RESEARCH ARTICLE

The North American leaf-mining moth *Coptodisca lucifluella* (Lepidoptera: Heliozelidae) reached Southern Russia and Abkhazia: genetic variability and potential for further spread

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Abstract

The invasive North American leaf-mining moth *Coptodisca lucifluella* (Clemens, 1860) (Lepidoptera: Heliozelidae) is reported for the first time in Russia and Abkhazia based on findings of the leaf mines on *Carya illinoinensis*, *Car. tomentosa*, *Pterocarya fraxinifolia*, *Juglans regia*, and *J. nigra* in Sochi, Sirius, Crimea, and Abkhazian settlements in 2023 and 2024. The species was identified by DNA barcoding of two larvae dissected from the leaf mines and by morphology of adults grown from the leaf mines in 2024. The analysis of DNA barcoding data of the Russian and Abkhazian specimens together with those from Europe (invaded range) and North America (native range), publicly available in BOLD and/or GenBank, defined relatively low (1.45%) intraspecific variability. High interspecific divergence

(over 9%) was detected when comparing *C. lucifluella* with another invasive North American heliozelid, *C. juglandiella*, currently spreading in Europe but not yet recorded in Russia and Abkhazia. The leaf mines of *C. lucifluella* (Chambers, 1874) were notably abundant on the North American pecan, *Car. illinoinensis*, widely cultivated in orchards and urban area in Southern Russia and Abkhazia. Around 162±23 mines (maximal of 216 mines) and 182±30 mines (maximal of 269 mines) per a compound leaf were documented in Sochi (Russia) and Gulrypsh District (Abkhazia), respectively. In Abkhazia, up to 35% parasitism rate was recorded, with unidentified Eulophidae as a main cause. The overview of the modern range of *C. lucifluella* and the note on its bionomics in the studied localities are provided. Furthermore, the potential of the species spread in Russia and beyond (in particular to Caucasus and Asian countries) is discussed.

Introduction

Leafmining moths (Lepidoptera) comprise one of the most diverse groups, with the representatives found on various plants across different biogeographic regions (Hering 1951; Connor and Taverner 1997; Kirichenko et al. 2019). Heliozelidae, or shield-bearer moths, are one of the smallest moths with 12 genera, encompassing a total of 125 described species and a number of potentially new species awaiting formal description (Milla et al. 2018). Some heliozelids are known as pests of several commercial crops, including vineyards, cranberry farms, and walnut orchards (van Nieukerken et al. 2012; Bernardo et al. 2015; van Nieukerken and Geertsema 2015). In the last decade, three North American heliozelids were detected in Europe, i.e., *Aspilanta* (*Antispila*) *oinophylla* (van Nieukerken & Wagner, 2012), *Coptodisca lucifluella* (Clemens, 1860) and *C. juglandiella* (Chambers, 1872) (Bernardo et al. 2012; van Nieukerken et al. 2012; Takács et al. 2020; van Nieukerken and Eiseman 2020).

In Russia, a total of 10 Heliozelidae have been known so far: *Antispila inouei* Kuroko, 1987, *A. metallella* (Denis & Schiffermüller, 1775), *A. tateshinensis* Kuroko, 1961, *A. petryi* Martini, 1899, *Heliozela biprominens* Lee, Hirowatari & Kuroko, 2006, *H. hammoniella* Sorhagen, 1885, *H. resplendella* (Stainton, 1851), *H. sericiella* (Haworth, 1828), *Holocacista rivillei* (Stainton, 1855), and *Tyriozela porphyrogona* Meyrick, 1931 (Sinev and Kozlov 2019). Until recently, none of alien shield-bearer moths has been recorded in the country.

In 2023, during woody plants surveys in Sochi (Southern Russia) and Abkhazia, we noted small transparent elongated blotches, often with an oval hole at the end of a mine, on the North American pecan, *Carya illinoinensis* (Wangenh.) K. Koch. Two larvae found inside the leaf mines were identified through DNA barcoding. More leaves with the mines were sampled in Sochi and Abkhazia, and the moth adults, which emerged from them in spring 2024, were used for morphological identification. Thus, a shield-bearer moth, *C. lucifluella*, originally known from North America, was documented on the territory of the Russian Federation and

Abkhazia for the first time. Additionally, in July 2024, it was also recorded by our team in Crimea.

Here, we provided data on the species findings in Russia, including Crimea, and in Abkhazia, the host plants (Juglandaceae), the infestation level of *Car. illinoinensis* (the most damaged tree), as well as some notes on the moth bionomics, and the parasitism. Furthermore, we analyzed DNA barcodes of the Russian and Abkhazian specimens together with those from Europe (invaded range) and North America (native range), including other *Coptodisca* representative, to clarify intra- and inter-specific genetic distances.

Materials and methods

### Study region and field surveys

The surveys of *Car. illinoinensis* plantings were performed in Sochi and the Federal Territory Sirius (Russia, Krasnodar Territory), and in all seven administrative districts of Abkhazia, i.e., in Gulrypsh, Ochamchira, Sukhum, and Gudauta Districts from mid-September to mid-October 2023 and in Gagra, Gudauta, Tkuarchal, and Gal Districts in mid July 2024. Overall, 37 localities were visited, i.e., 15 in Sochi, 2 in Sirius and 22 in Abkhazia, mainly along the Black Sea cost (Fig. 1). Few other tree species from Juglandaceae were surveyed for the presence of the mines: *Carya tomentosa* (Lam.) Nutt. and *Pterocarya fraxinifolia* (Lam.) Spach, in Sochi (the point Nos 2 and 13, respectively), *Juglans regia* L., *J. nigra* L. in Sirius (No. 13) and in Abkhazia, (Nos 26, 32, 33, 34, 35, 37). In 2024, an opportunistic seach of leaf mines on *J. regia* was performed in Sevastopol, Crimea (Fig. 1A). In each locality, from one to five trees were examined. On the trees, from 5 to 50 compound leaves (containing from 11 to 15 leaflets) were checked on four low branches around tree crown for the presence of leaf mines (i.e., tiny blotches, ending with an oval cut after larvae vacated the mines), characteristic for shield-bearer moths (van Nieukerken and Eiseman 2020; Takács et al. 2020; Lepiforum 2024a).

Leaf mine abundance

The abundance of leaf mines on *Car. illinoinensis* was assessed in seven localities, i.e., in three districts in Sochi (Nos 1, 10, 14) and in four districts in Abkhazia (Nos 16, 25, 26, 29) (Fig. 1, Table 1). By 50 compound leaves were randomly collected on up to five trees in each locality, labeled, placed in zip-lock bags, and transported to the laboratory of the Subtropical Scientific Centre of the Russian Academy of Sciences (RAS) (Sochi, Russia). On the compound leaves, the mines were counted. All mines were taken into account: abandoned (i.e., with oval cut) and inhabited by larva (i.e., the mines of different size, including tiny mines where, by some reasons,

larvae died). To distinguish tiny mines from damage caused by other organisms, the mines were checked against the light using magnifying glass (×10–20). The average number of mines per a compound leaf (±SD) was computed for every locality. The non-parametric Mann-Whitney U-test was used to compare leaf mine abundance in different localities (Mann and Whitney 1947).

