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Use of an Arboretum and DNA Barcoding for the Detection and Identification of Leaf-Mining Insects on Alien Woody Plants

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Abstract: Arboreta serve as effective tools for identifying alien insect pests and novel trophic associations. In this study, we used an arboretum in Slovenia to survey woody plants and identify both alien and native leaf miners. The leaves and twigs of 50 woody plant species and their cultivars were examined for characteristic damage. We used an integrative approach that combined identification based on leaf mines and DNA barcoding of the larvae and pupae found in the mines. In total, 62 leaf-mining species were identified, including eight alien species, of which the heliozelid *Coptodisca lucifluella* (Clemens, 1860) and the agromyzid *Cerodontha unisetiorbita* Zlobin, 1992 were documented for Slovenia for the first time. Additionally, three presumably native Gracillariidae moths *Phyllocnistis labyrinthella* (Bjerkander, 1790), *P. ramulicola* Langmaid & Corley, 2007 and *P. saligna* (Zeller, 1839) represented the first record for Slovenia. Furthermore, we documented 23 novel-to-science trophic associations, 20 of which involved native insects and alien woody plants, primarily from Asia. This study highlights the importance of arboreta and botanical gardens for the interception of invasive alien insects and the early detection of trophic shifts of native insects to alien plants, which can aid in predicting their potential spread.

Keywords: botanical garden; sentinels; leaf miners; alien species; non-native trees; novel trophic associations; DNA barcoding; Slovenia

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1. Introduction

Invasions of herbivorous organisms, particularly tree pests, are closely associated with anthropogenic activities and ongoing global environmental changes [1,2]. A growing number of invasive alien species pose a threat to biodiversity, ecosystem functioning, and regional economies [3]. The early detection and identification of emerging threats are essential for effective plant and ecosystem protection [4].

Botanical gardens and arboreta play an invaluable scientific and educational role in the conservation of plant diversity [5–7]. They are often located in urban areas and are significantly impacted by ongoing human activities such as the regular introduction and replacement of plants and disturbance by public visitors, and can thus be a target for invasive alien organisms that may be introduced unintentionally [8,9]. At the same time, botanical gardens and arboreta can serve as both recipients and distributors of pests and pathogens as their ranges expand [8,10]. On the other hand, these man-made plantings harboring a variety of alien and native plants can provide valuable opportunities for studying the trophic shifts of native pests and pathogens to alien plants and for identifying the biosecurity risks that these organisms may pose if they spread beyond their native range [11,12]. Therefore, arboreta and botanical gardens are often viewed as sentinel plantings and can be used for a variety of purposes in plant health issues [11,13,14].

Several studies have demonstrated the potential use of arboreta and botanical gardens as tools for identifying potentially damaging insects and pathogens on woody plants [11–13,15–17]. These studies have also described novel trophic interactions or potential host shifts in mixed plantings of phylogenetically related native and alien tree species [15,18,19]. In such mixed plantings, insects may move between phylogenetically related native and non-native plants [16]. Since many herbivore insects are trophically specialized and knowledge of their host plants is important for taxonomic identification [20–22], these inventories can help identify emerging pests on new hosts.

In immature stages where morphological identification is difficult or impossible, modern tools such as DNA barcoding can be used for reliable identification [20,21]. DNA barcoding is a powerful tool that allows rapid and accurate identification of insect species [23,24]. It has been shown to be important for detecting invasive alien species intercepted at ports of entry, found feeding on imported plants, and in man-made plantings. Its value is indisputable in identifying arthropod pests and pathogens in sentinel plantings, including botanical gardens and arboreta [13,19,21].

Leaf miners are a relatively large ecological group of insects, comprising the representatives of four orders (Lepidoptera, Coleoptera, Hymenoptera, and Diptera), whose larvae live and feed in cavities (mines) created in leaf tissues. The vast majority of these insects cause damage to leaves, while a few species can also mine the petioles, young twigs, flower petals, fruits, and seed boxes of plants. They are present in the mines throughout the entire larval stage or just at the beginning, after which they continue to feed as external defoliators [25,26]. The shape and other characteristics of the leaf mines and their location on the leaves/twigs provide important diagnostic information that can help identify the insect taxa, at least to the genus or family level [22,25]. These features, as well as the trophic specialization of these insects, make leaf miners an excellent model group for various ecological studies [16]. Furthermore, many alien species are known to attack woody plants and crops worldwide [27].

The aim of this study was to investigate the potential of arboreta for the detection and identification of native and alien leaf miners on woody plants. Using an integrative approach combining data on host plants, morphological analysis of the leaf mines, and DNA barcoding of the insects sealed in the mines, we explored the diversity of leaf miners and identified new leaf-mining species for Slovenia, including alien insect species, in the largest arboretum in the country. Furthermore, we identified new trophic interactions between native leaf miners and alien woody plants. To the best of our knowledge, this is the first study using an arboretum as a platform for investigating the diversity of leaf-mining insects and their trophic associations with alien woody plants in Southern Europe.

2. Materials and Methods

2.1. Surveyed Arboretum

The study was conducted at the Volčji Potok Arboretum in Slovenia (46.1873 N; 14.6113 E; 345 m a.s.l.) during the periods from 6 to 9 August 2022 and from 5 to 8 January 2023. The arboretum is located in a subalpine region 20 km northeast of Ljubljana and is the oldest botanical garden in Slovenia (Figure 1). Established in 1882 by the landowner Ferdinand Souvan I, it became a public institution in 1952 and presently covers an area of 85 ha. The arboretum holds the largest collection of alien and native woody plants in Slovenia, with about 2500 species and cultivars of deciduous and coniferous plants and shrubs originating mostly from Europe, Asia, and North America [28].

