Acta Biol. Univ. Daugavp. 15 (1) 2015
ISSN 1407 - 8953

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF *SYNANTHEDON TIPULIFORMIS* CLERCK FROM BLACK CURRANT FIELDS

Laura Ozoliņa-Pole, Jūlija Vilcāne

Ozoliņa-Pole L., Vilcāne J. 2015. Molecular identification and characterization of *Synanthedon tipuliformis* Clerck from black currant fields. *Acta Biol. Univ. Daugavp., 15 (1): 157 – 163.*

Currant clearwing moth *Synanthedon tipuliformis* Clerck belongs to order Lepidoptera, family Sesiidae, and is one of the most important pests of plants belonging to genus *Ribes* and, particularly, black and red currants. Species within genus are not always easily distinguishable by morphological features from closely related species, and some similar species cold be found in Latvia. Since currant clearwing moth inhabits perennial plantings of currants, which are located along the country, distinct populations may occur. To observe possible variability between populations of *Synanthedon tipuliformis*, larvae and imago were collected in three black currant plantations in geographically distinct parts of Latvia, in municipalities of Tukums (Western part), Saldus (South-Western part), and Pārgauja (Northern part). A 710-bp fragment of highly conserved regions of the mitochnodrial cytochrome c oxidase subunit I (COI) gene were amplified using primers LCO1490 and HCO2198, sequenced from 33 samples, at least ten insects from each location. All sequences after processing and search in NCBI BLASTn database and phylogenetic analysis resulted in the same species, *Synanthedon tipuliformis*. Samples from all three populations were relatively homogenous, but some polymorphisms were observed. Hypothetically these differences could be related to planting material used, as very often it came from different plant nurseries. All sequences were deposited at NCBI GeneBank, and this is first time when molecular data of *Synanthedon tipuliformis* is available from Baltics.

Key words: clearwing moth, SNP, population, similar species.

Laura Ozoliņa-Pole, Jūlija Vilcāne. Latvian Plant Protection Research Centre, Struktoru str. 14A, Riga, Latvia, LV-1039, University of Latvia, Faculty of Biology, Kronvalda blvd .4, LV-1010, Riga, Latvia, e-mail: laura.ozolina.pole@laapc.lv, julija.volkova@laapc.lv

INTRODUCTION

The currant clearwing moth *Synanthedon tipuliformis* Clerk. (synonym *Spinx tipula*) (Lepidoptera: Sesiidae) is a species that has been found all over the world, and it is considered as one of the most important pests for plants belonging to genus *Ribes* (Brock et al. 1964, Manko 1965, Leska 1966, Yakimova 1968, Scott & Harrison 1978, Hardy 1981, Gottwald & Künzel 1994) *S. tipuliformis* is a major pest in black currant plantations also in the Northern part of Europe, as well as Baltic states.

In Baltic countries, besides economically important species *S. tipuliformis,* some other

species of the genus have been described. In Lithuania, another species *Synanthedon mesiaeformis* (Herrich-Schäffer), as well as sallow clearwing moth *Synanthedon flaviventris* (Staudinger) listed in the Red Data Book of Lithuania as a rare species are reported (Karalius & Būda 2006, Mozuraitis & Būda 2013). *S. mesiaeformis* has been described in Lithuania, Estonia and Finland, but not yet in Latvia[1], as well *Synanthedon soffnerii* (*I*vinskis & Rimšaite 2012). Phenotypically similar species to *S. typuliformis* is *Synanthedon cephiformis* (Ochsenheimer), currently not reported in Baltics, but found in Poland (Bąkowski et al. 2011). The correct distribution of species from genus *Synanthedon* in Northern Europe could be unknown, due to hidden life style of some species, or because of the expanding of the living areal as related to climate change (Ulrich et al. 2011).

Studies about diversity of *Synanthedon* species found in Latvia are lacking. Earlier, distribution of economically important pest *S. typuliformis* has been described (Ozolina-Pole et al. 2013). As a continuation of the research, identification and characterization of *S. typuliformis* from different geographical locations by molecular methods was performed. The aim of this study was to clearly identify species of insects found in invaded stems of black currants, ensuring that closely related species are not occurring. In addition, possible genetic variation was characterized.

