

Genetic Diversity of the Invasive Sycamore Lace Bug (SLB), *Corythucha ciliata* (Say, 1832) (Tingidae, Hemiptera), in Its Native and Invaded Areas [†]

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Abstract: The sycamore lace bug (SLB) (*Corythucha ciliata*) is one of the most abundant and widespread pests on plane (*Platanus* spp.) trees. 38 geographic location of *C. ciliata* from Europe, Asia and North America were analysed by sequencing. Seventeen haplotypes were detected on 1356 bp long fragment of the COI gene from 327 individuals. *C. ciliata* populations from North America showed a higher haplotype diversity (12 HTs), than populations from Europe (6 HTs) or populations from Japan (2HTs). The haplotypes formed two haplogroups, one including only North American HTs and another one including HTs from all continents.

Keywords: *Corythucha ciliata*; population genetics; phylogeny; invasive insect

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1. Introduction

To understand the success of an invasive insect species we should to know the source and pathway(s) of invasion and the spatial distribution of intraspecific diversity [1–2]. Without that knowledge we will not be able to set up efficient control measures. In the recent decades several studies have been conducted with various genetic markers to answer these questions [3–7].

The native range of *C. ciliata* is located in North America, where its main hosts are *Platanus* spp. [8]. The invasion history of sycamore lace bug across Europe is well-documented. The first found was recorded in Italy (1964) followed by a high-speed spread across the continent: 1972: Yugoslavia (Zagreb, Rijeka and Ljubljana), 1975: South France, 1976: Hungary, 1978: Spain and Austria, 1983: Switzerland, Czechoslovakia, 1986: Bulgaria, 1988: Greece, 1997: Russia, 2007 Turkey, 2008: Georgia and 2011: Macedonia. No other continent remained free, South-America: Chile (1985); Asia China (2002), Japan (2006), Uzbekistan (2017); Australia (2006); Africa: South Africa (2014) [9–19]. It spread with anemochor and antropochor transportation [10–12, 17, 20–22].

Major host plants of *C. ciliata* are sycamore trees (*Platanus occidentalis*, *P. orientalis*, *P. acerifolia*) [8, 23], but feeding have been recorded on *Fraxinus* sp., *Morus alba*, *Brussonetia papyrifera*, *Carya ovata*, *Chamaedaphne* sp. too [8]. However, other publication [23] reports other possible host plant species as well (*Quercus laurifolia*, *Liquidambar*

sytraciflua and *Euphorbia pulcherrima*), but their list doesn't include *Broussonetia*, *Carya*, *Chamaedaphne* or *Fraxinus* as host.

In the invaded areas the sycamore lace bug was found mainly on *Platanus* species [11, 21–22, 24]. However, nymphs and adults were detected also on maples (*Acer*) and ash-trees (*Fraxinus*) from Georgia [18]. Sycamore lace bug individuals are sucking on the leaves causing aesthetical damage, but they may have a role as vector of various diseases as well (e.g., *Erysiphe platani*). Sycamore is a widely used ornamental tree species in the northern hemisphere [25–28].

A few numbers of genetic studies have been already conducted on *C. ciliata* in the last decade. One single individual was analysed with microsatellites markers from China [29]. The gene expression profiles were studied [14], as a part of DNA barcode library construction project [30–31]. The whole mitogenomes compared with the avocado lace bug (*Pseudacysta perseae*, Heidemann 1908) using a genome skimming approach was published by Kocher et al. [32]. Ten populations from China, including one outgroup population for Slovenia was analysed by Yang et al. [29]. There are currently 22 COI fragment data entries for *C. ciliata* in the GenBank. Some preliminary results on *C. ciliata* have been published in 2020 [18], but this subset of data incorporates only 22 locations, 117 individuals and a short fragment (546 bp) of the COI gene.

Our aims were (i) reveal the genetic structure of a *Corythucha ciliata*. (ii) exploration of the species' phylogeographic pattern and (iii) to track back the possible introduction events of the species.