Figure 1. Geographical situation of the Volčji Potok Arboretum in Slovenia (**A**,**B**); (**C**) one of examined woody plant collections. Photo: S. Gomboc. The maps were produced using ArcGIS Pro software and country layers [29–31].

The study area has a continental climate, with an annual average temperature of +8.5 °C (1961–1990) and annual average precipitation of 1396 mm [32]. The study year was warmer than previous years in Slovenia [32]. The average temperature in August was +21.2 °C (vs. the long-term average of +18 °C in 1961–1990), with a min of +10.8 °C and a max of +35 °C. Precipitation was 35.2 mm (vs. the long-term average of 142 mm). The average January temperature was +3.8 °C (vs. the long-term average of −2.3 °C), with a min of −0.6 °C and a max +11.8 °C. Precipitation was 134.8 mm (vs. the long-term average of 77 mm). In the arboretum, woody plants and shrubs grow naturally, and insecticides are rarely used; no insecticides were applied to the surveyed plots during the study year, except for a planned treatment of *Buxus* spp. against the box tree moth, *Cydalima perspectalis* (Lepidoptera: Crambidae) (Walker, 1859) [33].

The map of Slovenia and the enlargement of the studied arboretum were produced using the WGS 1984 coordinate system and ESRI ArcGIS Pro 3.0 software [29]. Layers of the Surveying and Mapping Authority of the Republic of Slovenia and Esri ArcGIS Online, available in the form of geographic web services, were used [30,31].

2.2. Woody Plants

We surveyed a total of 50 species and cultivars (forms, hybrids) of woody plants from 14 families (Adoxaceae, Anacardiaceae, Betulaceae, Cannabaceae, Ericaceae, Fabaceae, Fagaceae, Juglandaceae, Malvaceae, Oleaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae) and 10 orders (Dipsacales, Ericales, Fabales, Fagales, Lamiales, Malpighiales, Malvales, Proteales, Rosales, and Sapindales). In addition, one non-woody plant, the bamboo *Phyllostachys viridiglaucescens* (Bambusoideae, Poaceae), was also included in the analysis. A list of the plants surveyed, their origin, and native range as per various sources [34,35] can be found in Table S1. The majority of the examined plants (29 species and their cultivars, i.e., 58%) originated from Asia (16 species and cultivars), while a smaller number came from North America (9) and Eurasia (4). Furthermore, 42% of the plants surveyed were native to Slovenia (21 species and cultivars). In the study, 19 plant species and cultivars were each represented by one individual tree or shrub, while 31 species and cultivars were represented by 2–20 individual plants (Table S1).

2.3. Leaf Miner Sampling

The leaves, leaf petioles, and young shoots of plants were checked for damage caused by leaf-mining insects, i.e., mines of various shapes. The survey was conducted in early August 2022, when the majority of the mines could be found on leaves with larvae of the second generation or abandoned mines from the first generation (some still with the remnants of insects found inside leaf mines, i.e., larval molting exuvia, dead/parasitized larvae and/or pupae, which could still be used for identification). Occasional observations were also done in early January 2023 to detect leaf miners that develop later in the season on evergreen trees and shrubs.

The survey was performed on a minimum of six low branches (up to 2 m from the ground) around the tree crown. A random number of 100–500 leaves were examined on the branches, including the lower and upper sides of leaves and entire petioles and young shoots. The relative damage level was estimated as the number of mines per 100 leaves and expressed as a percentage. Four damage levels were taken into account: 1—low (1%–24% of leaves with mines per 100 leaves), 2—medium (25%–49%), 3—high (50%–75%), and 4—severe (>75%). The leaves with mines were collected for further examination under magnification and insect taxonomic identification.

2.4. Leaf Miner Identification

The insect species were identified based on their characteristic leaf mines [22,36]. The following features were taken into account: the shape of the mine, its position on the leaf/petiole/shoot, frass pattern, the presence of an exit hole, the characteristics of larval and/or pupal exuviae or dead larvae and pupae found in the mines, and trophic association (i.e., data on host plant species or genus). Leaf mines were examined under magnification using a binocular Olympus SZ51 stereomicroscope (Tokyo, Japan, Olympus Corporation). Leaf mines were dissected using a sterile needle, and insect specimens were then examined. The photographs of the leaf mines were taken through the stereomicroscope using a Xiaomi 11 Lite smartphone digital camera (Beijing, China, Xiaomi Corporation). The photographs of damaged leaves in the arboretum were taken with a Sony Alpha 7II camera (Tokyo, Japan, Sony Corporation).

All leaves with mines collected during the surveys (ca. 1000 specimens) were herbarized (including those that were dissected) and stored in the collection of the first author at the Sukachev Institute of Forest SB RAS (Krasnoyarsk, Russia).

The leaf-mining species identified in the study were cross-checked with early records in Slovenia using various literature sources [37,38]. Based on this cross-checking, a decision was made on the novelty of the leaf-mining species for the country. Further records of the novel to Slovenia leaf-mining species documented in the arboretum and found beyond the arboretum by our team were provided as additional material for insect species essays. The alien species found for the first time in Slovenia were communicated to the national NPPO.

2.5. DNA Barcoding

The insects requiring confirmation of their species and those detected for the first time in the country were barcoded with DNA. The mitochondrial cytochrome oxidase I gene (mtDNA COI, 658 bp) was sequenced in 13 larvae and pupae sampled from leaf mines in the arboretum. Additionally, three specimens of *Phyllocnistis saligna* (Zeller, 1839) and one of *Coptodisca lucifluella* collected from the mines on *Salix alba* and *Juglans regia* outside the arboretum in Slovenia (i.e., 46 km to the west and 66 km south of the arboretum, respectively), representing new records for the country, were DNA barcoded. Their typical mines were also collected in the arboretum, but they did not contain larvae or pupae for the genetic analysis (in the case of *P. saligna*) or were sampled on a different host (*J. regia* in the case of *C. lucifluella*, whereas in the arboretum it was collected solely on *Pterocarya fraxinifolia*).