By five compound leaves from different host plants from the studied districts in Sochi, Sirius, Crimea, and Abkhazia were herbarized, annotated and arranged in the herbarium collection following our early approach (Lopez-Vaamonde et al. 2021). Herbarized leaves are stored at the Subtropical Scientific Centre of RAS (Sochi, Russia) and Sukachev Institute of Forest (SIF) SB RAS (Krasnoyarsk, Russia).
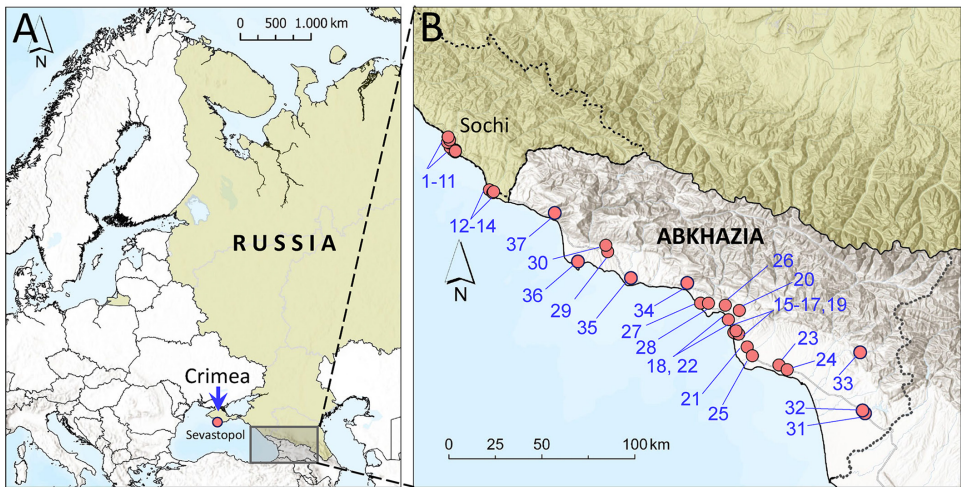
Figure 1. The study region in Southern Russia, Crimea and Abkhazia. **A** – surveyed area (in Crimea, only Sevastopol was explored); **B** – the main sampled territory (Sochi, Sirius and Abkhazia), where red circles and numbers (1–37) indicate examined localities. RUSSIA, the localities Nos 1–14 [i.e., Sochi, Khosta District: 1 – Gnilushka, 2 – Sanatorium Frunze, 2a – Sanatorium "Zolotoy Kolos"; 3 –Arboretum, 4 – Deputatskaya Str., park, 5-6 – Yana Fabritsiusa str. (different parts); Central District: 7 – Kurortny Avenue, park, 8 – Chaikovskiy Str., 9 – Vinogradnaya Str., 10 – Krasnoarmeyskaya Str., 11 – City cross-road; Adler District: 12 – Kalinina str.; Sirius: 13 – Yuznie Kultury park; 14 – Kaspiyskaya str.]. ABKHAZIA, the localities Nos 15–37 [i.e., Gulrypsh District: 15–18 – vill. Gulpysh (different streets), 19 – vill. Agudzera, 20 – vill. Bagmaran; 21 – vill. Dranga, 22 – vill. Kashtan; Ochamchira District: 23 – vill. Kyndyg, 24 – vill. Tamysh, 25 – vill. Adzubzha; Sukhum District: 26 – Sukhum, 27 – vill. Eshera, 28 – vill. Achadara; Gudauta District: 29 – vill. Barmysh, 30 – vill. Blabyrkhua; 34 – Novyi Afon, 35 – Gudauta; Gal District: 31–32 – Gal (different streets); Tkuarchal District: 33 – Tkuarchal; Gagra District: 36 – Pitsunda, 37 – Gagra].

Moth rearing and parasitism assessment

The moth adults were reared from seven localities: three in Sochi (Nos. 2, 3, 7) and four in Abkhazia (16, 19, 26, 27). In September-October, the larvae (presumably of the 3rd generation) were finishing feeding in the leaf mines and vacating them by cutting oval shields. From 10 to 20 leaf shields, randomly taken from the compound leaves from 1-3 trees in each locality, were placed in Petri dishes (90 mm in diameter) lined with filter paper. The dishes were kept at the constant conditions (temperature, +23°C; humidity, 50%) in the laboratory. The humidity in the dishes was maintained by moisturizing the segment of a cotton pad attached to the internal side of the dish lid. On the 5th week, we suspected that the larvae were entering the diapause because no single adult emerged from the shields. Thus, in late November 2023 the dishes with the shields were placed in a fridge at the temperature +3°C. In early March 2024, the dishes were moved to room temperature (+23°C). The shields were checked every second day and emerged adults collected.

The moths and all shields were transported to SIF SB RAS (Krasnoyarsk, Russia). The adults were pinned, the shields (except from which the moths emerged) were opened and examined under Zeiss Stemi DV4 Stereo Zoom Microscope (Zeiss, Germany). The presence of pupal exuvia of the moth inside the shields (meaning that the moths managed to emerged, but by some reason pupal exuvia remained in shields) and the presence of dead larvae and pupae were documented. Dead larvae and pupae without parasitism sign were assigned to the category "died from unknown reasons". The presence of a round hole in a shield or finding a parasitoid exuvium inside a shield were assigned to parasitism. The number of emerged moth, dead moth's larvae or pupae and parasitoid exuvia were counted. Their proportion were computed into relation to all shields examined, and expressed in %. Such estimation was possible as only one larva/pupa of *C. lucifluella* or one pupal exuvium of a parasitoid were present in a shield. The adult emergence and the parasitism rate were compared between Sochi and Abkhazia using Mann-Whitney U-test in STATISTICA 12 (USA, TIBCO Software Inc.).

Taxonomic identification, imaging, mapping

The adults of *C. lucifluella* were identified based on forewing pattern and male genitalia (Takács et al. 2020; Lepiforum 2024a). Two genitalia dissections were performed and the slides were prepared based on commonly used technique (Robinson 1976). The adults, their genitalia, and the examined shields are stored at SIF SB RAS (Krasnoyarsk, Russia).

In nature, affected trees and leaves and the typical leaf mines were photographed by the digital camera Fujifilm X30 (Japan, Fujifilm Holdings Corporation). The images of adults were taken through the Zeiss Stemi DV4 Stereo Microscope and male genitalia through the microscope Mikmed-5 (Russia, LOMO). Images were revised

in Adobe Photoshop (v. 23.5.0). ESRI ArcGIS Pro 3.1 software was used for mapping sampled localities (ESRI 2024).

DNA barcoding

Two larvae dissected from the leaf mines (by one from Sochi and Abkhazia, Table 1) were DNA barcoded. Sequencing of the mitochondrial cytochrome oxidase I gene (mtDNA COI, 658 bp) was performed to confirm the species identity, compare DNA data with the species representatives from the modern range, and specify intra- and interspecific divergences. The primer set C_LepFolF/C_LepFolR and the standard protocol for sequencing were used (de Waard et al. 2008). Molecular genetic analysis was carried out at the Canadian Center for DNA barcoding (CCDB, Canadian Center for DNA Barcoding) at the University of Guelph (Canada). The sequence, electropherograms, the specimen data, and the images of larvae from Sochi and Abkhazia were deposited to the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert, 2007) and GenBank (National Centre for Biotechnology Information).

For comparison, 46 sequences of *Coptodisca* spp. (i.e., *C. lucifluella*, *C. juglandiella*, *Coptodisca* sp.), publicly available in BOLD and GenBank, were involved to the analysis. Among them, there were 36 specimens of *C. lucifluella* (3 from the USA, 2 from Canada, 25 from Italy, 3 from Slovenia, 3 from Hungary), 7 specimens of *C. juglandiella* (2 from Canada, 1 from the USA, 4 from Hungary) and 3 specimens of *Coptodisca* sp. (all from the USA) (Table 1). All sequences are accessible in BOLD through the link dx.doi.org/10.5883/DS-COPTORUS.