1 http://www.lepidoptera.eu/show.php?ID=349&country=GB

MATERIAL AND METHODS

Insect sampling and preparation

Samples of *S. typuliformis* were collected in three distinct parts of Latvia, in Pūre (Western part, municipality of Tukums), Pārgauja (Northern part, municipality of Pārgauja) and Lutriņi (South-Western part, municipality of Saldus). Locations were chosen to be geographically distinct (Fig. 1), the farthest distance was 162 km, from Pūre to Pārgauja, the nearest distance – 92 km. Plantations of black currants were 7-10 years old, shoots visually damaged by clearwing moth were collected. Shoots were cut along, larvae were removed from damage, and preserved in 96% alchocol at 5 °C until needed. In the case if the larvae were alive, they were put into exiccators covered with cotton cloth, together with some black currant stems and leaves, and

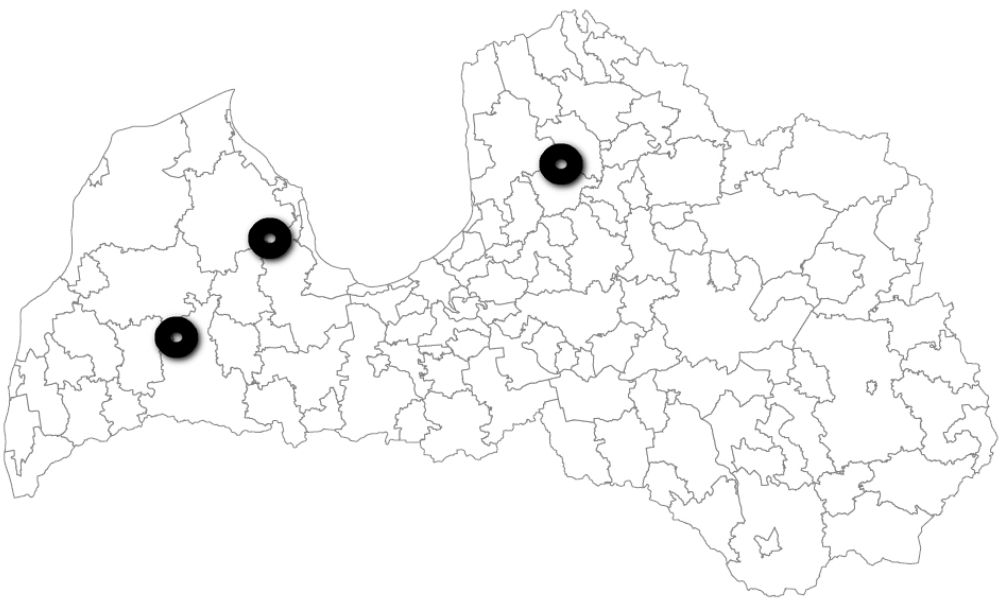

Fig. 1. Location of black currant plantations, where shoots damaged by *S. typuliformis were* collected.

Table 1. List of samples analyzed in this study. Submission ID is 1819040 deposited in NCBI GeneBank

Location	ID	Development stage	Collection date	Gene Bank accession number
Saldus	LO 1	imago	06. 06. 2013	
	LO 2	imago	06. 06. 2013	
	LO 3	imago	06. 06. 2013	
	LO 4	imago	06. 06. 2013	
	LO 5	imago	06. 06. 2013	
	LO 6	imago	06. 06. 2013	
	LO 7	imago	06. 06. 2013	
	LO 8	imago	06. 06. 2013	
	LO 9	imago	06. 06. 2013	
	LO 10	imago	06. 06. 2013	
	LO 31	imago	06. 06. 2013	
	LO 35	larvae	09. 09. 2013	
Pārgauja	LO 11	imago	06. 06. 2013	
	LO 12	imago	06. 06. 2013	
	LO 13	imago	06. 06. 2013	
	LO 14	imago	10. 06. 2013	
	LO 15	imago	06. 06.2013	
	LO 16	imago	07. 06. 2013	
	LO 17	larvae	09. 09. 2013	
	LO 18	larvae	09. 09. 2013	
	LO 20	larvae	10. 05. 2013	
	LO 21	larvae	09. 09. 2013	
Pūre	LO 19	larvae	13. 09. 2013	
	LO 22	larvae	13. 09. 2012	
	LO 23	larvae	13. 09. 2012	
	LO 24	larvae	10. 05. 2012	
	LO 25	larvae	10. 05. 2012	
	LO 26	imago	06. 06. 2013	
	LO 27	imago	06. 06. 2013	

