

MOLECULAR CHARACTERISATION
OF *THRIPS TABACI* LINDEMAN, 1889
(THYSANOPTERA: THIRIPIDAE) POPULATIONS
IN HUNGARY BASED ON THE ITS2 SEQUENCES

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Thrips tabaci is comprised of morphologically indistinguishable 'biotypes' or cryptic species with various host ranges, populations propagating by distinct modes and with different virus vector ability. *T. tabaci* 'communis-type' has wide host range while *T. tabaci* 'tabaci-type' is associated only with tobacco. Since tobacco was introduced to Europe only 500 years ago, we supposed that the differentiation of the *T. tabaci* 'tabaci-type' population had to begin on another host species that is native to the Palearctic region. To observe the interaction between host plant preference and molecular characteristics, maximum likelihood tree based on the sequences of the internal transcribed spacer 2 region of the rDNA (ITS2) of *Thrips tabaci* specimens collected on tobacco, onion, cabbage and distinct weed plants from various locations of Hungary was analysed. According to the results of the phylogenetic study the only common host for *Thrips tabaci* 'tabaci-type' and *Thrips tabaci* 'communis-type' was *Solanum nigrum*. This finding supported our hypothesis that the splitting process of the two main molecular clades could have happened on this solanaceous host species. To compare our results to that of the literature based on cytochrome oxidase I (COI) sequences further investigations with these markers (mitochondrial DNA markers) still needed.

Key words: *Thrips tabaci*, ITS2 sequence, phylogenetic tree, *Solanum nigrum*.

INTRODUCTION

Thrips tabaci Lindeman, 1889 (Thysanoptera, Thripidae) until recently was known as a polyphagous widespread insect pest. As the first reported vector of *Tomato spotted wilt virus* (TSWV) causes serious epidemics and heavy crop losses worldwide (PITTMAN 1927). Until the report of ZAWIRSKA (1976) it was known as an unambiguous unitary species. Investigating the reason of the variable virus vector activity ZAWIRSKA (1976) established the existence of two biological "types" of *T. tabaci*: namely *T. tabaci* Lindeman (communis-Typ Zawirska, 1976) and *T. tabaci* Lindeman (tabaci-Typ Zawirska, 1976). Ac-

According to the establishment of Zawirska, the populations of *T. tabaci* communis-type do not include males, propagate by thelytokous parthenogenesis, associated with a wide host range, breeding mainly on onion, garlic, leek and cotton and do not able to transmit TSWV. The populations of *T. tabaci* tabaci-type include males, propagate by arrhenotokous parthenogenesis, associated above all with tobacco and dead-nettle (*Lamium* sp.) and are effective vectors of TSWV. Zawirska has emphasized that her observations were carried out under Polish climatic conditions. The morphological characters of the adult *Thrips tabaci* specimens are constant through the world. The two proposed types cannot be distinguished based on morphological characters. The differences in the virus vector capability of the two "biotypes" were confirmed by WIJKAMP *et al.* (1995) and CHATZIVASSILIOU *et al.* (1999, 2002). Genetic variation based on the mitochondrial COI sequence in relation to the vector competency was linked with the reproduction mode of *Thrips tabaci* populations by JACOBSON *et al.* (2013a). The existence of two different "biotypes" was confirmed by molecular investigations (JENSER *et al.* 2001, BRUNNER *et al.* 2004). It is worthy to mention that these results are regarding specimens collected from tobacco and onion. These investigations confirmed that this morphologically constant species has (at least) three different biotypes and that host plant associated divergence occurred. *T. tabaci* communis-type populations have wide host range. *T. tabaci* tabaci-type according to Zawirska is associated only with tobacco and dead-nettle populations. Further detailed data regarding the host range are unknown. By reason of the Zawirska's statement the populations of *T. tabaci* breeding on tobacco have been differentiated from the polyphagous *T. tabaci* populations a long time ago. According to the opinion of BRUNNER *et al.* (2004) this split could be estimated 28 million years ago. Since tobacco was introduced to Europe only 500 years ago, the differentiation of this so called "tobacco associated" population had to begin on another host species that is native to the Palaearctic region.