The Barcode Index Numbers (BINs) (i.e., species proxy) were retrieved from BOLD (Ratnasingham and Hebert 2013). The sequences were aligned in BioEdit 7.2.5 (Jeanmougin et al. 1998). The phylogenetic tree was built and intra- and interspecific distances were assessed using maximum likelihood estimation, the Kimura 2-parameter model, and a bootstrap approach (1000 iterations) in MEGA11 (Tamura et al. 2021). The median-joining haplotype network was built in PopART (version 1.7 for Windows) with a parsimony algorithm (Leigh and Bryant 2015). The DNA barcode of *Phyllonorycter pterocaryae* (Kumata, 1963) (Lepidoptera: Gracillariidae) (host plant *Juglans mandshurica* Maxim., I. Oschima coll.) was used for rooting the tree (Table 1).

Table 1. *Coptodisca* specimens involved into the DNA barcoding analysis

No	Process ID	Country	State/region	Year	Host plant[1]	Collectors[2]	GenBank No.
				Coptodisca lucifluella			
1	NALAS058-23	Russia	Sochi	2023	*Car. illin.*	E. Zhuravleva	PQ100151
2	NALAS089-23	Abkhazia	Sukhum	2023	*Car. illin.*	I. Khuapshychu	PQ100148
3	GBGL40569-19	Italy	–	2014	–	–	KY937894

No	Process ID	Country	State/region	Year	Host plant[1]	Collectors[2]	GenBank No.
4	GBGL40568-19	Italy	–	2014	–	–	KY937893
5	GBGL40567-19	Italy	–	2014	–	–	KY937892
6	COPIN003-14	Italy	Veneto	2012	*J. regia*	P. Triberti	KJ427007
7	COPIN013-14	Italy	Lazio	2013	*J. regia*	R. Sasso	KJ427008
8	COPIN012-14	Italy	Lazio	2013	*J. regia*	R. Sasso	KJ427009
9	COPIN011-14	Italy	Lazio	2013	*J. regia*	R. Sasso	KJ427010
10	COPIN010-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427011
11	COPIN009-14	Italy	Tuscany	2013	*J. regia*	F. Delcore	KJ427012
12	COPIN008-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427002
13	COPIN007-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427003
14	COPIN006-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427004
15	COPIN005-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427005
16	COPIN004-14	Italy	Campania	2013	*J. regia*	U. Bernardo	KJ427006
17	HELA047-13	Italy	Verona	2012	*J. regia*	P. Triberti	KJ427013
18	GBGL40579-19	Italy	–	2014	–	–	MF002041
19	GBGL40578-19	Italy	–	2014	–	–	MF002040
20	GBGL40577-19	Italy	–	2014	–	–	MF002039
21	GBGL40576-19	Italy	–	2014	–	–	MF002038
22	GBGL40575-19	Italy	–	2014	–	–	MF002037
23	GBGL40574-19	Italy	–	2014	–	–	KY937898
24	GBGL40573-19	Italy	–	2014	–	–	KY937899
25	GBGL40572-19	Italy	–	2014	–	–	KY937897
26	GBGL40571-19	Italy	–	2014	–	–	KY937896
27	GBGL40570-19	Italy	–	2014	–	–	KY937895
28	LPEST284-22	Slovenia	Skoc. jame	2022	*J. regia*	NIK, SG	OQ413707
29	LPEST272-22	Slovenia	V. Potok	2022	*P. fraxinif.*	NIK, SG	OQ413695
30	LPEST271-22	Slovenia	V. Potok	2022	*P. fraxinif.*	NIK, SG	OQ413706
31	MN103407	Hungary	–	–	–	A. Takács et al.	MN103407
32	MN103408	Hungary	–	–	–	A. Takács et al.	MN103408
33	MN103409	Hungary	–	–	–	A. Takács et al.	MN103409
34	HELB005-19	Canada	Ontario	2015	*Car. ovata*	EJN	PQ100147
35	GBMNB43284-20	Canada	–	–	–	–	MK978220
36	HELA045-13	USA	Connecticut	2011	*Car. glabra*	EJN	KJ427014
37	HELA044-13	USA	Tennessee	2010	*Car. glabra*	EJN, CD	KJ427015
38	ANTVI012-11	USA	Tennessee	2010	*Car. glabra*	EJN, CD	JQ412564
			Coptodisca juglandiella				
39	MN103410	Hungary	–	–	–	A. Takács et al.	MN103410
40	MN103410	Hungary	–	–	–	A. Takács et al.	MN103410
41	MN103410	Hungary	–	–	–	A. Takács et al.	MN103410

No	Process ID	Country	State/region	Year	Host plant[1]	Collectors[2]	GenBank No.
42	MN103411	Hungary	–	–	–	A. Takács et al.	MN103411
43	HELA046-13	USA	N. Carolina	2010	*J. nigra*	EJN, CD	KJ427001
44	OPPQM1907-17	Canada	Ontario	2014	–	CBG Coll. Staff	PQ100149
45	RRSSC1277-15	Canada	Ontario	2015	–	BIO Coll. Staff	MG361333
			Coptodisca sp.				
46	HELA043-13	USA	Georgia	2010	*Carya*	EJN, CD	KJ427000
47	LNAUX059-18	USA	Maryland	2002	*Car. tom.*	R. Gagne	PQ100150
48	GBGL40585-19	USA	Georgia	–	*Carya*	–	MF118302

Notes: [1]*Car. – Carya, illin. – illinoinensis, tom. – tomentosa, J – juglans, P. fraxinif. – Pterocarya fraxinifolia*; [2]EJN – Erik J. van. Nieukerken, CD – C. Doorenweerd, NIK – Natalia I. Kirichenko, SG – Stanislav Gomboc. – no data available.

Result

I. Novel geographical records and the species bionomics

Coptodisca lucifluella (Clemens, 1860) (Lepidoptera: Heliozelidae)
Figure 1

Material examined. RUSSIA, Sochi, Deputatskaya str., 43.56 N, 39.73 E, 14 m alt., 1 larva, 7 females, 4 males, *Car. illinoinensis*, 17.IX.2023 coll., 25-27.03.2024 adults emerged, E. Zhuravleva & N. Karpun coll., N. Kirichenko & S. Gomboc det., 1 larva DNA barcoded (process ID NALAS058-23); Crimea, Sevastopol, Estonskaya str., 44.60 N, 33.54 E, 11 m alt., 3 larvae, *Juglans regia*, 19.VII.2024, E. Zhuravleva coll.; ABKHAZIA, Sukhum, Dzidzaria, 43.01 N, 41.01 E, 12 m alt., 1 larva, 2 males, *Car. illinoinensis*, 14.IX.2023 coll., 25-27.04.2024 adults emerged, I. Khuapshykhu coll., N. Kirichenko & S. Gomboc det., 1 larva DNA barcoded (process ID NALAS089-23).

Adult morphology. Forewing length (including fringe) 1.8-2.2 mm. The head with gray-silvery scales, the body dark with silver reflection. The forewings with gray-silvery scales at the basal half, with two silver triangles border at distal thirds of costa and dorsum (Fig. 2A-C). At the distal dorsal side, three black streaks with the area between them in yellow. At the distal costal side, dark area around silver triangle (Fig. 2A-C).

