



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

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- ✓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, capable of causing serious epidemics and crop losses
- ✓ The effectiveness of *T. tabaci* as a vector 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; transmitted efficiently IYSV on onion (Chatzivassiliou et al., 2002)



- ✓ *T. tabaci* is a very small insect that shows a high degree of similarity, particularly in preadult stages
- ✓ Difficult to identify at the species level
- ✓ 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



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 using the QIAGEN DNeasy extraction Kit
- ✓ The mitochondrial cytochrome oxidase subunit I gene (COI) was chosen as the appropriate gene with good genetic resolution for differentiation at species level
- ✓ 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 GenBank and used for phylogenetic analysis
- ✓ 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
- ✓ 500 bootstrap replicates were performed to assess branch support in the resulting tree topology



RESULTS



Phylogenetic analysis

- ✓COI gene of *T. tabaci* individuals collected from five host plants were successfully amplified and sequenced (acc. num. JX275861 to JX275865)
- ✓COI sequencing yielded a 617 bp long fragment for four specimens and 571bp long fragment for one individual of *T. tabaci*
- ✓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

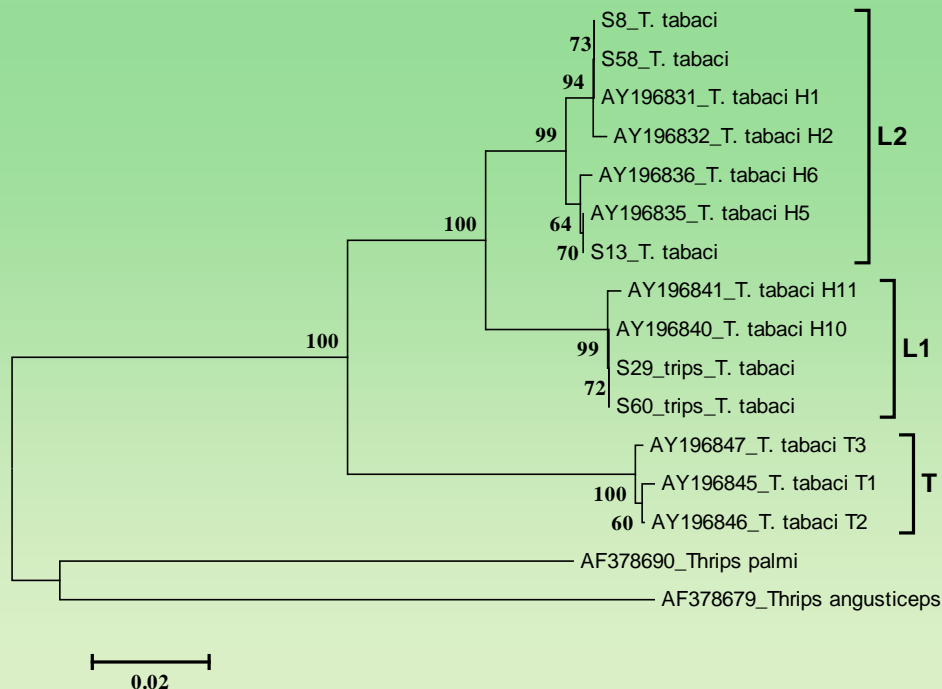
✓The COI sequences showed polymorphism at 18 nucleotide positions (0.4%) among the five Serbian samples. All variations were in the form of silent, single base-pair substitutions, resulting in no amino-acid replacement

