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The Invasive Horse-Chestnut Leaf Miner *Cameraria ohridella* (Lepidoptera: Gracillariidae) in the Southern Russia: Preliminary Molecular Genetic Characterization †

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Abstract: The horse-chestnut leaf miner *Cameraria ohridella* is a micromoth that in the last few decades invaded most of the European continent becoming a notorious ornamental pest. This species was also recorded in the European part of Russia, including the southern regions, where it causes impressive damage affecting health of its host plant, horse chestnut *Aesculus hippocastanum*. Our preliminary molecular genetic study based on the analysis of the gene COI mtDNA in a few individuals sampled in Moscow and the southern Russian (in the Republics of Crimea and Adygea) indicates the presence of one haplotype, widely distributed across Europe.

Keywords: alien micromoth; distribution; Russia; southern regions; DNA barcoding

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1. Introduction

The horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) is a micromoth species that is widely known by its rapid expansion across Europe and the impressive damage it causes to the horse chestnut *Aesculus hippocastanum* L. (Sapindaceae) [1]. The species was documented in high density in Macedonia, near Ohrid Lake in the 1980s, from where it was formally described in 1986 [2]. Soon after that, the horse-chestnut leaf miner started being recorded in other European countries, speedily occupying the most territory of the continent [1,3].

The moth has also expanded its range to Russia [4]. For the first time, it was detected in 2003 in Kaliningrad Oblast [5], the semi-exclave region bordering with Poland and Baltic countries where *C. ohridella* was already known [6]. Shortly after that, in 2005, the moth was recorded in Moscow [7]. In the following years, the leaf miner was found in a number of regions in the European part of Russia where its primary host, the horse chestnut, is grown in ornamental plantings. The easternmost point where the pest is nowadays found in Russia is the Volga region [8,9], which is around 900 km southeast of Moscow in a straight line. The moth has widely occupied the southern territory of the country, the regions situated along the Black Sea coast, where it provides spectacular outbreaks in resort areas along the cost adversely affecting tree health and the local landscapes [10].

The molecular genetic studies run in the present range of *C. ohridella* in Europe suggested that the leaf miner originates from the Balkans that is in agreement with its rich genetic diversity in this region [11,12]. On the contrary, in the other parts of Europe where the species is known as nonnative, it has low genetic diversity [11,12].

Our study aims at assessing the damage caused by the horse-chestnut leaf miner to the horse chestnut in the ornamental plantings of the Southern Russia and at characterizing its populations genetically in order to identify the invasive haplotypes in Russia. Here we present the preliminary results of this research.

2. Materials and Methods

2.1. Damage Documentation

The pest survey has been carried out in Sochi (Southern Russia). In 2014–2020, we opportunistically monitored horse chestnut plantations in the city in order to document the degree of damage caused by *C. ohridella*. We inspected up to 10 trees in the same city parks and streets (Riviera, Dendropark of Sanatorium named after M.V. Frunze, Victory Park in Adler, Sochi river embankment) and recorded number of leaves carried mines in the lower part of tree crown. We recorded the damage rate using the following scale: low (< 24% of leaves carrying mines), medium (25–49%), high (50–75%), total (> 75%).

2.2. DNA Barcoding

Earlier samplings of larvae and pupae done in Moscow and in the Southern Russia (in the Republics of Adygea and Crimea) were used for the molecular genetic analysis. *C. ohridella* individuals were collected in the Principal Botanical Garden of the Russian Academy of Sciences (Moscow) in June 2010 and in the city park in the Lublino district (Moscow) in September 2020, in the city planting of Feodosia (Crimea) in August 2017, and in Maykop (the Republic of Adygea) in June 2017. For this preliminary study, only a few individuals of the moth from each of these places were used for DNA barcoding, i.e. two pupae from Moscow, one pupa from Maykop, and one larva from Feodosia.

The COI mtDNA gene from these specimens was amplified and sequenced at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using a sequencing set C_LepFolF/C_LepFolR, following the standard high-throughput protocol [13]. The COI sequences (process IDs: GPRU090-21, GRPAL1099-13, GPRU092-21, GPRU091-21) and the voucher data were deposited in the Barcode of Life Data Systems (BOLD, www.barcodinglife.org) [14]. The identification of the sequenced individuals by their DNA barcodes was done using the identification engine in BOLD and the Barcode Index Number (BINs) was retrieved [15].