DNA barcoding was performed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) following the standard protocol [39]. Species identification was done using the identification engine in BOLD, Barcode of Life Data System, https://www.boldsystems.org/ (accessed on 3 January 2023). In all cases, the nearest neighbors were identified, and Barcode Index Numbers (BINs), i.e., the species code in BOLD [40], were retrieved. Twenty-six sequences of 10 leaf-mining species, *P. ramulicola* (5), *P. labyrinthella* (5), *P. saligna* (3), *P. unipunctella* (4), *P. valentinensis* (2), *P. xenia* (2), *Cameraria ohridella* (1), *Phyllonorycter issikii* (1), *Ph. distentella* (1), *Ph. pastorella* (1), and *Captodisca lucifluella* (1), freely available in BOLD, were borrowed for the joint analysis. Additionally, two sequences of *Chrysocharis* sp. from Italy were used for comparison with that of the parasitoid collected from *Cerodontha unisetiorbita* in the Slovenian arboretum. The specimen data involved in the study are provided in Table S3. The original DNA sequence, along with the voucher data, image files, and trace files, as well as the sequences included in the study and their BIN and GenBank accession numbers, can be accessed at https://dx.doi.org/10.5883/DS-ARBOLM (accessed on 5 February 2023). The sequences were aligned using BioEdit 7.2.5 [41]. A maximum likelihood (ML) tree was constructed in MEGA X [42] using the maximum likelihood method, the Kimura two-parameter model, and the bootstrap method (2000 iterations); intra- and interspecific genetic distances were assessed using the same approaches.

2.6. Analysis of Trophic Associations

All trophic associations documented in the Slovenian arboretum were analyzed against known data on host plants of leaf miners using literature sources and electronic databases in order to define novel trophic associations [22,37,43].

All cases of infestation by leaf-mining insects in the arboretum are listed in Table S2. An infestation case refers to a documented incidence of a leaf-mining species attacking an individual plant. For all these cases, leaf miner attacks were documented as individual infestation incidences and thus provided per individual plant examined (Table S2).

Data on trophic associations documented in the botanical garden were used for analyzing the "host plant—leaf miner" network. In these analyses, the cultivars of the same plant species were treated as one plant species, resulting in the analysis of 42 woody plant species in the trophic network. The following indices were calculated: nestedness, web asymmetry, specialization, links per species, and number of compartments. A detailed description of the metrics can be found in [44]. The trophic network with plant species at the lower level and insect species at the higher level was constructed using R statistical software (2021, version) [45]. The indices were calculated with the R package "bipartite" [46] for host plants at both the species and genera levels.

3. Results

3.1. Leaf Miner Diversity on Woody Plants in the Arboretum

Using an integrative approach, a total of 62 leaf-mining insect species were identified on woody plants in the arboretum (Figure 2, Table S2). The majority of the insects, i.e., 56 out of 62 species (90%), were represented by Lepidoptera, followed by Coleoptera (three species, 5%), Diptera (two species, 3%), and Hymenoptera (one species, 2%) (Figure 2).