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

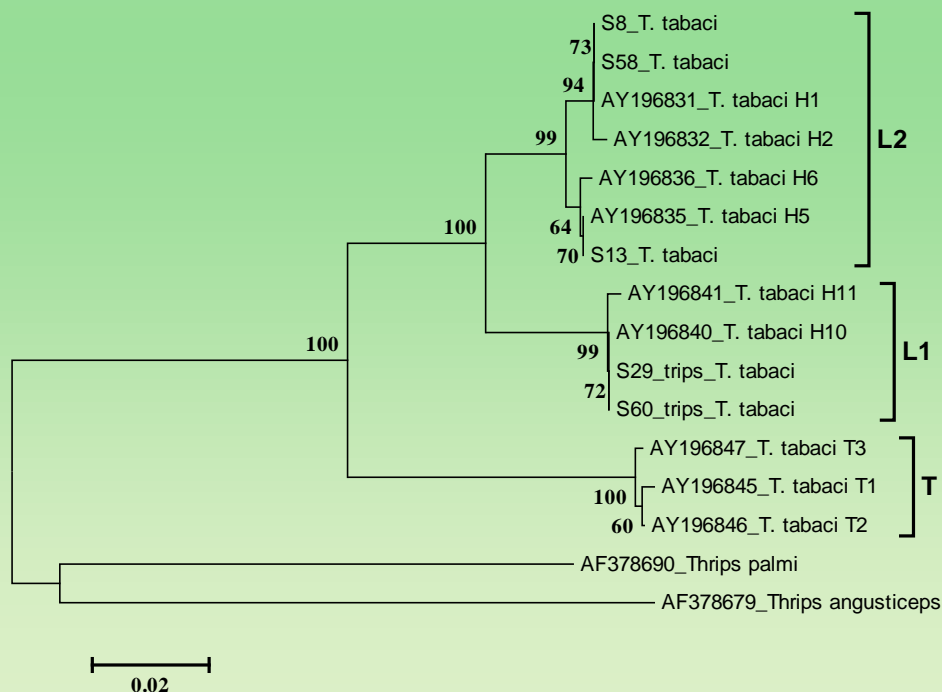
- ✓ Phylogenetic trees were estimated using the neighbor-joining and maximum likelihood method
- ✓ For both analyses, trees showed the same pattern of phylogenetic clustering





Phylogenetic analysis

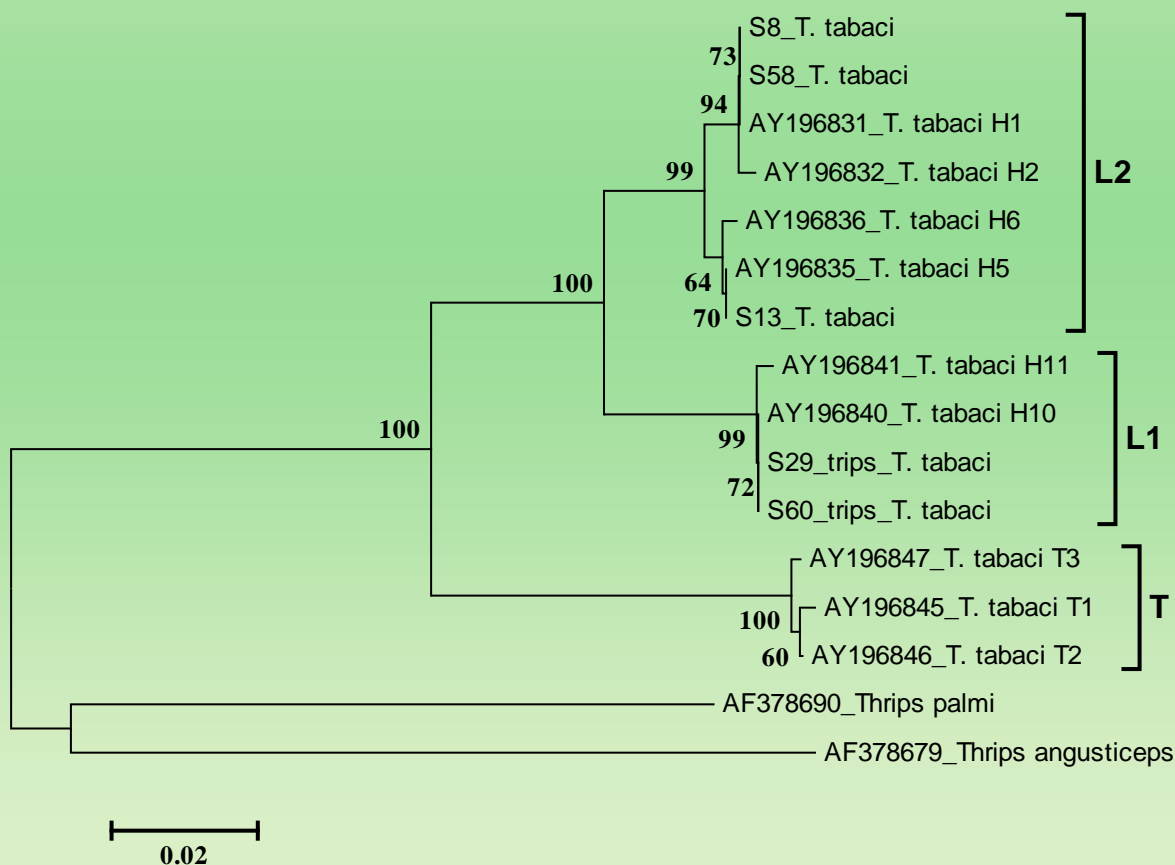
- ✓ Three different haplotypes were identified among COI sequences from Serbia
- ✓ All samples belong to the leek-associated lineage (L1 and L2), proposed by Brunner et al. (2004)
- ✓ None of them belongs to tobacco-associated lineage (T)