mature imago were grown, to identify species. Afterwards mature imagoes were preserved in the same way, as larvae.

DNA extraction, PCR and sequencing

Larvae and imago were grinded in mortar with pestle before DNA extraction. DNA was extracted by E.Z.N.A. Insect DNA Kit (Omega Bio-Tek, USA), by using the standard protocol. Extracted DNA was stored at –20 °C until needed. PCR reaction was set up in a 25 µl reaction mixture containing 1 µl target DNA, 12.5 µl Maxima™ Hot Start *Taq* DNA Polymerase Master mix (Thermo Scientific), 1 µl of each forward and reverse primers LCO1490 and HCO2198

(Folmer et al. 1994), manufactured by IDT DNA (Germany), which amplify 710-bp fragment of highly conserved region of the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) and nuclease-free water (Thermo Scientific) to 25 µl. PCR products were purified with Exo I/ FastAP (Thermo Scientific) reaction according to manufacturer's instructions prior to sequencing. Sequencing was performed with the same primers as used for PCR reaction at Macrogen Europe (Netherlands).

Data analysis

DNA sequences were assembled using Contig Editor tool in Gene Studio 2.0.4.4. software (GeneStudio Inc. 2011) and aligned using multiple sequence alignment tool Clustal X2 (Larkin et al. 2007). Before further analysis, batch of assembled contigs was trimmed, to avoid influence of sequencing errors that may occur at the begging and end of sequences. Each 710-bp-long sequence was trimmed before 86-bp and after 638-bp, 552-bp-long segment left for further single-nucleotide polymorphism (SNP) analysis. Phylogenetic analysis of the sequencing data was performed with the MEGA 5.1. software (Tamura et al. 2011) using Maximal Likelihood Method, and the quality of dendrograms was assessed by 100 bootstrap replicates. In addition, sequences of *S. tipuliformis* available from other countries were retrieved from NCBI GeneBank and added to study. The list of sequences used is provided in Table 2.

RESULTS AND DISCUSSION

Phylogenetic analysis revealed that all sequences from *S. tipuliformis*, except the ones from USA and Greece, clustered together with sequences retrieved from NCBI GeneBank, in a separate distinct clade, supported by bootstrap value 98% (phylogenetic tree not shown). Sequence data from Australia clustered in a separate sub clade within *S. tipuliformis* clade, but other European data, e.g. from Finland, Austria, Germany, were uniformly distributed within the *S. tipuliformis* clade.

Analysis of polymorphisms showed an association between geographical location or distinctness of populations and variability in it. Sequences from Latvia were identical in 55.2% cases, and distributed within all three parts of populations analyzed. Rest of the insects (44.8%) had some polymorphisms in part of *COI* gene. All SNPs observed were transitions, in 27.6% cases the particular sequence contained just one polymorphic site, in 13.8% two transitions, but in one case transition in three sites of the same analyzed sequence was evident (Table 2). When the placement of polymorphisms within analyzed part of population was compared, three to five different polymorphic sites were observed for each representative population, and transitions were partially placed in the same places for sequences containing polymorphisms, or were placed at completely distinct sites and SNPs observed were not common for all analyzed population.

The most significant SNP was located at 186-bp, where adenine was replaced by guanine. This SNP was found at the same site in samples from Latvia, Germany and Australia, as well as in samples coming from very distinct populations. Other SNPs found were observed at different sites, more specific for location of population. Samples from Finland without any polymorphisms were observed, and were identical to 55.2% of samples from Latvia.