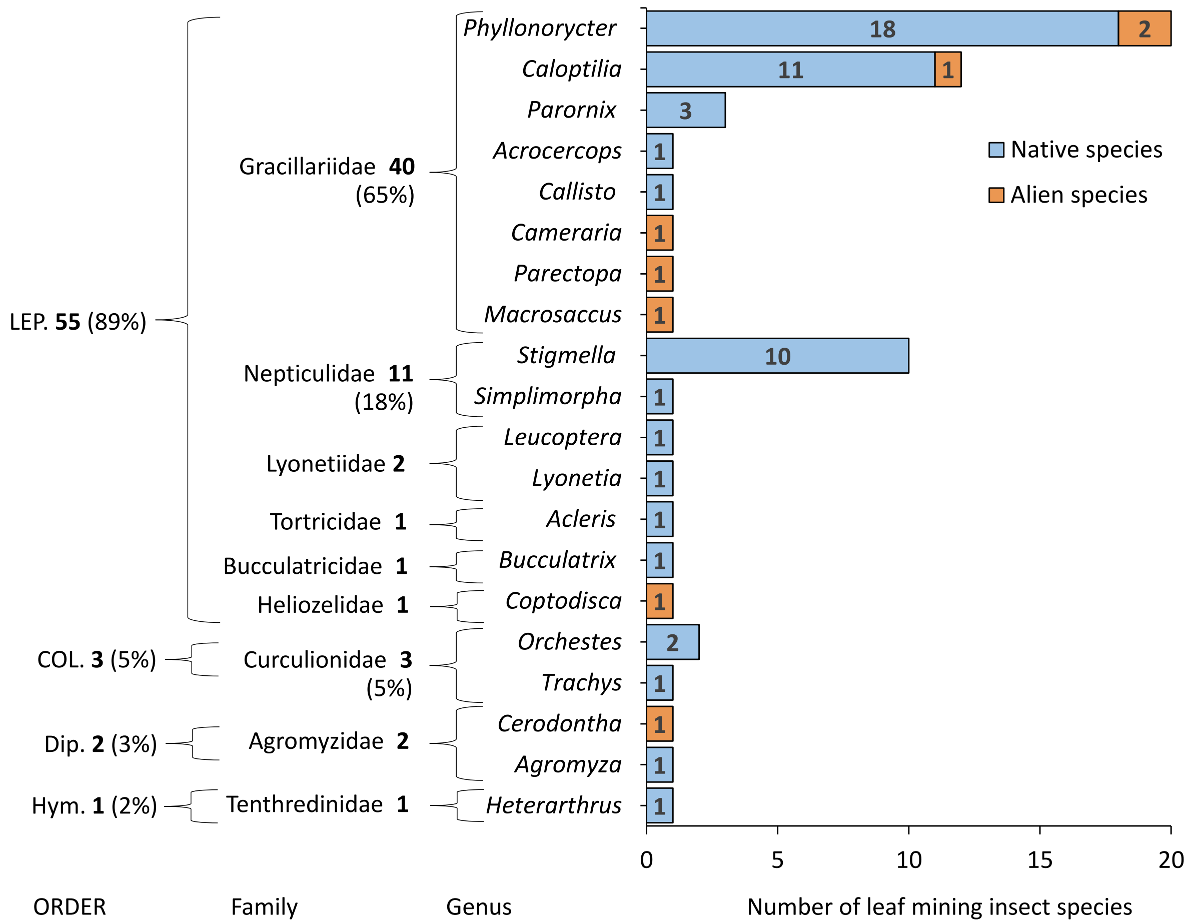

Figure 2. Number of leaf-mining insect species from various genera, families, and orders recorded in the Volčji Potok Arboretum (Slovenia) in the period 2022–2023. Orders: Lep.—Lepidoptera, Col.—Coleoptera, Hym.—Hymenoptera, Dip.—Diptera. The number of species in families and orders is given in bold in the figure.

Among Lepidoptera, the Gracillariidae were the most diverse group in the arboretum. A total of 40 gracillariid species (65% of all recorded leaf miners) were documented, with *Phyllonorycter* and *Caloptilia* species being the most dominant (20 and 12 species, respectively), followed by Nepticulidae (11 species, 18%) (Figure 2). Four different *Caloptilia* from *Acer* spp., one *Phyllonorycter* from *Malus* spp., one *Stigmella* from *Aesculus glabra* var. *arguta*, and one *Bucculatrix* from *Quercus* spp. were impossible to identify to the species level. The remaining four moth families were represented by 1–2 species only (Figure 2). Three beetle species were identified: two from the *Orchestes* genus and one from the *Trachys* genus. Leaf-mining flies were represented by one *Cerodontha* and one *Agromyza* species. Only one sawfly species was noted (Figure 2).

A total of eight alien leaf miners were detected in the arboretum: seven species of moths and one species of leaf-mining fly (Figure 2). Six alien gracillariid species were previously known in Slovenia: *Caloptilia azaleella* (Brants, 1913), *Cameraria ohridella* (Deschka and Dimić, 1986), *Phyllonorycter issikii* (Kumata, 1963), *Ph. platani* (Staudinger, 1870), *Macrosaccus robiniella* (Clemens, 1859), and *Parectopa robiniella* (Clemens, 1863). The leaf-mining moth, *Coptodisca lucifluella* (Lepidoptera: Heliozelidae), and a fly, *Cerodontha unisetiorbita* (Diptera: Agromyzidae), are new alien species for the country (see Section 3.5).

It should be noted that the list of leaf miners identified during the course of this study in the Slovenian arboretum (provided in Table S2) is not exhaustive, as the goal was not to cover all woody plants and all leaf miner species on those plants in the arboretum.

3.2. DNA Barcoding Data

In total, 12 DNA barcodes were generated for leaf-mining specimens collected in the arboretum, which enabled the identification of nine species (Figure 3). The majority of focus was given to *Phyllocnistis* spp., as the representatives of this genus are challenging to identify morphologically and, to some extent, genetically.

