

Barcoding data of the first *Harmonia axyridis* (Pallas, 1773) invaders in Lithuania

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An invasive lady beetle *Harmonia axyridis* (Pallas, 1773) was recorded for Lithuania (Curonian spit) in 2011 for the first time. It was collected in Vilnius (Eastern Lithuania) in 2014 in the colony of an alien aphid *Brachycaudus divaricatae* Shaposhnikov, 1956. However, *Harmonia axyridis* (Pallas, 1773) appeared extremely rare in monitored regions of Eastern Lithuania in 2014. Samples from Vilnius (Eastern Lithuania) belong to the most common COI haplotype of *Harmonia axyridis* (Pallas, 1773), detected both in its native (Russian Far East, Korea, China, Japan) and invasive areas, including five European countries. Probably, Lithuania is being colonized by the widely distributed and highly invasive lineage of *Harmonia axyridis* (Pallas, 1773), representing the non-melanic *succinea* form, which was reported as one of the most common in Europe.

Keywords: *Harmonia axyridis*, invasivity, COI, distribution, Europe

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INTRODUCTION

The lady beetle *Harmonia axyridis* (Pallas, 1773) is taken for one of the most impressive insect invaders of the last century (Koch 2003; Brown et al. 2011). Its native distribution area is Eastern Palearctic region and Central Asia (Lombaert et al. 2014). This effective predator was introduced intentionally for pest aphid control on several crops around the world. Together with accidental introductions, the invasivity of the species

itself contributed to significant expansion of its range (Michie et al. 2010; Laugier et al. 2013; Lombaert et al. 2014; Maes et al. 2014). As a result, the distribution of *Harmonia axyridis* (Pallas, 1773) has already reached West Palearctic, Nearctic, Neotropical, Oriental, and Afrotropical regions (Brown et al. 2011). Starting from 1968, it also has been effectively established in Europe (Ukrainsky & Orlova-Bienkowskaja 2013). Negative impacts of *Harmonia axyridis* (Pallas, 1773) on local ecosystems in invasive areas have been

reported: competition with the native coccinellid species, feeding on fruits, invading housing estates, causing allergic reactions to people and staining parts of the household with a yellow defensive fluid they release when agitated (Koch & Galvan 2008; Grez et al. 2013; Vilcinskis et al. 2013; Comont et al. 2014).

The first record of *Harmonia axyridis* (Pallas, 1773) in the countries surrounding Lithuania was from Wielkopolska-Kujawy Lowland of Poland in 2006 (Przewozny et al. 2007). In 2009, numerous larvae, pupae and adults were observed in the colonies of mealy plum aphid in Līvāni municipality in the Southeastern part of Latvia (Barševskis 2009). From 2010, numerous records came from the Kaliningrad region of Russia (Alekseev et al. 2012). For Lithuania, the only record so far was from Curonian spit (Nagročkaitė et al. 2011). In 2014, two adult specimens and one pupa were collected in Vilnius (Eastern Lithuania).

The aim of the present study was to summarize the most recent data concerning *Harmonia axyridis* (Pallas, 1773) in Lithuania, and to assess the barcoding markers of Lithuanian samples, to check the relationships between these beetles and other Palearctic samples already characterized.

MATERIAL AND METHODS

Beetles were collected in Lithuania, Vilnius, V. Pietario str. (N 54°102' 39", E 25°14' 53" WGS), in July 3-8, 2014, on *Prunus cerasifera* Ehrhart, 1784 in the colony of *Brachycaudus divaricatae* Shaposhnikov, 1956 during the study of the lady beetle guild associated with the plum inhabiting aphids. Dry specimens were identified using the key of Przewozny et al. (2007), and then attributed to the particular colour form in accordance with Michie et al. (2010). Samples are stored at the Department of Zoology, Vilnius University (Vilnius, Lithuania).

Total genomic DNA was extracted from legs using the DNeasy Blood & Tissue kit (Qiagen). For the amplification of mitochondrial COI fragment primers LCO-1419 and HCO-2198 (Folmer et al. 1994) were used. The cycling parameters were from Magro et al. (2010). PCR products of approximately 700 bp length were excised from the gel and purified using GeneJet Gel extraction Kit (Thermo Scientific). Samples were sequenced at Macrogen Europe (Amsterdam, the Netherlands) with amplification primers used as sequencing primers. Sequences were submitted to GenBank, their Accession numbers are KP859535-KP859537.

In addition, 261 partial sequences of mitochondrial COI were downloaded from BOLD systems (their list may be provided by the corresponding author). To minimize the data set sequences were collapsed into haplotypes using FaBox 1.41 (Villesen 2007). Uncorrected p-distances were calculated using MEGA 5 (Tamura et al. 2011). Bayesian analysis was conducted in MrBayes 3.2.1 (Ronquist & Huelsenbeck 2003) using General Time Reversible model with Gamma distribution (GTR+G) which was selected by jModeltest (Posada 2008). One run for 2 000 000 generations with tree sampling every 1000 generations was performed using the coalescence model of molecular clock.