At the same time we intended to look for further host range of the *T. tabaci* tabaci-type population. To study the very complex interaction/correlation between host plant preference, vector competency, reproduction forms and population genetics, DNA barcoding proves to be a suitable tool. For this purpose, most of the publications deal with mitochondrial COI (and COII) sequence analysis (BRUNNER *et al.* 2002, 2004, KOBAYASHI & HASEGAWA 2012, NAULT *et al.* 2014, TODA & MURAI 2007, JACOBSON *et al.* 2013). Internal transcribed spacer 2 region of the rDNA is mostly used at interspecific level (GLOVER *et al.* 2010, BUCKMAN *et al.* 2012) but hence *Thrips tabaci* seems a more complex taxon that comprises of two or more subspecies or even cryptic species (BRUNNER *et al.* 2004), we chose this marker to differentiate the populations according to host plant and to compare our results to the analysis of the COI sequences completed by others.

MATERIAL AND METHODS

Sample collection

Thrips tabaci specimens were collected from tobacco, onion, cabbage and distinct weed plants from the tobacco production region and from other parts of Hungary (Table 1) and identification was confirmed using a compound light microscope. One sample originated from Israel was collected by Dr. David Ben-Yakir and samples from the USA were collected by Dr. József Fail. The 29 thrips specimens were preserved in 96% ethanol and stored at -70°C until the molecular investigation.

DNA extraction, amplification, cloning and sequencing

Total genomic DNA was extracted from single thrips individuals using REDExtract-N-AmpTMTissue PCR Kit (Sigma) according to the manufacturer's instructions. To amplify the ITS2 sequence CAS5p8Fc and CAS28sB1d primer pair (KIM & LEE 2008) was used for PCR. PCR was performed using *Taq* DNA polymerase (Fermentas) in a thermo-cycler (Eppendorf Mastercycler gradient) as follows: initial denaturation at 96°C for 4 min, followed by 40 cycles of 95°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 60 sec; final extension at 72°C for 10 min. The PCR products were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid). Purified PCR products were cloned into CloneJet (Fermentas) or pGEM-T Easy vectors and inserted into *Escherichia coli* DH5 α competent cells. All cloning steps were based upon standard molecular biology protocols (SAMBROOK *et al.* 1989). The recombinant plasmids isolated from selected colonies were sequenced using pJET1.2 forward and reverse primers, the PCR products were sequenced by CAS5p8sFc and CAS28sB1d primers by an automated DNA sequencer (Applied Biosystem Gene Analyzer 3100). Sequences were analyzed with the CLC Sequence Viewer 6.8.1. or MEGA 6.06 program (TAMURA *et al.* 2007). DNA sequences in the ITS2 region of the thrips specimens were deposited to the GenBank (Table 1). Estimates of evolutionary divergence between ITS2 sequences were conducted using the Kimura 2-parameter model (KIMURA 1980). The rate variation among sites was modeled with a gamma distribution (shape parameter = 5.58). The analysis involved 29 nucleotide sequences (see the [Electronic Supplement](#)). Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 413 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (TAMURA *et al.* 2013).

Maximum likelihood (ML) tree was constructed and used to evaluate taxonomic relationships among the tested 29 *Thrips tabaci* individuals. Bootstrap support for ML trees was assessed using 1000 replicates. Maximum-likelihood tree was composed using Tamura-Nei model (TAMURA *et al.* 2011) according to MEGA model selection; *Thrips urticae* was chosen for outgroup species.

RESULTS

The length of the ITS2 sequences varied between 481–487 bp. Phylogenetic tree of ITS2 sequences of *Thrips tabaci* specimens collected from different host plants and distinct geographical locations was constructed as shown in Figure 1.

Table 1. List of *Thrips tabaci* individuals, host plants, origins, GenBank accession numbers and sample name (see Fig. 1)