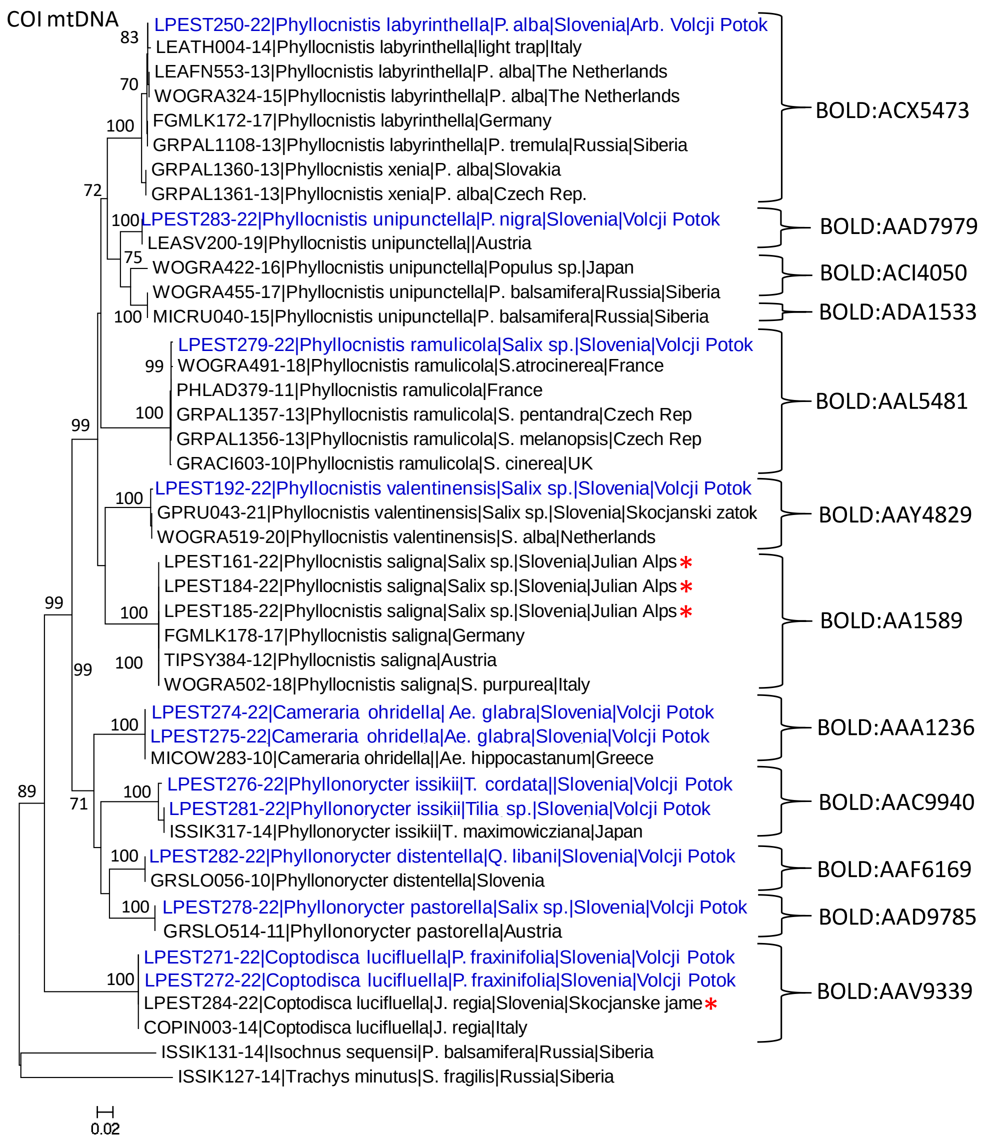

Figure 3. The maximum likelihood tree illustrating the relationship between the leaf-mining moths sampled in the Volčji Potok Arboretum, Slovenia (specimens indicated in blue), and those from other parts of Europe. Species BINs are indicated next to the species clusters on the right. Each specimen is identified by its process ID, leaf miner species name, host plants (genera: *Ae.—Aesculus*, *J.—Juglans*, *P.—Populus*, *P.—Pterocarya*, *Q.—Quercus*, *S.—Salix*, *T.—Tilia*), and country. Additional specimens from Slovenia collected during the course of the study are indicated by a red asterisk (*). Bootstrap values > 70% are shown next to the branches.

The Slovenian specimen identified genetically as *Phyllocnistis labyrinthella* (Bjerkander, 1790) showed a 0.46% maximum divergence when compared against representatives of the species from other European countries (Italy, Germany, the Netherlands), and Russia (Siberia) (Figure 3, Table 1). The Slovenian specimen and those from the Netherlands were reared from mines on *Populus alba*, the Siberian specimen was reared from *Populus tremula*, and the host plants of the others were unknown. When compared against *Phyllocnistis xenia* M. Hering, 1936, from Europe (Slovakia and the Czech Republic), the minimum pairwise distance in the complex *P. labyrinthella—P. xenia* increased to 1.08% (Table 1). Notably, the representatives of *P. labyrinthella* and *P. xeni* formed a single cluster on the phylogenetic tree (Figure 3). Moreover, these two species share the same BIN in BOLD (Figure 3).

Table 1. Intra- and interspecific genetic divergences in DNA barcodes between *Phyllocnistis* species documented in the Volčji Potok Arboretum (Slovenia) assessed based on Kimura 2-parameter (K2P) distances (%). For each species pair, the minimum pairwise distance is provided; values in square brackets show maximum intraspecific distances.

No.	Species [1]	Species					
		P. labyrinthella	***P. xenia****	***P. unipunctella***	***P. valentinensis***	***P. saligna***	***P. ramulicola***
1	*Phyllocnistis labyrinthella*	[0.46]					
2	*Phyllocnistis xenia* *	1.08	[0]				
3	*Phyllocnistis unipunctella*	8.21	7.85	[5.81]			
4	*Phyllocnistis valentinensis*	11.19	11.19	11.47	[0.31]		
5	*Phyllocnistis saligna*	12.09	12.27	12.09	10.84	[0]	
6	*Phyllocnistis ramulicola*	12.63	12.82	12.42	14.87	13.57	[0.31]

[1] Species involved in the analyses were represented by the following number of replications: *Phyllocnistis ramulicola* (7), *P. saligna* (6), *P. labyrinthella* (5), *P. unipunctella* (5), *P. xenia* (4), and *P. valentinensis* (3). * *Phyllocnistis xenia* was not sampled in the arboretum, but its DNA barcodes were retrieved from BOLD and used for comparative analysis.

The Slovenian specimen identified as *Phyllocnistis unipunctella* (Stephens, 1834) displayed the greatest genetic proximity to the specimen from Austria (100% identity) in BOLD (Figure 3). However, when compared against other representatives of the genus from Asia, particularly from Siberia and Japan, high intraspecific divergence reaching 5.81% was detected (Table 1), suggesting the presence of a species complex across Eurasia. The specimens of *Phyllocnistis valentinensis* M. Hering, 1936, and *P. ramulicola* from Slovenia showed high similarity to the species representatives from other parts of Europe, with only 0.31% intraspecific divergence (Table 1).

A specimen of *Phyllocnistis saligna* (Zeller, 1839) from the Slovenian arboretum was not sequenced, but instead, three additional specimens of this species collected at about the same time in Slovenia (but outside the arboretum, 46 km west) were analyzed. Their sequences were identical to those from Germany, Austria, and Italy (Figure 3, Table 1).

The minimum interspecific divergence (excluding the *P. labyrinthella—P. xenia* species pair mentioned above) varied from 7.79% in the species pair *P. unipunctella—P. xenia* to 14.78% in *P. ramulicola—P. valentinensis* (Table 1).