RESULTS

Two adult beetles and one pupa (merged out in 4 days after collecting) matched the non-melanic form of *Harmonia axyridis* (Pallas, 1773) f. *succinea* (Fig. 1). GenBank sequence HQ658148 representing haplotype No 9 appeared the most distant one and definitely belonging to another species. BLAST search clustered it together with sequences referred as *Brumoides suturalis* (Fabricius, 1798) (HQ694829), *Hippodamia variegata* Goeze, 1777 (JN580802) and *Harmonia octomaculata* (Fabricius, 1787) (EU392412). Therefore, sequence HQ658148 was used as an outgroup in the phylogenetic analysis. The range of the pairwise sample divergences between re-

maining haplotypes of *Harmonia axyridis* (Pallas, 1773) were from 0.00 to 3.59 % (average 0.80%).

Partial COI sequences were collapsed into 32 haplotypes (Fig. 2, Table 1). All sequences of Lithuanian samples of *Harmonia axyridis* (Pallas, 1773) were identical and represented the most abundant haplotype No 5 distributed both in native and invasive areas (Fig. 2, Table 1). This haplotype appeared in the same weakly supported (posterior probabilities less than 0.50) cluster of the Bayesian phylogenetic consensus tree containing the majority (n=16) of haplotypes. This group contained samples both from native and invasive distribution areas of *Harmonia axyridis* (Pallas, 1773) (228 sequences altogether, including Lithuanian ones).

DISCUSSION

For now, *Harmonia axyridis* (Pallas, 1773) seems to be rare in Lithuania. The beetle specimens used for DNA analysis in this study come from the same collection site. It was the only record of this species during the four month (April – July, 2014) monitoring of 195 colonies of 7 aphid spe-

cies in Vilnius city (four monitoring sites) and Elektrėnai district (five monitoring sites) of Lithuania (Danilov et al. unpublished). Only one specimen of *Harmonia axyridis* (Pallas, 1773) was recorded during the previous faunistic study of beetles in the Lithuanian part of Curonian spit in 2011 (Nagrockaitė et al. 2011). Such a rarity of this highly invasive lady beetle species is rather surprising, because it has been already recorded in the surrounding territories: Poland, Latvia, Belarus, and Kaliningrad region of Russia (Przewozny et al. 2007; Barševskis 2009; Ukrainsky & Orlova-Bienkowskaja 2013). Further faunistic investigations on larger scale are necessary for documenting the distribution of *Harmonia axyridis* (Pallas, 1773) in Lithuania.

Samples from Lithuania belong to the most common COI haplotype of *Harmonia axyridis* (Pallas, 1773), detected both in its native (Russian Far East, Korea, China, Japan) and invasive areas, including five European countries (Fig. 2, Table 1). Morphologically, it is the non-melanic *Harmonia axyridis* (Pallas, 1773) f. *succinea* form, which was reported as one of the most common in Europe (Michie et al. 2010).

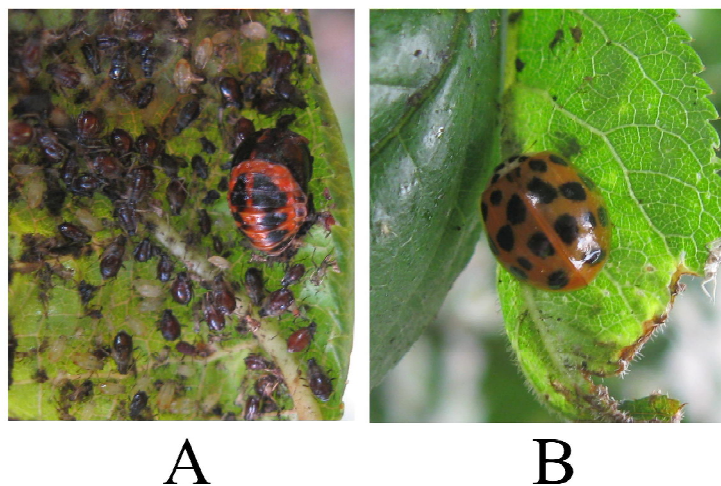


Fig. 1 *Harmonia axyridis* (Pallas, 1773) in the colony of *Brachycaudus divaricatae* Shaposhnikov, 1956 on *Prunus cerasifera* Ehrh. (Lithuania, Vilnius, V. Pietario str., N 54°102' 39", E 25°14' 53" WGS) A – pupa (July 3, 2014) and B – adult (July 8, 2014)