Host plant	Location ¹	GeneBank acc. no.	Sample name
<i>Allium cepa</i>	Pottersville, New York, USA	JF968504	Onion USA-1
		JF968503	Onion USA-2
<i>Allium porrum</i>	Beg Dagan, Israel	KP216405	Onion Israel-1
<i>Allium schoenoprasum</i>	Felcsút	JF968500	Onion Felcsut-1
		JF968499	Onion Felcsut-2
		JF968505	Onion Felcsut-3
<i>Asclepias syriaca</i>	Pilismarót		Asclepias Pilismarot-1
<i>Brassica oleracea</i>	Tordas	KP216398	Cabbage Ocsa-1
	Geneva, New York, USA	JF968493	Cabbage USA-1
		JF968492	Cabbage USA-2
		JF968502	Cabbage USA-3
<i>Capsella bursa-pastoris</i>	Encsencs*		Capsella Encsencs-1
<i>Nicotiana tabacum</i>	Nyíregyháza*	KP216403	Tobacco Nyiregyhaza-1
		Debrecen*	JF968495
		JF968496	Tobacco Debrecen-2
		JF968497	Tobacco Debrecen-3
		JF968498	Tobacco Debrecen-4
		JF968501	Tobacco Debrecen-5
<i>Solanum nigrum</i>	Nagycsérkesz*	KP216402	Snigrum Nagycsérkesz-1
		KP216404	Snigrum Nagycsérkesz-2
	Csengersima	KP216397	Snigrum Csengersima-1
	Nagykovácsi	KP216396	Snigrum Nagykovacsi-1
		KP216399	Snigrum Nagykovacsi-2
<i>Solanum tuberosum</i>	Tiszanagyfalu*	KP216407	Potato Tiszanagyfalu-1
	Keszthely	KP216395	Potato Keszthely-1
<i>Stellaria media</i>	Szada	KP216406	Stellaria Szada131-1
<i>Urtica dioica</i>	Nagykovácsi	KP216394	Thripsurticaet706-L1.1
			Thripsurticaet706.1 (organism: <i>Thrips urticae</i>)
			Thrips sp.T1.2 (organism: <i>Thrips</i> sp.)

¹where country is not indicated means Hungary; * tobacco production region

The phylogenetic tree was divided into strongly supported (99% support) two main clades; one of them composed of the tobacco associated 8 *T. tabaci* individuals. All the tobacco associated clones originated from *N. tabacum* or