In male genitalia, valvae with five teeth and 5-6 sensilla on pectinifers in the specimens from Sochi and Abkhazia, whereas up to 7 sencilla were recorded by other authors (Bernardo et al. 2015). Aedeagus with two spine rows apically. The detained species description is provided in Bernardo et al. (2015), Takács et al. (2020).
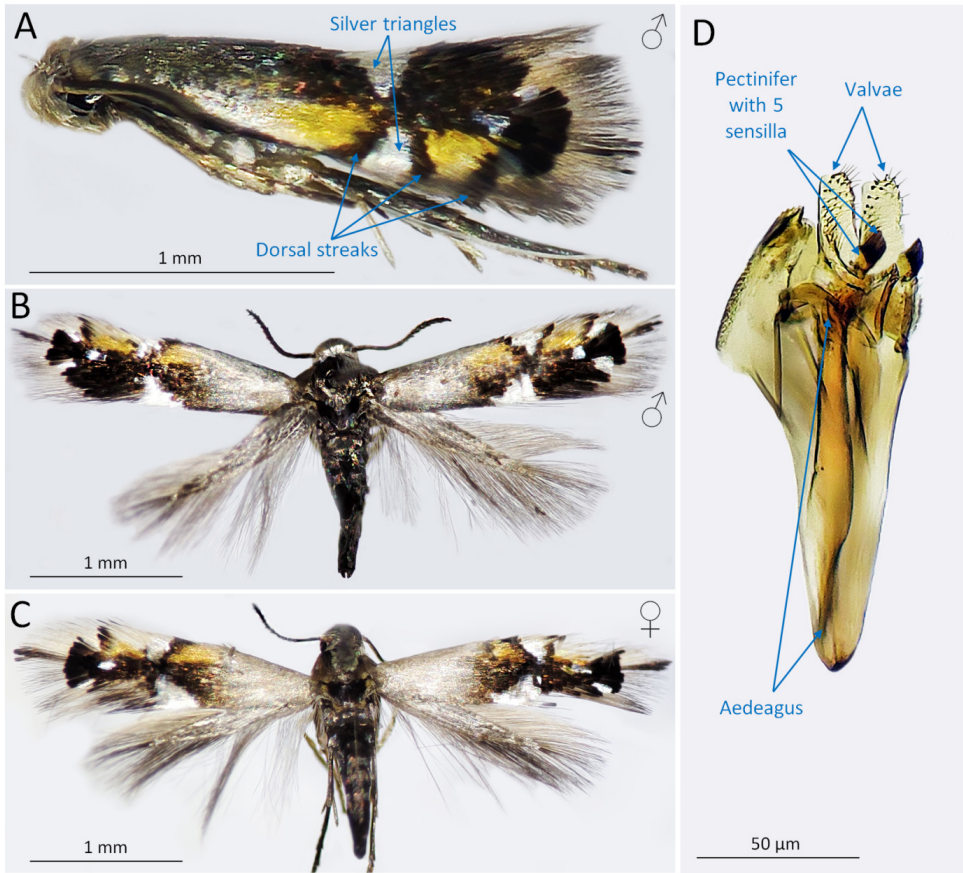
Figure 2. The adult of *C. lucifluella* grown from the leaf mines on *Car. illinoinensis*, Sochi, Russia. **A** – lateral view; **B**, **C** – dorsal view; **D** – male genitalia. Photos: N. Kirichenko, S. Gomboc.

Leaf mines. The leaf mines were recorded on the trees in urban plantings (Fig. 3A). The mine is a tiny transparent oval full-depth blotch (up to 10 mm in length), notable on both sides of the leaf (Fig. 3B-D). In Sochi and Abkhazia, the leaf mines were mostly concentrated along the main vein (i.e., about 90% of such mines), rarely elsewhere on the leaf lamina (Fig. 3B, C). In the mine placed against the light, a pale brown larva and black grains of frass are seen; frass grains are accumulated in the mine along its edge (Fig. 3F). Before pupation, a larva constructs an oval shield at the distal part of a mine by making a cut from upper and lower epidermis joining their edges by silk (Fig. 3E,F). Wrapped in such shield, larvae vacate the mine and pupate elsewhere on the tree. The abandoned mines dry and become brownish; eventually their epidermis cracks.

Leaf mine of *C. lucifluella* and another invasive North American species *C. juglandiella* can be distinguished by following features: (1) mine shape (oval vs. round); (2) mine position on the leaflet (anywhere on the leaf surface vs. along the midrib,

in particular in the angle of lateral and main veins, and (3) frass placement in the mine (dispersed within the mine vs. compactly placed in a small area of the mine) (Takács et al. 2020). However, in Sochi and Abkhazia the majority of *C. lucifluella*'s mines were situated on pecan's leaves along the main vein and frass was often present along the mine edge. If same *C. lucifluella*'s mines are found in Russia on *Juglans*, it will be tricky to reliably distinguish these two species by leaf mines.

Host plants. *C. lucifluella* feeds exclusively on Juglandaceae. In the native range (North America), it develops on *Carya* spp. (common name: hickory) (BugGuide 2015). In the invaded range (Europe), it was documented on *Carya cordiformis* (Wangenh.) K. Koch, *Car. glabra* (Mill.) Sweet, *Car. illinoinensis*, *Car. laciniosa* (F. Michx.) Loudon, *Juglans ailantifolia* Carrière, *J. nigra* L., *J. regia* L., *Pterocarya fraxinifolia* (Bernardo et al. 2012; Takács et al. 2020; Kirichenko et al. 2022; Lepiforum 2024a). In 2023 and 2024 in Sochi (Russia), the leaf mines were found on *Car. illinoinensis* (most damaged host), *Car. tomentosa*, *Pterocarya fraxinifolia*, *Juglans regia*, and *J. nigra*; in Abkhazia, on *Car. illinoinensis* (most damaged host), *J. regia* and *J. nigra*; in 2024 in Crimea on *J. regia* (present paper).

Damage. In 2023, the leaf mine were documented on *Car. illinoinensis* in all studied localities, i.e., in 15 localities in Sochi and in 16 localities in Abkhazia. The most northern locality was the City alleyway in Sochi, No. 11 (43.62 N, 39.72 E), and the most southern was the city Gal in Abkhazia, No. 31 (42.63 N, 41.44 E), situated in about 12 km from the border with Georgia (Fig. 1; Fig. 4B).

The leaf mines abundance was estimated in seven localities. In two localities, the leaf mines were most numerous: 182±30 mines (with the maximal value of 269 mines) per compound leaf in Gulrypsh District (Abkhazia) and 162±23 mines (max 216 mines) per compound leaf in Sochi, Khosta District (Russia, Krasnodar Territory) (Fig. 4, red stars). In the majority of other places, less than 100 leaf mines per compound leaf were documented (Fig. 4, yellow stars), whereas only in one out of seven localities, less than 50 leaf mines per a compound leaf were noted (Fig. 4, green star). Additionally, in Khosta District in Sochi, the mines were also documented on the leaves of another North American pecan, *Car. tomentosa*, but they were much least abundant (23±5 mines per compound leaf) compared to what was observed on *Car. illinoinensis*. In Sochi, on other Juglandaceae representatives (i.e., *Pterocarya fraxinifolia*, *Juglans regia* and *J. nigra*), the leaf mines were as abundant as on *Car. tomentosa*. In Crimea, the damage was very low on *J. regia*, only 11 mines were recorded overall on 4 examined trees.

Moth emergence, larval and pupal parasitism. Moth emergence from the shields was evident as the pupal exuvia protruded the shield "mouth" (Fig. 5A); less often, the pupal exuvia were lost or, conversely, remained inside the shields.