Part of mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) is a core of global bio identification system, and has been used as an universal barcode for animal and also insect species (Lunt et al. 1996, Hebert & Cywinska et al. 2003, Hebert & Ratnasingham et al. 2003). *COI* sequences have an ability to correctly identify both diverse orders and with low number of sequence divergence among families in order (Hebert & Cywinska et al. 2003). The ability of *COI* to describe genetic diversity and population structure of single species has been reported (Li et al. 2014). In Finland by using *COI*, cryptic species of *Baetis vernus* group (*Ephemeroptera, Baetidae*) were identified and resolved, and it was found that divergence within the each

Table 2. Mitochondrial DNA polymorphic sites in the three different populations of *S. tipuliformis* from geographically distinct plantations and some other countries

Gene Bank accession nr.	ID	Location	Position									
			93	113	186	207	304	357	498	426	525	543
			T	C	A	T	T	C	T	C	C	G
	LO7	LV: Saldus	.	.	.	C	.	.	.	.	.	.
	LO8	LV: Saldus	.	.	.	.	C	.	.	.	.	.
	LO9	LV: Saldus	.	T	.	.	.	.	.	.	.	A
	LO31	LV: Saldus	.	.	.	.	.	.	.	.	T	.
	LO35	LV: Saldus	.	.	.	.	.	.	.	.	T	.
	LO14	LV: Pārgauja	.	.	.	.	.	.	.	T	.	.
	LO20	LV: Pārgauja	.	.	.	C	.	.	.	.	.	.
	LO21	LV: Pārgauja	.	.	.	C	.	.	.	.	.	A
	LO22	LV: Pūre	.	.	.	C	.	.	.	.	.	A
	LO23	LV: Pūre	.	T	.	C	.	.	.	.	.	A
	LO24	LV: Pūre	.	T	.	.	.	.	.	.	.	A
	L025	LV: Pūre	.	.	G	.	.	.	.	.	.	.
	LO27	LV: Pūre	.	.	G	.	.	.	.	.	.	.
HM870940.1	MM00117	Finland	.	.	.	.	.	.	.	.	.	.
JF853822.1	MM17410	Finland	.	.	.	.	.	.	.	.	.	.
KM572234	TLMF Lep 09870	Austria	.	.	.	.	.	T	C	.	.	.
GU706627.1	BC Lep 24884	Germany	.	.	G	.	.	.	.	.	.	.
HQ922203.1	10ANIC-02606	Australia	C	.	G	.	.	.	.	.	.	.
HQ922201.1	10ANIC-02604	Australia	C	.	G	.	.	.	.	.	.	.

resolved group ranged from 0.3 to 1.4% (Ståhls & Savolainen 2008). Order Lepidoptera is considered as complicated for taxonomic studies, because of low genetic variation within the group (Hebert & Cywinska et al. 2003, Huemer et al. 2014). By analysis of *COI*, 98.8% of species could be recognized, and maximum divergence within species was slightly below 2% (Huemer et al. 2014).

In this study we found polymorphisms within single species *S. tipuliformis*, as well as specific transition characteristic for sequences from three different countries. Non-random regional variation has been shown to occur in mtDNA (Roe & Sperling 2007), but reasons of mutations

are not always clear.

Variation between sequences of *S. tipuliformis* from three different locations was between 0.2 - 0.5%, and this could be considered as intraspecific variation within species. Therefore, we conclude that all species of *Synanthedon* found in black currant plantations are *S. tipuliformis,* since higher divergence could be necessary to consider the samples as different species.

AKNOWLEDGEMENTS

The study was financed by the Latvian Ministry of Agriculture project "Sustainable development of fruit growing, using environment-friendly and water saving, as well as the rural landscape retaining integrated production technologies to reduce climate changes and to maintain biodiversity" Nr. 080410/c-32.

REFERENCES

Bąkowski M., Celadyn R., Hołowiński M., Zajda W. 2011. Clearwing moths (Lepidoptera: Sesiidae) of Małopolska Province. Polish Journal of Entomology / *Polskie Pismo Entomologiczne,* 80(2): 411–421.

Brock A.M., Collingwood C.A., White J.M. 1964. The currant clearwing moth (*Aegeria tipuliformis* (Clerk)) as a pest of blackcurrants. *Annals of Applied Biology*, 53(2): 243–249.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299.