Additionally, four COI sequences of *C. ohridella* adults from Europe, publicly available in BOLD, were involved in the study. The two sequences originated from Belarus, Minsk, botanical garden, 07.07.2016 coll., T. Lipinksaya coll., sample ID BIOUG36740-C07, GenBank accession number MW214209; same place, same collector and the date of collection, BIOUG36740-H05, MW214334; one from Macedonia, Kicevo, 01.08.1985 coll., G. Deschka coll., DLCO112, KX044715 [16]; one from Greece, Thessaly, 06.06.2008 coll., Lopez-Vaamonde C. & Augustin S. coll., CCDB-02228-H06, HM379297 [12]. Macedonia and Greece, together with other Balkan countries, are considered to be the primary range of the species [11,12].

The alignment of sequences (658 bp length) was performed in BioEdit 7.2.5 [17]. The phylogeny was analyzed in MEGA X [18] using Kimura 2-parameter model and displayed as Maximum Likelihood (ML) tree with 2000 iterations and bootstrap support. The intraspecific genetic distance was assessed using the same model. The assignment of the obtained COI sequences of the horse-chestnut leaf miner individuals from Russia to a certain haplotype was done by comparing with the haplotype set identified for the species in early study [11].

The sequences of the two specimens of East Asian *Cameraria niphonica* Kumata (Primorsky Krai, the Russian Far East, hosts: *Acer caudatum* subsp. *ukurundense, A. pseudosieboldianum* (Sapindaceae), 22–25.07.2016 coll., N. Kirichenko coll.), earlier obtained by the collector and deposited in BOLD (dx.doi.org/10.5883/DS-GRARFE) [19] were used to root the phylogenetic tree.

3. Results and Discussion

3.1. Increasing Impact of C. ohridella

Sochi is the largest resort city and the important touristic region (the region itself is called the Greater Sochi) in the country and is situated along the Black Sea in Krasnodar Krai. In 2014, Sochi hosted the XXII Winter Olympic Games [20]. This event preceded by the significant modernization of the region and landscaping improvement [10]. For that, many ornamental plants were imported there that directly or indirectly promoted the introduction of unwanted pests to the region through increased interregional traffic [10]. The horse-chestnut leaf miner was documented in the region of Sochi in 2014 [21]. Since then, it promptly spread across the Black Sea coast and started causing severe damage to the horse chestnut in ornamental plantings in the resort areas (Figure 1).

Figure 1. The horse chestnut *Aesculus hippocastanum* affected by *Cameraria ohridella* in Sochi (Southern Russia), in 2020: trees with significantly damaged leaves, early August (**a**); the tree crowns with restarted vegetation, early September (**b**); leaf lamina with numerous mines (individual mines merged into the big whitish spots) (**c,d**); leaves infected by the fungus *Guignardia aesculi* causing leaf browning and distortion, resulting in premature leaf drop (**a,d**). Photos taken by N. Karpun.

In seven years after its first detection in Sochi, *C. ohridella* became a major pest of *A. hippocastanum*. In 2014–2015, less than 25% of leaves carried mines on the examined trees in the Sochi urban area. In the following years, we observed the sharp growth of the pest population. In 2016–2017, the *C. ohridella*'s damage was classified as being at the level from medium to high, whereas in 2018–2020, it was severe across the biggest part of the city, with more than 75% of leaves carrying numerous mines. The significantly damaged leaves lose their color and start falling already in late July – early August giving the impression of sudden autumn arrival at the pick of the touristic season (Figure 1a). Later in the season, in late August – early September, some trees refoliate partially, which, however, does not improve tree health neither the landscape outlook (Figure 1b). In severely attacked leaves, we counted up to 230 mines per a leaf lamina. Growing in size, the mines merged into big whitish spots covering most part of the leaf lamina (Figure 1c). Moreover, the pathogenic fungus *Guignardia aesculi* (Peck) V. B. Stewart (Ascomycota: Dothideomycetes, Botryosphaeriaceae), that causes leaf browning in the horse chestnut (Figure 1a,c,d), worsened the situation by added damage and acceleration of leaf fall in late July – early August.