The relative number of the moth emerged was significantly higher in Sochi than in Abkhazia: 80±11% vs. 52±13% (Z = 2.56, p = 0.01, N = 7). The highest rate of adult emergence, reaching 90%, was documented in the Sochi Arboretum (locality No. 3) (Fig. 6).
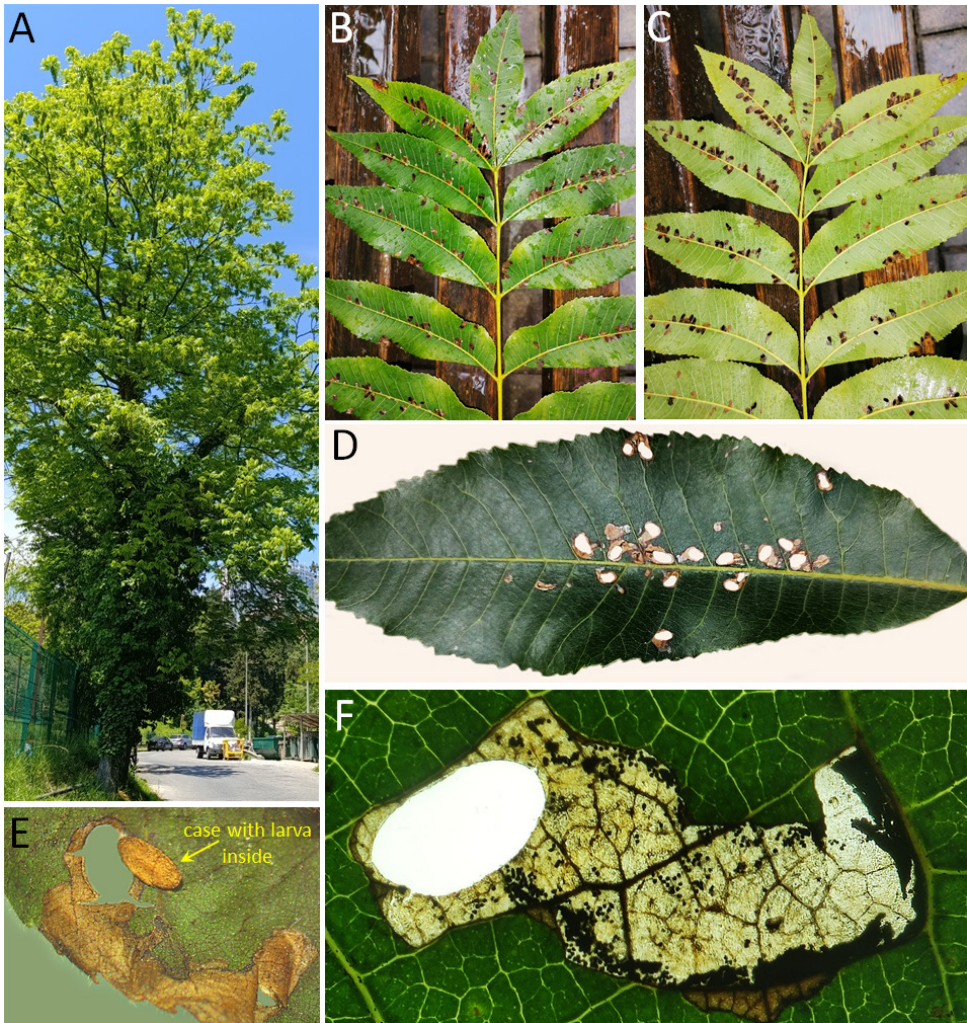
Figure 3. The damage caused by *C. lucifluella* in Sochi (Russia) in 2023. **A** – a tree of *Car. illinoinensis* in the city planting; **B**, **C** – a compound leaf with numerous mines, upper side (**B**) and lower side (**C**) views; **D** – an individual compound leaf with the vacated mines along the main vein; **E** – a shield with a larva inside cut from leaf mine; **F** – the vacated leaf mine with an ovel hole remained after larva cut out the shield. Photo: E. Shoshina (**A**), E. Zhuravleva (**B**, **C**, **E**), N. Karpun (**D**), N. Kirichenko (**F**).

The lowest adult emergence rate (40%) was documented in Abkhazia in Gulpysh village (No. 16) (Fig. 6). The mortality of larvae and pupae of the moth caused by unknown reasons did not differ between Sochi and Abkhazia: 15±5% vs. 16±12% ($Z = 0$, $p = 1$, $N = 7$), and it varied from 10 to 30% across the studied localities (Fig. 6).The parasitoid presence in the moth shields was determined by finding a round hole on shield wall (not far from the shield "mouth" or bottom) (Fig. 5B,C)

or, if not lost after the parasitoid emergence, by finding its pupal exuvium inside the shield (Fig. 5C,D). The parasitism was significantly higher in Abkhazia than in Sochi: 31±3% vs. 5±8% (Z = -2.03, p = 0.04, N = 7). The maximal parasitism rate (35%) was recorded in Sukhum, Abkhazia (locality No. 26) (Fig. 6). No one parasitoid adult was obtained indoor as the parasitoid adults vacated the host shields prior our samplings. According to the characteristic parasitoid exuvia found inside the shields of *C. lucifluella*, the emerged parasitoids were from Eulophidae (Hymenoptera) (Fig. 5D).

Distribution. The moth's range covers North America (USA: Kentucky, Maryland, Missouri, North Carolina, Ohio, Oklahoma, Pennsylvania, Tennessee, Texas, Wisconsin) (North American Moth Photographers Group 2013). In 2012, the moth was newly documented in Mexico (Ávila-Rodríguez et al. 2015). For the first time outside the Nearctic, the moth was recorded in Europe (Italy) in 2010 (Bernardo et al. 2012, 2015). In the following years, its occurrence was documented in following European countries: Hungary in 2017 (Takács et al. 2017, 2020; Szabóky and Takács 2018), Ukraine in 2019 (Pályi et al. 2019), Bulgaria and Czech Republic in 2020 (Tomov 2020; Laštůvka and Laštůvka 2020), Austria, Slovakia, France in 2021 (Huemer 2021; Tokár et al. 2021; Rennwald 2022), Romania, Germany, Slovenia, Switzerland, and Serbia in 2022 (Chireceanu et al. 2022; Fuchs et al. 2022; Kirichenko et al. 2023a; Lepiforum 2024a). In 2023, we documented the moth for the first time in Russia (Krasnodar Region: Sochi, Sirius) and Abkhazia, whereas in 2024, in Crimea (present paper). We believe that the moth is already present in Georgia as we found the leaf mines in 12 km from the border.
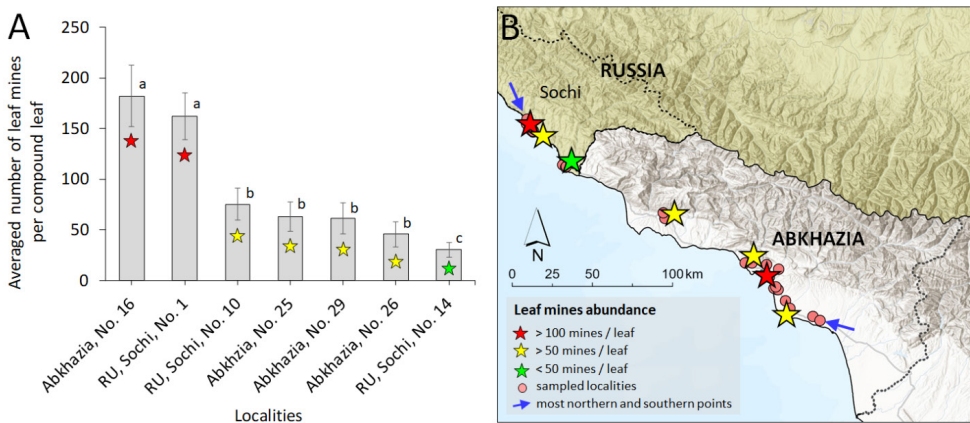
Figure 4. Averaged number of *C. lucifluella*'s mines (±SD) on compound leaves of *Car. illinoinensis* in Sochi (Russia, RU) and Abkhazia. The columns with different letters are statistically distinct (Mann-Whitney U-test, $p<0.05$). The sampled localities (Nos 1, 10, 14, 16, 26, 29) are coded in figure A, and indicated by the stars in figures A, B. The stars of different colors reflect leaf mine abundance (per compound leaf). Sampled localities are provided in Fig. 1.
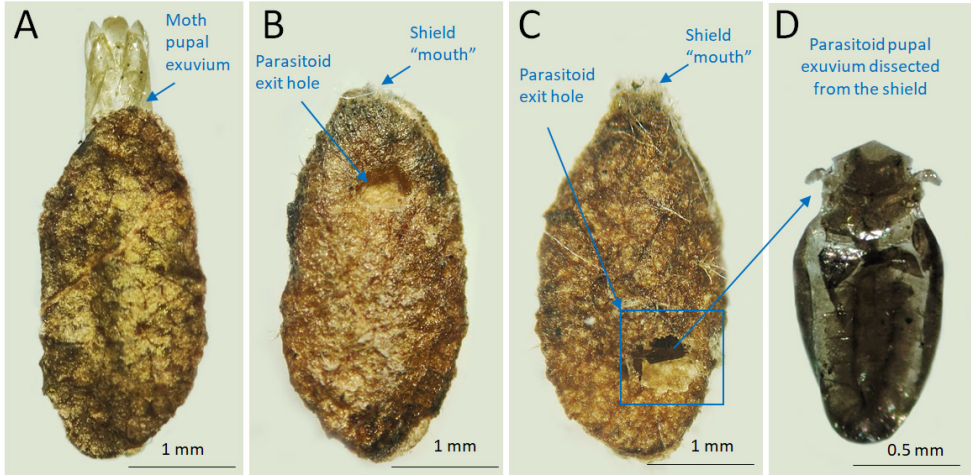
Figure 5. Abandoned shields of *C. lucifluella*. **A** – the shield with moth pupal exuvium protruding from the shield "mouth"; **B**, **C** – the shields with parasitoid exit hole; **D** – the pupal exuvium of an eulophid dissected from the shield. **A** – Sochi, **B**–**D** – Abkhazia, 2023. Photos: N. Kirichenko.
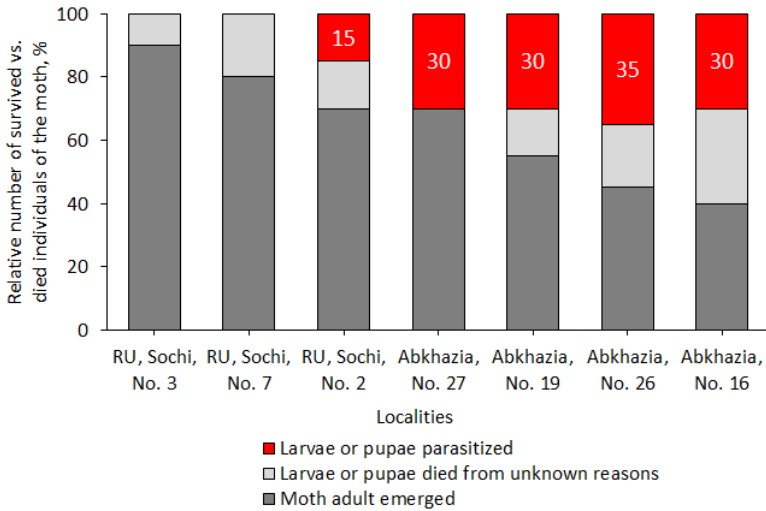
Figure 6. Relative number of survived vs. died (including parasitized) individuals of *C. lucifluella* in Sochi (Russia, RU) and Abkhazia, (%).