Phylogenetic analysis

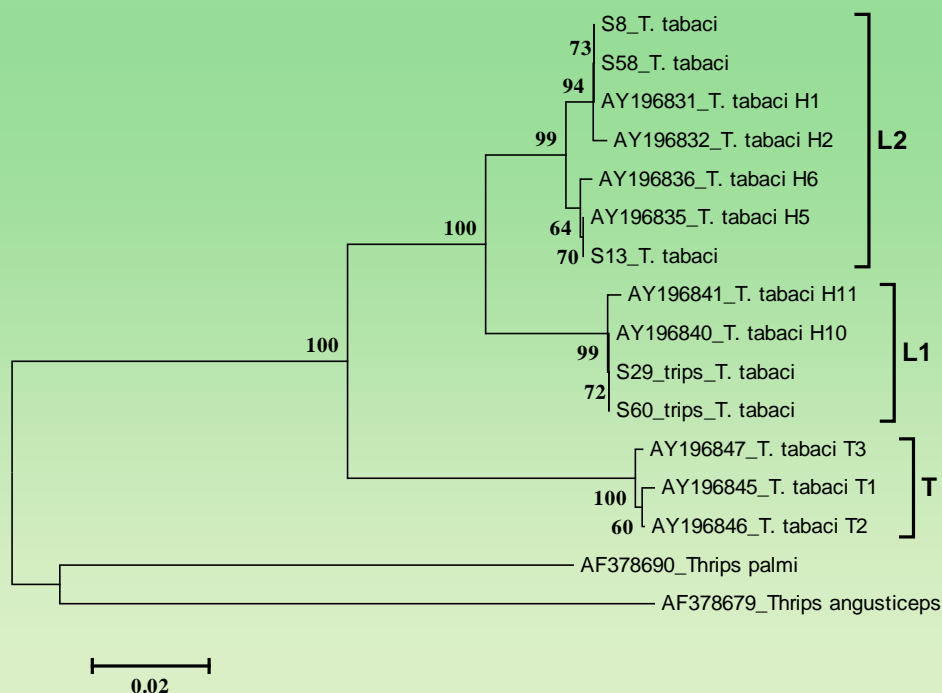
✓ Two haplotypes belong to L2 group, while the third haplotype belongs to L1 group





Phylogenetic analysis

- ✓ Two samples S8 and S58, collected on cucumber and pepper, showed 100% similarity between each other and with H1 of L2 group
- ✓ Specimen S13 collected on bean indicate 100% similarity with H5 of L2 group
- ✓ Samples S29 and S60, collected on leek and onion, showed 100% homology between each other and with H10 of L1 group





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 very complex
- ✓The worldwide distribution of the onion thrips 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 (Bulajić et al, 2008, Bulajić et al, 2009, Stanković et al, 2012)
- ✓The complex relationship between tospoviruses and *T. tabaci* as well as the infection mechanisms involved in this pathosystem remain to be elucidated
- ✓Further testing is required to correlate virus transmission with the presence of different populations in *T. tabaci*.

*THANK YOU
FOR
ATTENTION*