2. Materials and methods

2.1. Sampling and molecular methods

We collected nymphs and imagoes from 38 populations of *C. ciliata* from Europe, Central-Asia, Japan and North America and one of *C. arcuata* (Table S1). All samples were stored in 96% ethanol at 4°C. DNA was extracted from entire bodies using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich), following the manufacturer's protocol. Eluted DNA was stored at -20°C.

A 1356bp long region of the COI gene was amplified for 327 individuals by using Pat (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3'), and LCO1490-J-1514 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') primers [35–36]. PCR conditions included an initial denaturation step at 94 °C for 2 minutes, followed by 34 cycles at 94 °C for 30 s, 46 °C for 1 minute and 72 °C for 1 minute 30 s with a final extension step that lasted 10 minutes at 72 °C. Sequences were generated at the Eurofin's Laboratory (Ebersberg, Germany).

2.2. Data analysis

327 individuals were used for mitochondrial DNA (COI) analyses (Table S1). Sequences were visualized using FinchTV 1.4.0 (Geospiza Inc. <http://www.geospiza.com/finchtv>) and then aligned using ClustalX [37]. After haplotypes were identified, those represented by only a single individual were verified by additional sequencing of an independent amplicon. *C. arcuata* sequences were used as outgroups. Genetic distances were estimated using the Kimura 2-parameter, and computations were done in MEGA 5.02 [38].

2.3. Phylogenetic analyses

Maximum likelihood (ML) analysis was performed under GTR+I model with MEGA 5.02. The level of support for individual nodes was evaluated by bootstrapping with 5000 replicates. We used jModeltest 2.1.2 [39–40] to select the best model of nucleotide substitution with Akaike Information Criterion (AIC) [41].

Population structure

Patterns of molecular diversity based on the mtDNA sequences between and within populations were assessed by estimating: nucleotide diversity (π) [42], transition/transversion ratio, haplotype diversity (h) [43–44] using the software Arlequin version 3.5.1.2 [45].

QGIS 2.18.11 (QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>) were used to project haplotype distributions and frequencies onto maps.

3. Results

Seventeen haplotypes were detected on 1356 bp long fragment of the COI gene from 327 individuals, from 38 localities (Figure 1, Table S1). Number of the variable sites was 26 (1.92%), approximately half of them were located on the barcoding part of the gene. Haplotypes were differed from each other by 1–10 polymorphic sites.

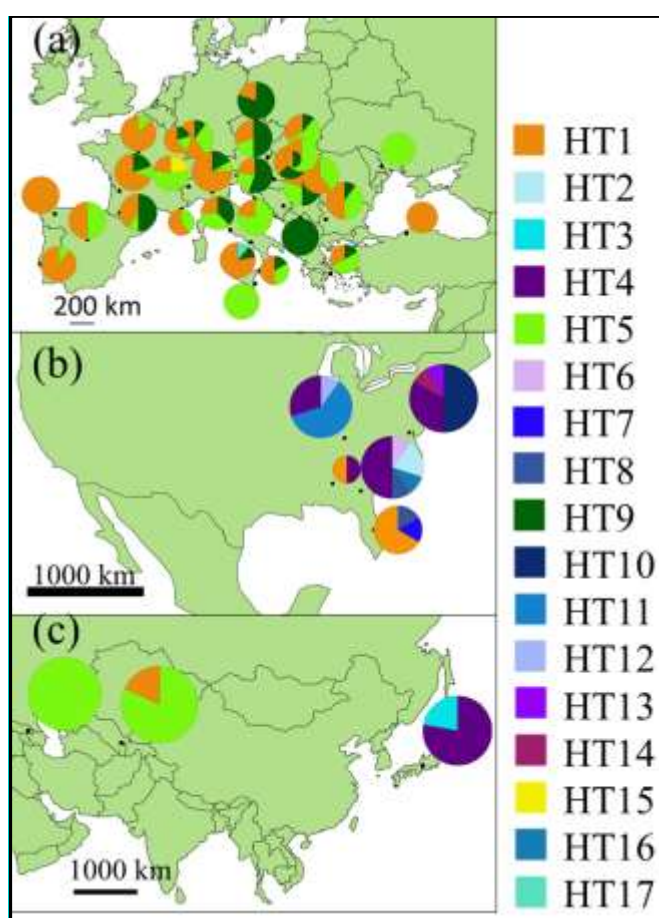


Figure 1. Distribution of *Corythucha ciliata* mitochondrial haplotypes: (a) in Europe; (b) in North America; (c) in Asia.