II. The genetics of *C. lucifluella* in the Holarctic

In BOLD, two sequenced specimens (one from Sochi and one from Abkhazia) were confidently identified as *C. lucifluella*. In the DNA barcode fragment of the COI gene, the specimen from Sochi was identical to five specimens from Italy (GenBank accession number: KY937892, KY937893, KJ427003, KJ427012, KJ427009) (Bernardo et al. 2015). The DNA barcode of the Abkhaz specimen had the highest similarity (99.39%) with the Canadian specimen (MK978220). The specimen from Sochi and Abkhazia had 0.8% divergence and were different in five nucleotide substitutions in the positions 439 (C → T), 448 (C → T), 496 (A → G), 514 (C → T), 550 (A → G) in 658 bp COI gene fragment.

On ML tree, *C. lucifluella* formed one cluster with the specimens from native range (USA, Canada) and invaded range (European countries, Russia, Abkhazia), assigned to one BIN: BOLD: AAV9339 (Fig. 7). The neighbor cluster (BIN BOLD: AAV9439) was formed by another invasive species, *C. juglandiella*, which included the specimens from the native range (USA, Canada) and invaded range (Hungary). The third cluster (BIN BOLD: AAV8691) was shaped by undescribed *Coptodisca* sp. from North America, also associated with *Carya* (Fig. 7).

In *C. lucifluella*, intraspecific divergence reached 1.45% (N = 38 specimens) (Table 2). In *C. juglandiella*, it was below 1% (N = 7), and in *Coptodisca* sp. it was nil (N = 3) (Table 2). The distances between the species were pronounced, i.e., over 8% (Table 2). In particular, between *C. lucifluella* and *C. juglandiella*, the minimal genetic divergence was 9.38%. The cluster of *Coptodisca* sp. was slightly more distant from *C. lucifluella* (10.34%) than from *C. juglandiella* (8.81%) (Table 2).

Table 2. Intra- and interspecific distances in COI gene among *Coptodisca* spp. in the Holarctic

| Species*	Species		
	C. lucifluella	*C. juglandiella*	*Coptodisca* sp.
C. lucifluella (38)	[1.45]	–	–
C. juglandiella (7)	9.38	[0.71]	–
Coptodisca sp. (3)	10.34	8.81	[0]

Note: * For each species pair, minimal pairwise distances are shown; in square brackets, maximal intraspecific distances are provided. The number of specimens of each species involved to the analysis is indicated in round brackets next to the species name.

Overall, eight haplotypes were recorded in *C. lucifluella* based on the analysis of the limited sampling set (38 specimens) (Fig. 8). Four haplotypes (H1, H3, H4, H5) were recorded exclusively in the invaded range (Europe, Russia, Abkhazia), three haplotypes (H2, H6, H7) were documented in the native range (USA, Canada), and only one haplotype (H8) was shared between native and invaded ranges (USA and

