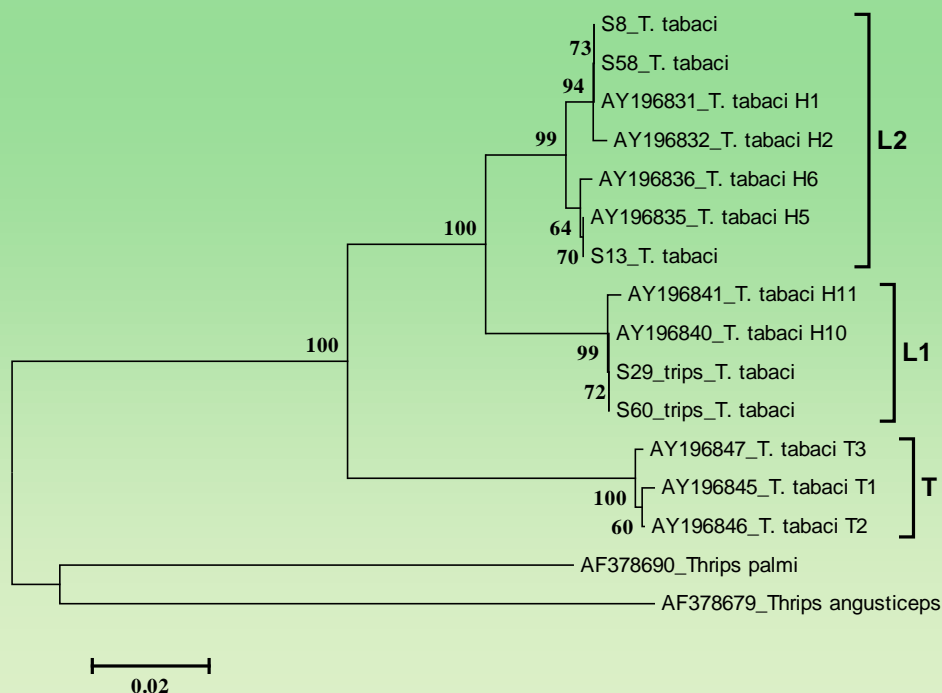
- ✓ Three different haplotypes were identified among COI sequences from Serbia
- ✓ All samples belong to the leek-associated lineage (L1 and L2), proposed by Brunner et al. (2004)
- ✓ none of them belongs to tobacco-associated lineage (T)





Phylogenetic analysis

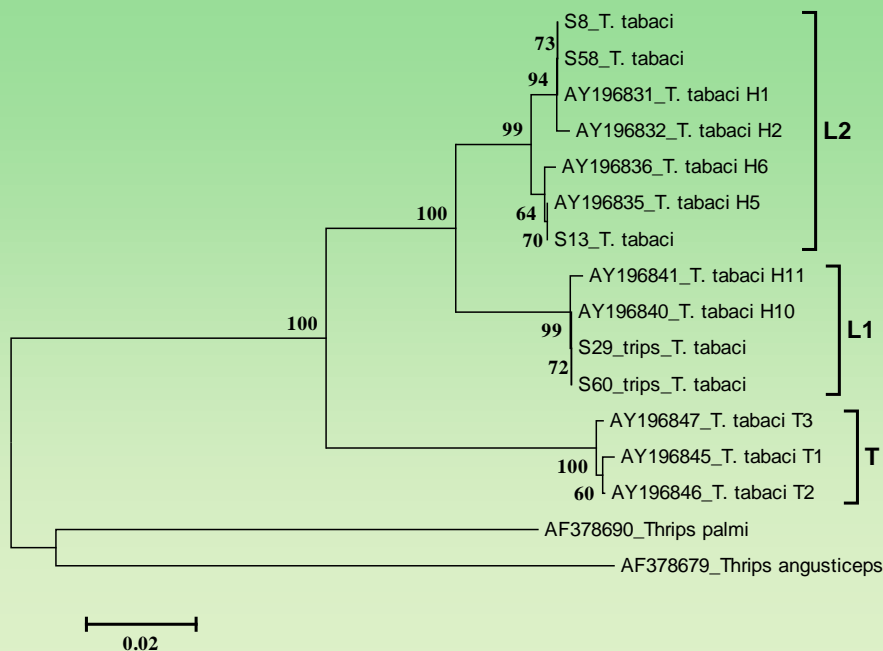
- ✓ Two haplotypes belong to Group 2 which corresponds to L2 group, while the third haplotype belongs to Group 1 which corresponds to L1 group
- ✓ Uncorrected pairwise distance between these two groups are about 0.4%, and between haplotypes inside Group 2 are 0.7%





Phylogenetic analysis

- ✓ Two samples S8 and S58, collected on cucumber and pepper, respectively, showed 100% similarity between each other and with H1 of leek-associated group (AY 196831)
- ✓ Specimen S13 collected on bean indicate 100% similarity with H5 (AY 196835)
- ✓ Samples S29 and S60, collected on leek and onion, respectively, showed 100% homology between each other and with H10 of leek-associated group (AY196840)





DISCUSSION AND CONCLUSIONS



- ✓ Brunner et al. (2004), proposed three distinct major lineages (T, L1 and L2) in *T. tabaci*
- ✓ They suggested that T is the tobacco-associated lineage, while L1 and L2 are the leek-associated lineages
- ✓ We found three mitochondrial COI haplotypes inside the leek-associated lineage, while none of the analyzed samples belonged to the tobacco-associated lineage (no sample was collected from host plants of the tobacco type)
- ✓ Our analyses clearly indicate that genetic differentiation is not correlated with host plant preference inside leek-associated group



- ✓The biological relationship of *T. tabaci* with TSWV and IYSV seems to be more complex than previously believed
- ✓The worldwide distribution of the *T. tabaci* populations in an extensive range of hosts may have generated specific associations with the tospoviruses that had an impact in their transmission
- ✓Both tospoviruses (IYSW and TSWV) are widely distributed in different host plants in Serbia
- ✓The complex relationship between tospoviruses and *T. tabaci* as well as the infection mechanisms involved in this pathosystem remain to be elucidated
- ✓Understanding the factors which determine the vector competence of *T. tabaci* populations may provide useful knowledge to manage tospovirus epidemics
- ✓Further testing is required to correlate virus transmission with the presence of different populations in *T. tabaci*

*THANK YOU
FOR
ATTENTION*