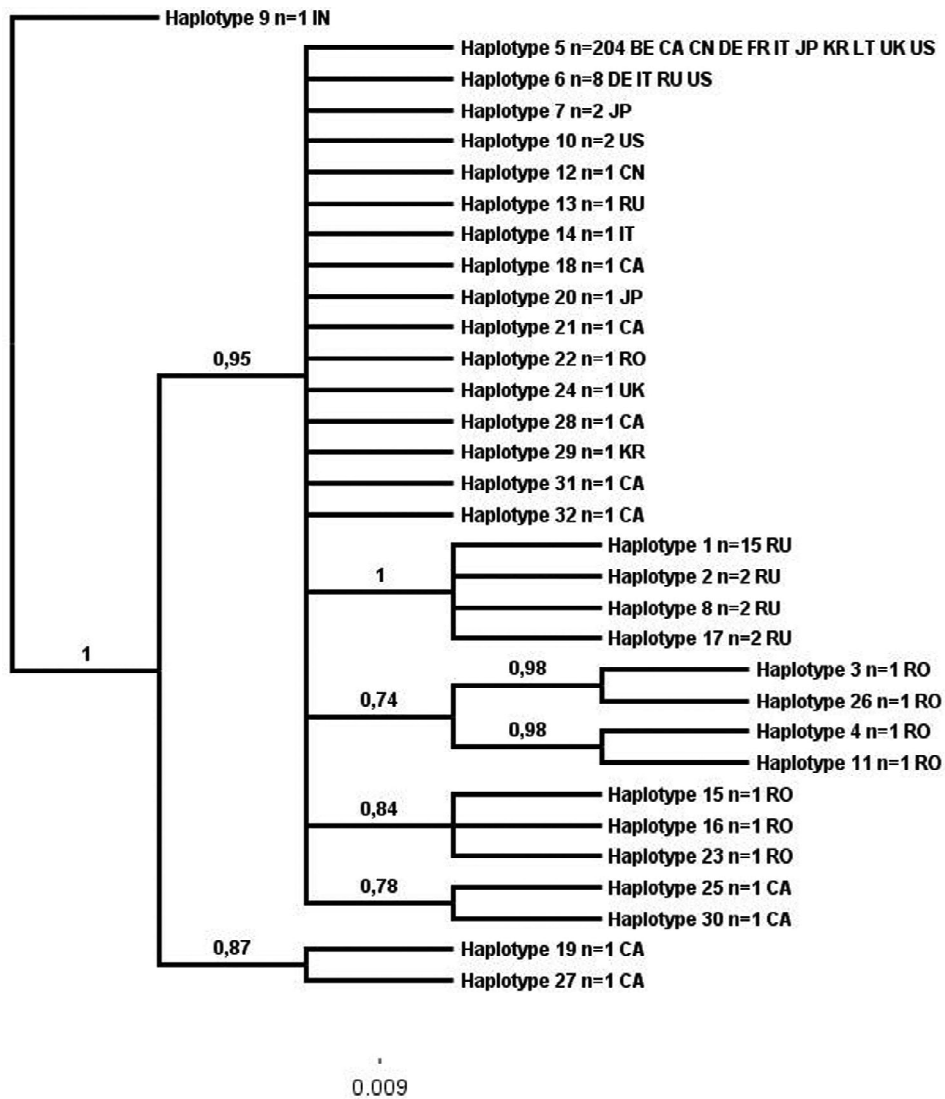


Fig. 2 Bayesian inference (BI) consensus tree showing phylogenetic relationships among *Harmonia axyridis* (Pallas, 1773) haplotypes based on partial sequences of mitochondrial *COI* (474 positions in final set) Numbers above branches indicate posterior probabilities of BI analysis over 0.50. Country abbreviations: BE – Belgium, CA – Canada, CN – China, DE – Germany, FR – France, IN – India, IT – Italy, JP – Japan, KR – Korea, LT – Lithuania, RO – Romania, RU – Russia (Vladivostok and Altai region), UK – United Kingdom, US – United States of America

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Table 1. COI haplotypes of *Harmonia axyridis* (Pallas, 1773) revealed by the construction of haplotype network analysis 474 bp data set

Haplotype label	Total number of individuals	Country	Number of individuals
No 1	15	Russia Altai region	n=15
No 2	2	Russia Altai region	n=2
No 3	1	Romania	n=1
No 4	1	Romania	n=1
No 5	204	Belgium	n=1
		Canada	n=22
		France	n=1
		Germany	n=22
		China	n=4
		Italy	n=15
		Japan	n=16
		Korea	n=1
		Lithuania	n=3
		Russia: Altai region	n=13
		Russia: Vladivostok	n=21
		United Kingdom	n=33
		United States	n=52
No 6	8	Germany	n=1
		Italy	n=3
		Russia: Vladivostok	n=2
		United States	n=2
No 7	2	Japan	n=2
No 8	2	Russia Altai region	n=1
No 9	1	India	n=1
No 10	2	United States	n=2
No 11	1	Romania	n=1
No 12	1	China	n=1
No 13	1	Russia: Vladivostok	n=1
No 14	1	Italy	n=1
No 15	1	Romania	n=1
No 16	1	Romania	n=1
No 17	2	Russia Altai region	n=2
No 18	1	Canada	n=1
No 19	2	Canada	n=2
No 20	1	Japan	n=1
No 21	1	Canada	n=1
No 22	1	Romania	n=1
No 23	1	Romania	n=1
No 24	1	United Kingdom	n=1
No 25	1	Canada	n=1
No 26	1	Romania	n=1
No 27	1	Canada	n=1
No 28	1	Canada	n=1
No 29	1	South Korea	n=1
No 30	1	Canada	n=1
No 31	1	Canada	n=1
No 32	1	Canada	n=1

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