Hungary) (Fig. 8). So far, the haplotypes revealed in Russia (Sochi) (H5) and Abkhazia (H4) were not detected in the species' native range. No geographic structure was revealed in the haplotype network. A low frequency polymorphisms, indicating population expansion, is supported by a negative Tajima's D value (-0.75) at $p = 0.48$.
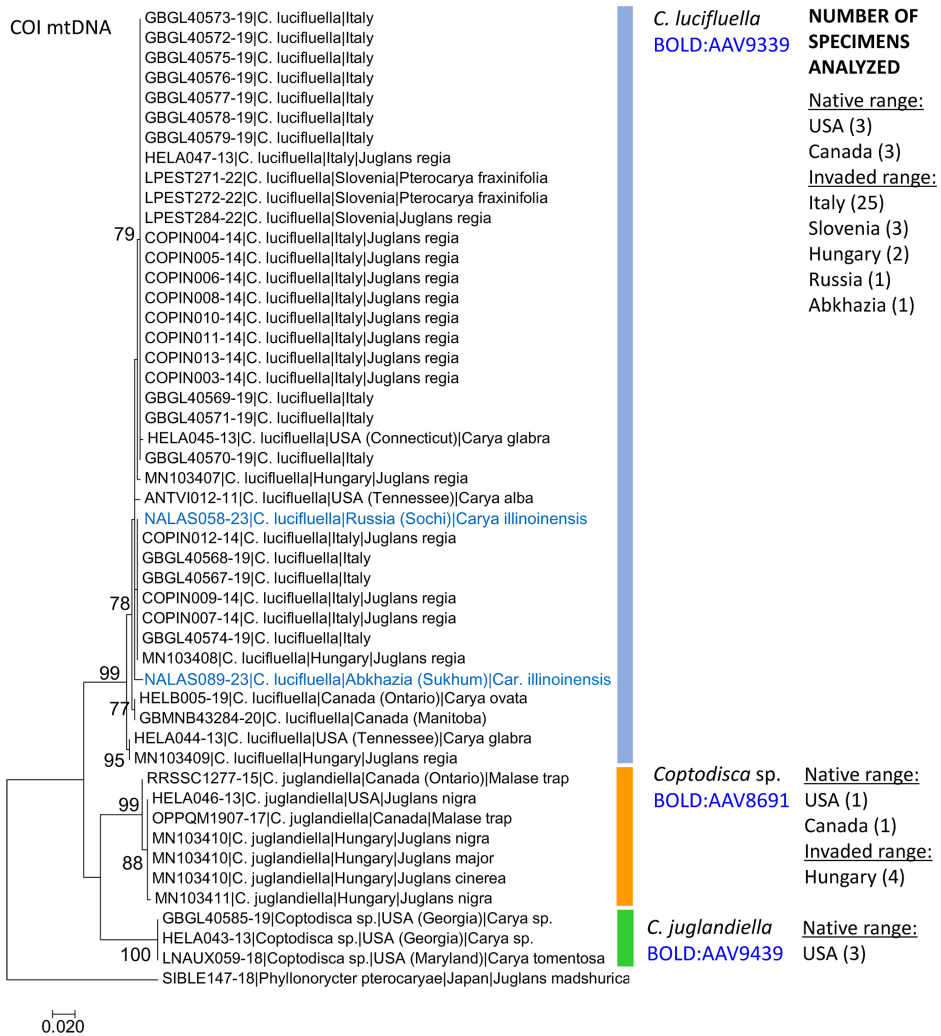
Figure 7. Maximum likelihood COI tree showing the relation of *C. lucifluella* with other *Coptodisca* spp. in the Holarctic. The clusters highlighted by different colors (blue, orange and green) correspond to different BINs (indicted next to each cluster), followed by the number of specimens examined and their origin. In the tree, the specimens are indicated by the BOLD process ID (begins with GBGL etc.), species name, country, locality (for North America, Russia and Abkhazia). Bootstrap values > 70 are shown next to the corresponding clusters.
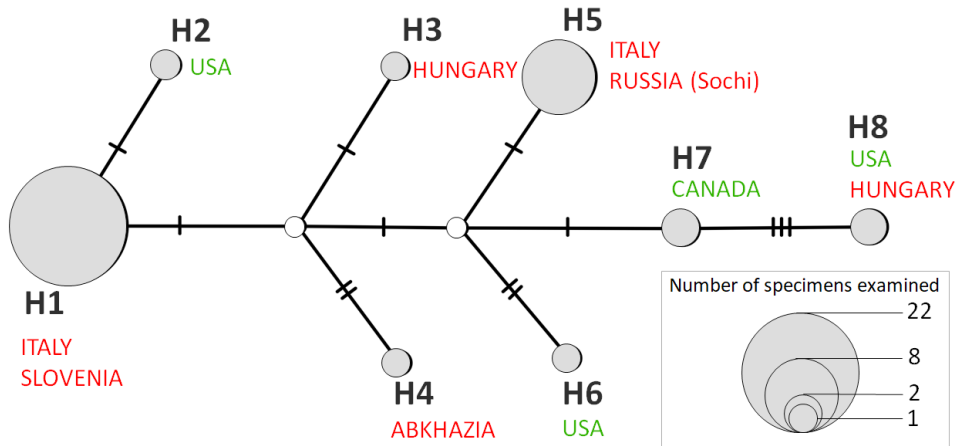
Figure 8. The haplotype network of *C. lucifluella* in the Nearctic. The circles correspond to the haplotypes (H1-H8). The circle size represents the number of specimens examined (indicated in the legend). Next to each circle, the countries are listed where certain haplotype was documented: the countries where the species is native are provided in green colour and where it is invasive in red. The short strokes on the connection lines and two white circles represent hypothetical haplotypes, not observed in the study. The haplotypes are linked with a 95% confidence level.

Discussion

The transcontinental invasion and further progressive spread of the North American *C. lucifluella* on the European continent (Fig. 9) suggests that the moth can become widespread in Eurasian regions where walnuts (*Juglans* spp.), hickory (*Carya* spp.) and wingnuts (*Pterocarya* spp.) are cultivated. As we showed, the moth has already penetrated Southern Russia and neighboring Abkhazia notably attacking leaves of the North American pecan, *Car. illinoinensis*, and to a less extend other Juglandaceae, *Car. tomentosa*, *P. fraxinifolia*, *J. regia* and *J. nigra*. Furthermore, we highlighted that the moth also occurred in Crimea on *J. regia*.

The introduction routes of the moth from the North American to the European continent remain unexplored. Bearing in mind that *C. lucifluella* hibernates outside the leaf mines, i.e., as the late-stage larva in the shields hidden in bark crevices and other discrete places, the shields could be translocated with plant material. Also, the adults of *C. lucifluella* can be hidden in cavities and unintentionally transported by humans. Indeed, leafminers are great travelers and their invasions are often associated with human activity (Gilbet et al. 2004; Kirichenko et al. 2019). Furthermore, tiny moths can also spread by wind currents (Ovsyannikova and Grichanov 2009; Kirichenko et al. 2019). Within Europe, the moth could spread by these or other

means through so-called bridgehead effect (Bertelsmeier and Keller 2018). This phenomenon explains the distribution of the invasive through intermediate harbors (Lombaert et al. 2010; Kirichenko et al. 2022, 2023b, 2023c).

We believe that the moth arrived to Southern Russia not directly from North America but from Europe through bridgehead effect. Furthermore, we suspect that Italy served a donor region of *C. lucifluella* for Sochi. Prior hosting the XXII Olympic Winter Games in 2014, intense landscaping programs were realized in Sochi (Kelina et al. 2016; Klemeshova et al. 2019; Karpun 2019). For that, many ornamental plants were imported from Italian nurseries, which serve as global commerce hubs (Volkovitsh and Karpun 2017; Karpun 2019). Due to multiple introductions of ornamental plants Sochi unintentionally received many alien tree pests, for instance, *Cydalima perspectalis* (Walker, 1859), *Paysandisia archon* (Burmeister, 1880), *Rhynchophorus ferrugineus* (Olivier, 1791), *Lamprodila festiva* (Linnaeus, 1767), *Pochazia shantungensis* (Chou & Lu, 1977), *Haritalodes derogata* (Fabricius, 1775), *Ceroplastes ceriferus* (Fabricius, 1798), *Stephanitis pyrioides* (Scott, 1874), *Glycaspis brimblecombei* Moore, 1964, and others (Karpun et al. 2014, 2022; Volkovitsh and Karpun 2017; Karpun 2019; Musolin et al. 2022; Zhuravleva et al. 2023a,b). Likely, *C. lucifluella* arrived to Sochi by the same way.

Despite we have no data available on the introduction of Juglandaceae plants from Italy to Sochi around the period of 2010-2014, we cannot exclude the fact that the moth could travel from Italy as "hitchhiker" with other plants and vehicles. The presence of the same haplotype in Italy and Sochi fuels this hypothesis. How the pest could distribute to Abkhazia remains puzzling for us. We may guess that vehicles circulating between Sochi and Abkhazia and the intensive year-around tourist traffic could facilitate further spread of *C. lucifluella*. Genetically, however, we cannot prove this suggestion, as the haplotype detected in Abkhazia differs from the common one in Italy. However, we sequenced only one individual from Abkhazia. Evidently, large sampling would provide more data for exploring the species invasion pattern.

If the insect arrived to Sochi around the year 2014, why it remained undiscovered for nearly a decade? In fact, the detection of newly arrived invasive species at low population density is a daunting task (Walsh et al. 2018). Indeed, miniature leaf-mining moths are hard to detect when their mines are scarce on the leaves. It explains why they remain overlooked until insects's populations begin growing (Mustățea and Chireceanu 2023). Bearing in mind early species record in Italy and about 10-year delay of the moth discovery in other countries (Fig. 9), one can speculate, that the moth may need about a decade to develop outbreaking populations. In Sochi and Abkhazia, we seemed to observe such a scenario. On the other hand, to Crimea, the moth likely arrive from Ukraine, where the species was for the first time documented in 2019 (Pályi et al. 2019).