No issues were encountered in identifying other leaf-mining species through DNA barcoding. The representatives of *Cameraria ohridella* and *Phyllonorycter distentella* (Zeller, 1846) sampled in the arboretum were identical to the sequences obtained from BOLD from Slovenia (previously collected by the second author and confirmed by adult morphology) and Austria (Figure 3). The specimens of *Phyllocnistis issikii* from the arboretum, when compared to the representative of the species from Japan (native species range), showed 0.90% genetic divergence (Figure 3). Two larvae of *Captodisca lucifluella* from the arboretum, dissected from leaf mines on *Pterocarya fraxinifolia*, and one larva sampled outside the arboretum on *Juglans regia* were genetically identical to a specimen from Italy (Figure 3).

The DNA barcode of a specimen of parasitoid that emerged from the pupa of *Cerodontha unisetiorbita*, sampled from a fly mine on the bamboo *Phyllostachys viridiglaucescens* in the arboretum (not shown in Figure 3), was identified through DNA barcoding as *Chrysocharis* sp. (process ID LPEST280-22). Two specimens from Italy (GenBank accessions: KY973634 and KY973633) determined to the genus level only were the nearest neighbors in BOLD to the Slovenian specimen (1.1% divergence) (Figure S1).

3.3. Leaf Miner Damage

The majority of native and alien leaf-mining insects caused insignificant damage to their host plants in the arboretum (Figure 4; Table S2).

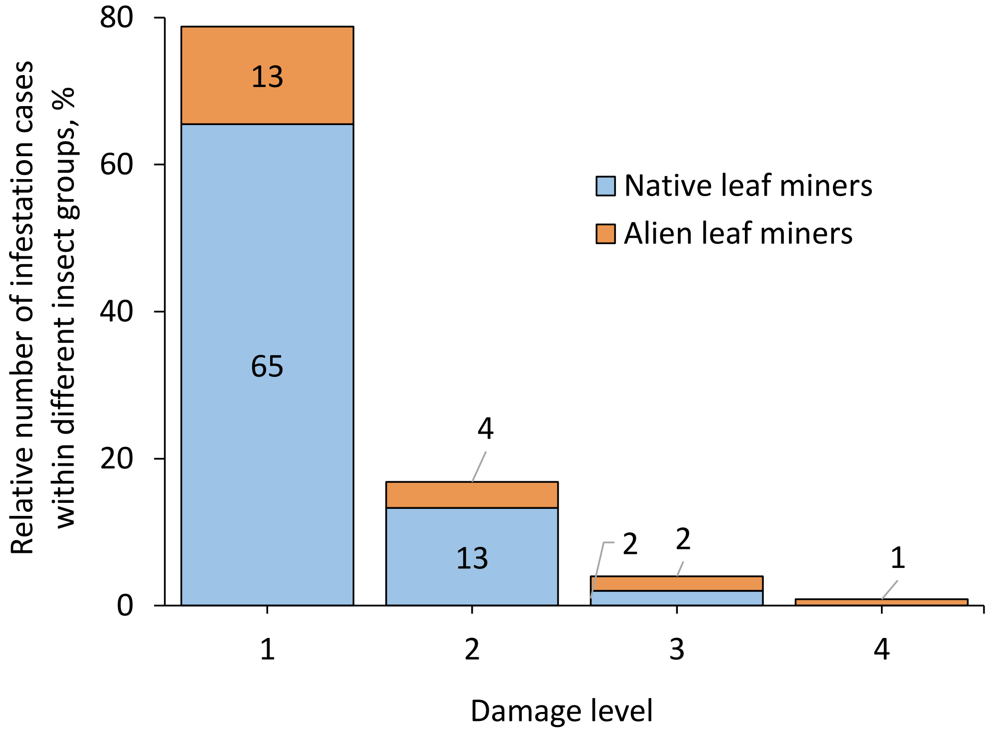

Figure 4. Relative number of infestation cases (%) among native and alien leaf-mining insects based on damage level: low (1%–24% of leaves with mines per 100 leaves), 2—medium (25%–49%), 3—high (50%–75%), and 4—severe (>75%).

Overall, a low damage level was recorded in 78% of infestation cases, in which the relative number of infestations by native leaf miners was five times higher than that of alien leaf miners (Figure 4). In another 22% of infestation cases, medium-to-severe damage levels were recorded (Figure 4). In all of these cases, both native and alien leaf miners were involved (Table S2). In cases of medium damage, attacks by native leaf miners were 3.3 times more frequent than those by alien leaf miners (Figure 4).

A high damage level was recorded in two cases in both native and alien leaf miners (Figure 4). Severe damage was recorded in only one case (Figure 4), i.e., for *Phyllorycter issikii* on European lime, *Tilia cordata* (Table S1).

3.4. Trophic Associations

A total of 113 infestation cases of leaf miners were recorded on woody plants in the arboretum (Table S2). Among them, 106 trophic associations were defined, while the remaining seven cases were repeated records on the same hosts but on different plant individuals. Twenty-three out of 106 trophic associations (22%) were novel records to science (Table 2). Of these twenty-three novel associations, twenty (87%) involved native leaf miners on alien plants from Asia (Table 2), while the remaining three (12.5%) involved plant species originating from North America (Table 2).

Table 2. Novel trophic associations between leaf-mining insects and woody plants based on records in the Volčji Potok Arboretum (Slovenia) in the period 2022–2023.