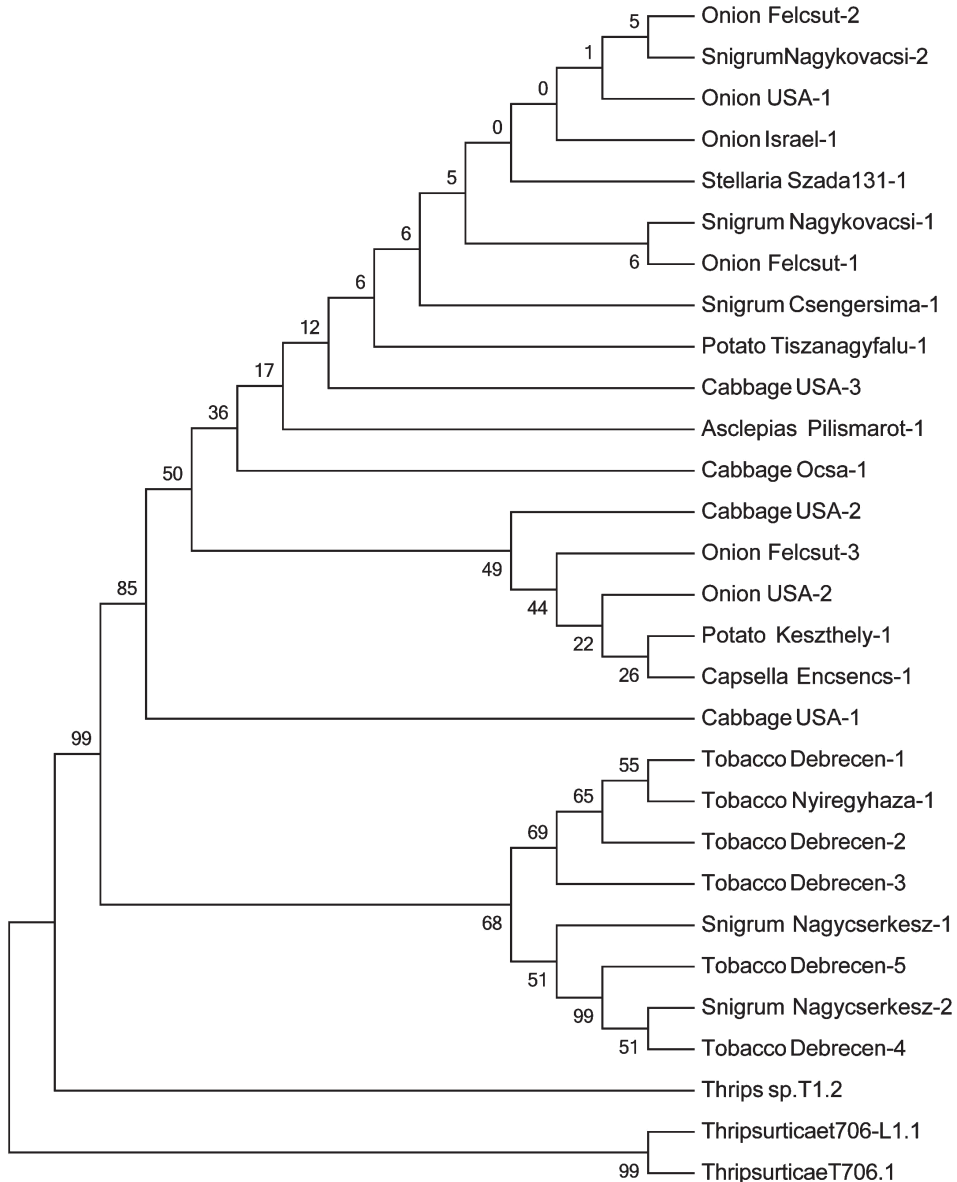


Fig. 1. Maximum likelihood tree constructed from ITS2 sequences of *Thrips tabaci* specimens collected from different plants and region of Hungary, USA and Israel (see Table 1).

S. nigrum from the tobacco production region in Hungary. The other clade of the onion associated 18 specimens formed two subclades (support 85%), one is represented by one specimen (Cabbage USA-1). Out of the other 17 clones 12 could be found in one branch (I) and 5 in another branch (II) (support 50 %), but the differences were not well supported. Three thrips specimens of *S. nigrum* from this branch originated outside of the tobacco production region belonged to this formerly mentioned branch I composed individuals collected on onion, cabbage, potato from the tobacco production region, *Asclepias syriaca*, *Stellaria media*. The other branch II contained individuals collected on cabbage, onion, potato outside the tobacco production region and *Capsella bursa-pastoris*. Among all the host plants only *S. nigrum* served host plant for *Thrips tabaci* in both clades.

DISCUSSION

Our results gained by phylogenetic tree analysis of ITS2 sequences of *Thrips tabaci* specimens collected from different host plants showed the same pattern as BRUNNER *et al.* (2004) observed on phylogenetic analysis of the mt-COI sequences about the molecular distinction of three main clades. In the literature molecular investigations of *Thrips tabaci* populations focus on the two or three main agricultural host plants, such as tobacco, leek/onion and cabbage. JACOBSON *et al.* (2013a,b) analysed the interactions between the molecular characteristics of *T. tabaci* collected from *Allium* spp., *Brassica* spp., *Raphanus* spp. *Secale cereale* and vector competency of various TSWV isolates in North Carolina, where *T. tabaci* does not play a role in TSWV transmission in tobacco (JENSER *et al.* 2011). Phylogenetic studies concerning different reproductive modes and geographically distinct thrips populations revealed similar tree composition to the host plant preference: thelytokous and arrhenotokous haplotypes divided into two or three main clades (JACOBSON *et al.* 2013, TODA & MURAI 2007, NAULT *et al.* 2014). In our experiments we extended the number of host plant species in order to support our hypothesis that the split between the two main clades or biotypes could have happened by an adapting mechanism to tobacco across an intermediate breeding host that might be a relative taxon of *Nicotiana tabacum*. Therefore we chose *Solanum nigrum* as a possible intermediate host and based on our results we found that among the host plant species we have collected *Thrips tabaci* specimen, from this host plant proved the sole host for both thrips biotypes. BRUNNER *et al.* (2004) estimated the phylogenetic split of the leek-associated and tobacco-associated lineages to 28 million years ago. Hence the nucleotide sequence differences in the hypervariable ITS2 region among closely related species are pronounced, the nucleotide sequence distances (Electronic Supplement) not higher than 0.05

in our experiment indicate that the phylogenetic division process might have not finished yet, rather it has been still in progress. It can be supposed, that the two taxa are not distinct cryptic species, but more likely biotypes that may have been still differentiating and the evolution of the *Thrips tabaci* taxon complex is still on progress. The fact that we found a plant species belonging to the *Solanaceae* family that was the only common host for *T. tabaci* tabaci-type and *T. tabaci* communis-type supported our hypothesis that in Hungary the splitting process of the two main molecular clades could have happened on the host species *Solanum nigrum*. To compare our results to that of the literature based on COI sequences further investigations needed with these markers (mitochondrial DNA markers).

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