The haplotypes formed two haplogroups (A & B), which were separated by at least seven mutation steps (Figure 2.). The fourteen intermediate haplotypes were not presented in our data set. The topology of the phylogenetic tree was similar to the haplotype network. Most abundant haplotypes are HT1 (38.23% of the total dataset), HT5 (29.66%), and HT9 (17.74%). HT5 and HT9 were only detected from Europe and Central Asia. HT3 is unique from Japan. Most of haplotypes were detected from North America only (HT2, HT6–8, HT10–14, HT16; six of these are singletons). HT4 (6.12%) was found both in Japan and North America. HT15 and HT17 are unique haplotypes from Europe. The divergence between the haplotypes were 0.07–1.04% much lower than the interspecific divergence between *C. arcuata* and *C. ciliata* 8.49–8.93%.

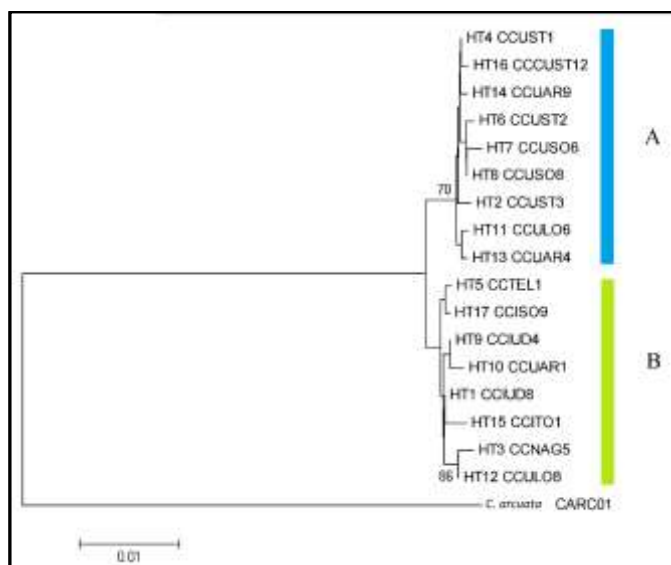


Figure 2. Phylogenetic relationship of *Corythucha ciliata* mitochondrial haplotypes. ML consensus tree of all COI haplotypes. Numbers above branches indicate ML probabilities (>0.60).

The genetic distance between populations 0.00-0.653%; within populations 0.00-0.519%, overall mean distance (TOTAL DATASET) 0.195%. Overall, haplotype diversity (h) was 0.7320 +/- 0.0136, and nucleotide diversity (π) was 0.1945 +/- 0.1152% (Table 1.).

3.1. Genetic diversity and structure in the native range - North America

Altogether twelve haplotypes were detected among the sequences of the 40 specimens collected in North America (five sampling location) and ten of them (HT2, HT6-8, HT10-14 and HT16) were unique. HT4 was the most common one (32.50%), which were found in all populations except Orlando. This haplotype was found also in Japan. HT1 and HT8 revealed two populations, all the other haplotypes were detected at single locations. Both of the two haplogroups A & B were represented in this continent (Figure 1-2). Haplotype diversity (h) was 0.8462 +/- 0.0378, and nucleotide diversity (π) was 0.3630 +/- 0.2005% (Table 1.).

Table 1. Summary of genetic diversity indices for the COI gene: (n) number of individuals sampled; (S) number of polymorphic sites; (No) number of haplotypes; (h) haplotype diversity; (π) nucleotide diversity; (Eur) Europe; (Asi) Asia; (NAm) Nort America.

group	n	No.	S	ts/tv	h ± SD	π (%)± SD
Eur	250	5	6	6/0	0.6542 +/- 0.0123	0.0937 +/- 0.0655
Asi	37	4	12	10/2	0.6562 +/- 0.0555	0.2401 +/- 0.1406
NAm	40	12	21	20/1	0.8462 +/- 0.0378	0.3630 +/- 0.2005
total	327	17	26	25/2	0.7320 +/- 0.0136	0.1945 +/- 0.1152