No.	Plant Species	Plant Origin [1]	Insect Family	Insect Species [2]	Damage Level [3]	Sampling Date
1	*Acer saccharinum*	NA	Gracillariidae	*Caloptilia* sp. 3	❶	9 August 2022
2	*Aesculus glabra var. arguta*	NA	Nepticulidae	*Stigmella* sp. 1	❶	6 August 2022
3	*Betula utilis*	EA	Nepticulidae	*Stigmella lapponica*	❶	9 August 2022
4	*Betula utilis*	EA	Gracillariidae	*Parornix betulae*	❶	9 August 2022
5	*Celtis julianae*	EA	Gracillariidae	*Phyllonorycter millierella*	❶	9 August 2022
6	*Celtis julianae*	EA	Gracillariidae	*Caloptilia fidella*	❶	9 August 2022
7	*Crataegus persimilis*	NA	Lyonetiidae	*Lyonetia clerkella*	❶	6 August 2022
8	*Malus × scheideckeri* 'Red Jade'	EA	Gracillariidae	*Callisto denticulella*	❶	6 August 2022
9	*Malus × scheideckeri* 'Red Jade'	EA	Lyonetiidae	*Leucoptera malifoliella*	❶	6 August 2022
10	*Malus × scheideckeri* 'Red Jade'	EA	Lyonetiidae	*Lyonetia clerkella*	❶	6 August 2022
11	*Malus × scheideckeri* 'Red Jade'	EA	Nepticulidae	*Stigmella incognitella*	❶	6 August 2022
12	*Malus floribunda*	EA	Lyonetiidae	*Lyonetia clerkella*	❶	6 August 2022
13	*Malus tschonoskii*	EA	Lyonetiidae	*Lyonetia clerkella*	❶	6 August 2022
14	*Phyllostachys viridiglaucescens*	EA	Agromyzidae	*Cerodontha unisetiorbita*	❶	9 August 2022
15	*Prunus incisa* 'February Pink'	EA	Lyonetiidae	*Lyonetia clerkella*	❶	6 August 2022
16	*Quercus libani*	WA	Gracillariidae	*Phyllonorycter distentella*	❶	9 August 2022
17	*Quercus libani*	WA	Gracillariidae	*Caloptilia alchimiella*	❶	9 August 2022
18	*Quercus libani*	WA	Gracillariidae	*Acrocercops brongniardella*	❶	9 August 2022
19	*Salix acutifolia*	WA	Gracillariidae	*Phyllocnistis valentinensis*	❶	9 August 2022
20	*Salix acutifolia*	WA	Gracillariidae	*Phyllocnistis ramulicola*	❷	9 August 2022
21	*Salix acutifolia*	WA	Gracillariidae	*Phyllonorycter pastorella*	❶	9 August 2022
22	*Salix magnifica*	EA	Gracillariidae	*Phyllonorycter salicicolella*	❶	6 August 2022
23	*Viburnum rhytidophyllum*	EA	Tortricidae	*Acleris schalleriana*	❶	9 August 2022

[1] Plant origin: NA—North America, EA—East Asia, WA—West Asia; [2] Insect species: all species are native to Slovenia; species and origin of *Caloptilia* sp. 3 and *Stigmella* sp. remain unclear; [3] Damage level: ❶—low (1%–24% of leaves with mines per 100 leaves), ❷—medium (25%–49%).

The majority of novel trophic associations were formed by lepidopteran leaf miners, among which 12 cases were represented by Gracillariidae, with the involvement of

11 species: *Acrocercops brongniardella, Callisto denticulella, Caloptilia alchimiella, Caloptilia fidella, Parornix betulae, Phyllocnistis ramulicola, Phyllocnistis valentinensis, Phyllonorycter distentella, Phyllonorycter millierella, Phyllonorycter pastorella*, and *Phyllonorycter salicicolella*. Six cases were represented by Lyonetiidae (two species: *Lyonetia clerkella* and *Leucoptera malifoliella*), three cases by Nepticulidae (three species: *Stigmella* sp. 1, *Stigmella lapponica,* and *Stigmella incognitella*), and one case by Tortricidae (*Acleris schalleriana*) (Table 2). A leaf-mining fly was recorded for the first time on *Phyllostachys viridiglaucescens* from China, making it a new alien species for Slovenia. Two leaf miners sampled on North American trees remain unidentified: *Stigmella* on *Aesculus glabra* and *Caloptilia* sp. 3 on *Acer saccharinum* (Table 2).

In the novel associations, the damage caused to trees by native insects was low, with only 1%–24% of leaves with mines per 100 leaves (Table 2). The exception to this was the association between *Phyllocnistis ramulicola*, a newly-recorded species for Slovenia, and Asian willow, *Salix acutifolia*, which had moderate leaf damage (25%–49%) (Table S1).

Among the previously known trophic associations, seven cases showed notable damage to alien species. The East Asian lime leaf miners *Phyllonorycter issikii* and *Cameraria ohridella* caused pronounced damage to North American *Tilia americana* and *Aesculus glabra* var. *arguta*, with up to 75% of the leaves carrying mines (Table S1). The invasive *Phyllonorycter platani* also caused notable damage to the hybrid of North American and Asian plane trees, *Platanus* × *acerifolia*, and oriental plane trees, *Platanus orientalis*, with up to 50% of the leaves carrying mines. Similar damage levels, with up to 50% of the leaves carrying mines, were recorded for three moth species: the alien *Caloptilia azaleella* on *Rhododendron* sp., *Coptodisca lucifluella* on the non-native *Pterocarya fraxinifolia*, and the native *Caloptilia roscipennella* on the non-native *Juglans regia*.

The studied complex of leaf-mining species on the woody plants in the arboretum had a highly structured trophic network (Figure 5, Table 3). This was true for both the "plant species—insect species" (Figure 5) and "plant genus—insect species" systems (not shown). Notably, of the 42 analyzed woody plant species in the trophic network, 25 (60%) were of alien origin, while the remaining 17 (40%) were native (Figure 5).