We believe that in Sochi and Abkhazian regions, the moth develops at least three generations per year: the last generation start diapausing in October. Same could be the true for Crimea. Similarly, in European countries, the moth develops

several generations. For instance, in Italy, it provided from three to four generations (Bernardo et al. 2012, 2015), in Hungary, three generations were documented (Takács et al. 2020). Each generation contributes to the host plant damage, and late-season generations produce more mines than spring generations so that the most pronounced damage is accumulated by September–October (Takács et al. 2020). This is exactly what we documented in our late-season surveys performed in Sochi and on Abkhazian territory.

The parasitoids, which may switch to the moth from local leafminers in Europe, together with those that might arrive with its host from North America, could be a promising controlling factor of *C. lucifluella* in the invaded range. In Italy, up to 18% parasitism of *C. lucifluella* on common and black walnuts (*J. regia* and *J. nigra*) was documented (Bernardo et al. (2012). In our study, we recorded up to 35% parasitism in Sukhum (Abkhazia). The representatives of Eulophidae (Hymenoptera) seem to be the most common parasitoids of the moth in Europe, especially the representatives of the genera: *Cirrospilus* Westwood, 1832, *Chrysocharis* Förster, 1856, *Neochrysocharis* Kurdjumov, 1912, *Pnigalio* Schrank, 1802, and *Closterocerus* Hartig, 1847 (Bernardo et al. 2012). Most of parasitoid species, which were documented in European countries, remain undetermined. In our study, we collected only parasitoid exuvia, as the parasitoids emerged in nature prior we sampled the leaf mines.

Another related North American shield-bearer moth, *C. juglandiella*, which has already invaded Europe (Takács et al. 2020), attacks *Juglans* spp., as *C. lucifluella*. Both species make highly similar leaf mines, and it can be tricky to distinguish these two species reliably based on their damage. However, morphologically and genetically, these two species are well separable. The adults differ by forewing pattern and male genitalia (Takács et al. 2020). In COI gene, these two species are highly divergent (>8%) (present study).

C. juglandiella shows tendency to spread on the territory of Europe (Lepiforum 2024b). We expect that this species may also arrive (if has not done already) to Southern Russia and distribute further with *C. lusifluella* across Caucasus, Asian countries, and other parts of the world where the host plants are available. In Caucasus, West and Central Asia, *Juglans* is economically valuable orchard tree (Molnar et al. 2011; Shigaeva and Darr 2020). In Asia, native *Juglans*, *Carya* and *Pterocarya* species are found in China, Korea, the Russian Far East, Laos, Northern Vietnam, Thailand (Aradhya et al. 2007; Trees and shrubs… 2024). China holds the richest diversity on Juglandaceae in Asia: *Juglans ailantifolia*, *J. mandshurica*, *Carya cathayensis* Sarg., *Car. hunanensis* W.C. Cheng & R.H. Chang, *Car. kweichowensis* Kuang & A.M. Lu, *Car. poilanei* (A. Chev.) Leroy, *Car. tonkinensis* Lecomte, *Pterocarya hupehensis* Scan., *P. macroptera* Batalin, *P. rhoifolia* Siebold & Zucc., *P. stenoptera* C. DC., *P. tonkinensis* (Franch.) Dode etc. (Aradhya et al. 2007; Trees and shrubs… 2024). It seems that host shifting is possible for *Coptodisca*. In North America *C. lucifluella* was known only on *Carya*, whereas in Europe it has been also recoded on *Pterocarya* and *Juglans* spp. (Bernardo et al. 2011; Takács et al. 2017) Thus, if the moths continue range expansion eastwards, host shifting to the Asian Juglandaceae species may not be ruled out, at least in case of *C. lucifluella*.
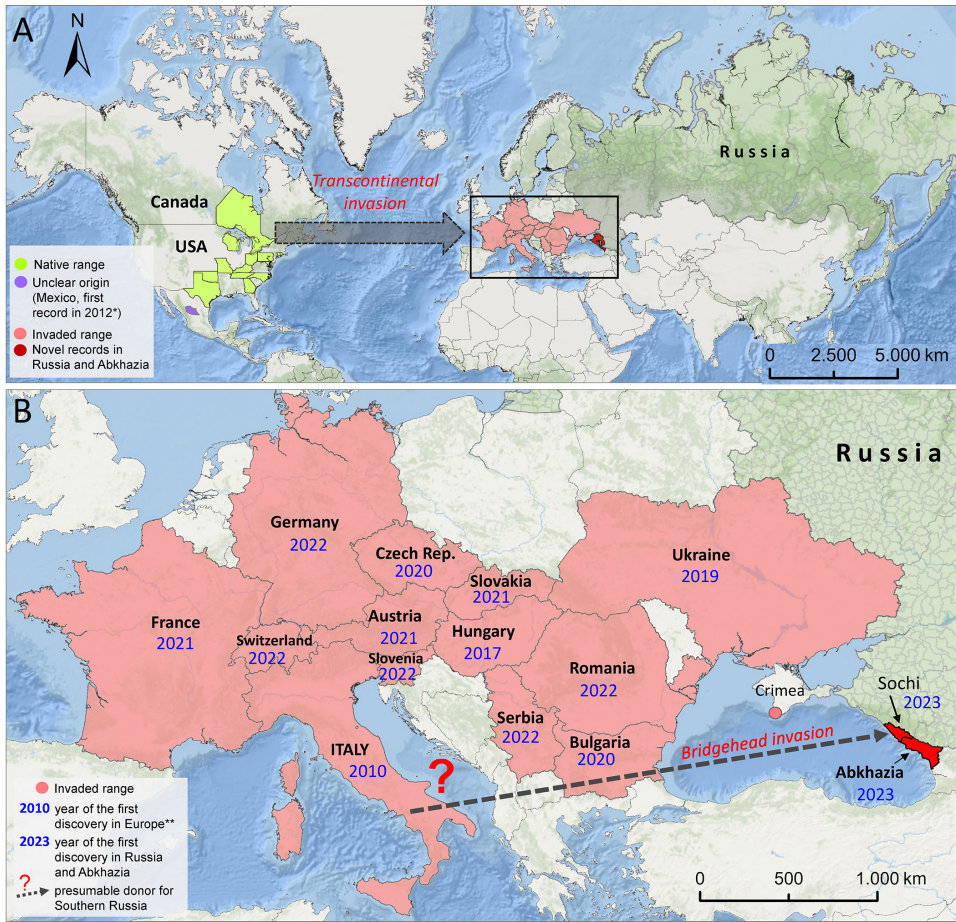
Figure 9. The present range of *C. lucifluella*. The native and invaded areas in the Holarctic (**A**); the detection years in Europe and beyond and possible introduction route to Sochi (Russia). In the legends: *(Ávila-Rodríguez et al. 2015), **(Bernardo et al. 2012, 2015).

Conclusions

Our study demonstrates another curious example of an ongoing invasion of the North American leaf-mining moth on the European continent and highlights its potential to occupy large territory in Eurasia, where the potential host plants, i.e., *Juglans*, *Carya* and *Pterocarya* species, are present. Further studies would be needed to clarify if *C. lucifluella* can shift on Asian Juglandaceae plants to predict its further spread eastwards. More molecular genetic data would be needed to reconstruct the modern species phylogeography and explore the pattern of the moth invasion. The last but not the least, a deeper study of parasitoid assemblages and parasitism rate in the invaded range would be essential to understand whether or not the natural factors will play a noticeable role in controlling the invasive moth.

Acknowledgements

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