3.2. Genetic diversity and structure in the invaded range – Europe and Asia

Europe

Five haplotypes were detected among the sequences of the 250 specimens collected Europe (29 sampling location), two of them were common (HT1 44.40% and HT5 31.20%) and HT9 (23.20%), HT15 (0.80%), HT17 (0.40%) were unique for Europe. HT9 were common in the populations from Central Europe and the Balkan Peninsula. Europe is represented in the haplogroup B only. Haplotype diversity was (h) 0.6542 +/- 0.0123, and nucleotide diversity (π) was much lower than in North America 0.0937 +/- 0.0655 (Table 1.).

Asia

Four haplotypes were observed among the 37 specimens collected in Asia (four locations) and one of them is unique (HT3). The population from Japan differ from the other Asian populations unambiguously, because HT3, HT4 were observed only here and no other Asian or European haplotype was detected here. HT1 (24.32%) and HT5 (51.35%), which were common also in Europe, were found from Asia Minor, the Caucasus and Central Asia. Population from Japan represents (together with the north American populations) the haplogroup A, while the other three locations represent the haplogroup B. Diversity indices differ from North American values (0.6562 ± 0.0555 , $\pi=0.2401 \pm 0.1406$).

4. Discussion

Interspecific divergence of the COI gene in the plant bugs (Miridae) was reported 6.30% [46]. For the lace bugs (Tingidae), where *C. ciliata* also belongs to, Park et al. [47] detected more than 3% interspecific divergence. In our study the interspecific divergence values between *C. arcuata* and *C. ciliata* varies 8.49–8.93%.

Intraspecific distances for other Heteropteran species were reported 0–7.72% (mean distance 0.74%) [47], and for Apolygus species (Miridae) 0.40% [46]. In our study the overall mean distance was 0.195%, and the distance between populations was 0.00–0.65%. Jung et al. [46] revealed in some cases the average interspecific genetic distance between closely related species 32 times higher than the average intraspecific distance (e.g., genus *Scolopocelis*). In our cases we also detected 44 times higher interspecific divergence.

COI sequences of SLB showed higher genetic differentiation than avocado lace bug (*P. perseae*), where altogether 9 haplotypes from 469 individuals with 16 polymorphic sites were found [48].

On the total dataset the haplotype diversity is relatively high ($h=0.7320 \pm 0.0136$), with low nucleotide diversity ($\pi=0.1945 \pm 0.1152\%$) which predict a population bottleneck followed by rapid population growth and accumulation of mutations [49]. We found high haplotype diversity with moderate high nucleotide ($h=0.8462 \pm 0.0378$; $\pi=0.3630 \pm 0.2005$) diversity in North America, which suggest a large and stable population with long evolutionary history or secondary contact between differentiated lineages [49]. In the invaded regions we found relative high haplotype diversity with low nucleotide diversity (Europe $h=0.6542 \pm 0.0123$, $\pi=0.0937 \pm 0.0655$; Asia $h=0.6562 \pm 0.0555$, 0.2401 ± 0.1406) which also suggest a population bottleneck followed by rapid population growth and accumulation mutations. Several authors [1, 7, 33, 50] report the loss of genetic diversity for invasive species under the process of biological invasion.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: COI haplotypes distributions.

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Conflicts of Interest: The authors declare no conflict of interest.

Kosovo	Pejë	10									10								
Serbia	Belgrad	10	6				4												
Greece	Olympus	6	2				3				1								
Moldova	Trisapol	8					8												
Turkey	Karabük	7	7				0												
Georgia	Telavi	10	0				10												
Uzbekistan	Samarkand	11	2				9												
Japan	Nagoya	9			2	7													
USA	Arlington	12				4						6	0		1	1			
USA	Louisville	10				3						0	6	1					
USA	Montgomery	2	1			1													
USA	Orlando	6	4						1	1									
USA	Tifton	10		2		5		1		1									1
total		327	125	2	2	20	97	1	1	2	58	6	6	1	1	1	2	1	1

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