3.2. Molecular Genetic Characterization

All four specimens collected in Russia (in Moscow, the Republics of Crimea and Adygea) were unmistakably identified in BOLD by their DNA barcodes as *C. ohridella* (100% identity to this species) and were assigned to the only known BIN of the species (BIN ID: BOLD:AAA123). The analyzed DNA barcoding fragments (658 bp length) did not show any divergence in these four analyzed Russian specimens. Furthermore, they were 100% identical to that of the European specimens involved in the study (Figure 2).

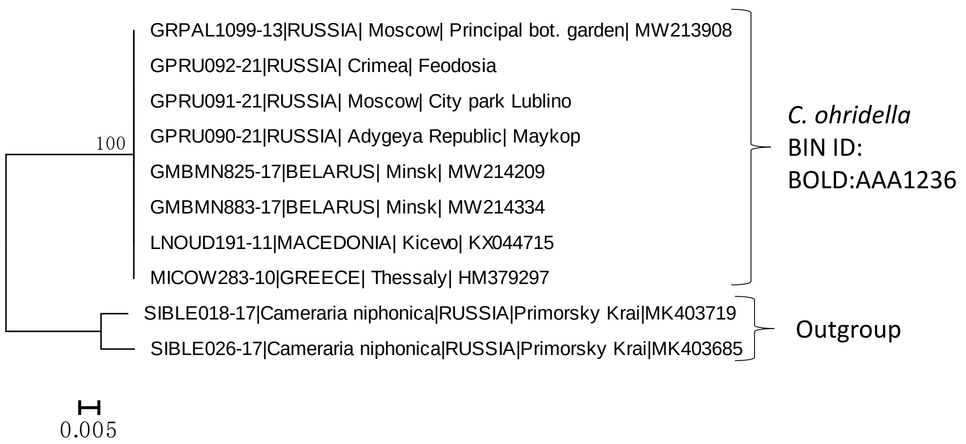

Figure 2. COI Maximum likelihood tree showing the proximity of *Cameraria ohridella* specimens from Russia to those originating from Europe; generated with the K2P nucleotide substitution model. Each specimen is supplied by its Sample ID code, followed by the country and the region of sampling, and by GenBank accession number where applicable. Branch lengths represent the number of substitutions per site. BIN number was retrieved from BOLD.

The DNA barcodes from Russia together with the European ones illustrated in the tree (Figure 2) all corresponded to the haplotype A, most commonly found in the modern range of the species in Europe, as per [11]. Interestingly, this haplotype was the dominant in both the presumable original range (the Balkans) and the invaded regions across Europe [11]. Furthermore, it was one of the two haplotypes detected in the invaded locations in European countries, whereas in the Balkans, 25 geographically structured haplotypes were revealed in the moth's populations [11]. High haplotype diversity and low value of nucleotide diversity altogether signal about rapid expansion of *C. ohridella* in Europe [11,12]. It can also be the case for the European part of Russia, where the pest colonized the significant area in less than two decades [4,5,7–10, 21]. Our preliminary study based only on four individuals of the moth randomly sampled in the three geographically distant regions (the distance from 375 km in a straight line between Feodosia and Maykop, to 1200 and 1250 km between Moscow and Feodosia and Moscow and Maykop respectively), so far revealed only one, the most invasive haplotype. We believe that further sampling and detailed analysis will return similar phylogeographic pattern as it was identified in the neocolonized regions of Europe.

4. Conclusions

Our preliminary molecular genetic study based on the analysis of COI mtDNA in a few individuals sampled in Russia (in Moscow, the Republic of Crimea and Adygea) indicates the presence of one haplotype, largely found across the secondary range of the species in Europe. The vectors facilitating the pest distribution in Russia, as well as the moth's ability to establish the novel trophic associations with the exotic Sapindaceae plants extensively cultivated in the botanical gardens and parks in the southern Russia (in particular East Asian maples) remain unexplored. The study we are presently running in the Southern Russia will help to clarify these and other questions about *C. ohridella* invasion in the country.

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

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