Gottwald R., Künzel K. 1994. Neue Erkenntnisse zur Populationsökologie des Johannisbeerglasflüglers (*Synanthedon tipuliformis* Clerck). *Gesunde Pflanzen*, 46: 131–136.

Hardy R.J. 1981. Field observations on the effect of currant borer moth, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Aegeriidae), on the yield of blackcurrants produced in Tasmania. *Scientia Horticulturae,* 15(2): 165–172.

Hebert P.D.N., Cywinska A., Ball S., de Waard J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B*, 270(1512): 313–321.

Hebert P.D.N., Ratnasingham S., de Waard J.R. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B*, 270 (S1): S96–S99.

Huemer P., Mutanen M., Sefc K.M., Hebert P.zD.N. 2014. Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. *PLoS ONE,* 9(12): e115774.

Ivinskis P., Rimšaitė J. 2012. Lepidoptera species new for Lithuanian fauna. *New and Rare for Lithuania Insect Species*, 24: 24–29.

Karalius V., Būda V. 2006. The investigation of *Synanthedon mesiaeformis* and *S. flaviventris* (Lepidoptera, Sesiidae) distribution in Lithuania using sex attractants. *Acta Zoologica Lituanica*, 16(3): 204–209.

Larkin M., Blackshields G., Brown N., Chenna R. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21): 2947–2948.

Leska W. 1966. Studies on the biology of currant clearwing (*Synanthedon tipuliformis* Cl.) (Lep. Aegeriidae, syn. Sesiidae). *Polskie Pismo Entomologiczne,* B19: 245–261 (in Polish).

Li J., Ye Y., Wu C., Guo B., Gul Y.2014. Genetic diversity and population structure of *Sepiella japonica* (*Mollusca: Cephalopoda: Decapoda*) inferred by mitochondrial DNA

(COI) variations. *Biochemical Systematics and Ecology*, 56(1): 8–15.

Lunt D.H., Zhang D.-X., Szymura J.M., Hewitt O.M. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology*, 5(3): 153–165.

Manko V.V. 1965. On biology of currant clearwing moth under Byelorussian conditions. *Newsletter of Academy of Sciences Belarus SSR. Ser. Agr. Studies,* 4: 70–76 (in Belarusian).

Mozūraitis R., Būda V. 2013. Intra- and interspecific activities of semiochemicals from the sex pheromone gland of the Welsh Clearwing, *Synanthedon scoliaeformis*. *Journal of Chemical Ecology*, 39(8): 1066–1069.

Ozolina-Pole L., Apenīte I., Ciematnieks R. 2013. Distribution and invasion of clearwing moth *Synanthedon tipuliformis* Cl, a pest of currant in Latvia. *Proceedings of the Latvian Academy of Sciences B*, 67(2):188–191.

Roe A.D., Sperling F.A.H. 2007. Patterns of evolution of mitochondrial cytochrome *c* oxidase I and II DNA and implications for DNA barcoding. *Molecular Phylogenetics and Evolution*, 44(1): 325–345.

Scott R.R., Harrison R.A. 1978. Sampling plan for population dynamics studies on currant clearwing, *Synanthedon tipuliformis* (Lepidoptera: Sesiidae). *New Zealand Journal of Zoo*logy, 5(1): 177–184.

Ståhls G., Savolainen E. 2008. MtDNA COI barcodes reveal cryptic diversity in the *Baetis vernus* group (Ephemeroptera, Baetidae). *Molecular Phylogenetics and Evolution*, 46: 82–87.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10): 2731–2739.

Ulrich W., Bakowski M., Lastuavka Z. 2011. Spatial distributions of European clearwing moths (Lepidoptera: Sesiidae). *European Journal of Entomology*, 108(3): 439–446.

Yakimova N.L. 1968. Some factors influencing the population dynamics of *Synanthedon tipuliformis* Cl. (Lepidoptera, Aegeriidae). *Entomological Review*, 47(1): 19–30.

Received: 27.04.2015.
Accepted: 08.06.2015.