Table 3. Statistic indices reflecting different characteristics of the trophic network organization and specialization in the studied complex of leaf-mining insects on woody plants in the Volčji Potok Arboretum (Slovenia).

Trophic Systems	Indices				
	Nestedness	Web Asymmetry	Specialization	Links per Species	Number of Compartments
Insects species—plant species	6.74	0.18	0.86	1.37	24
Insects species—plant genus	9.40	0.45	0.98	1.03	18

The nestedness index was slightly lower in the interaction system "plant species—insect species", indicating that such a system has a high degree of orderliness when plants are analyzed by genus, i.e., 6.74 vs. 9.40 (Table 3). The web asymmetry index, reflecting the organization of the trophic network, was positive (i.e., 0.18 and 0.45 for both systems, respectively), meaning there was a relatively high balance between the number of taxa in the two levels (Table 3).

The number of compartments, i.e., sub-sets of the web that are not connected, was slightly higher in the "plant species—insect species" system than in the "plant genus—insect species" system, i.e., 24 and 18, respectively (Figure 5; Table 3).The specialization index was high in both systems, though slightly higher when considering plants at the genus level rather than species level, i.e., 0.86 and 0.98, respectively (Table 3), indicating pronounced monophagy in the studied complex of leaf miners.

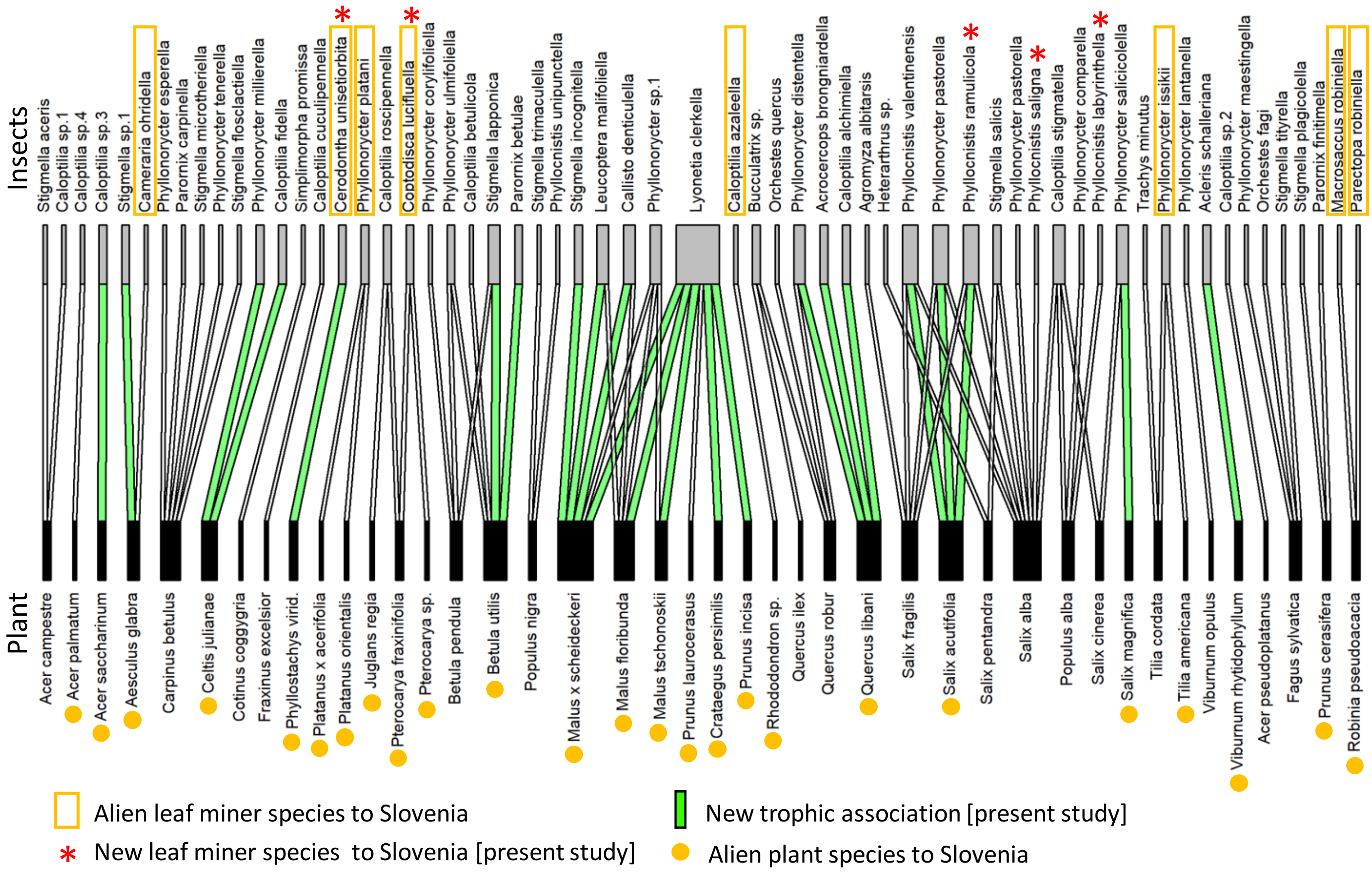

Figure 5. Trophic relationships between leaf-mining insect species and woody plants in the Volčji Potok Arboretum (Slovenia). Cut name in the figure: *Phyllostachys virid.*—*Phyllostachys viridiglaucescens*.

On the other hand, the "plant species—insect species" system had slightly more trophic links per insect species than the "plant genus—insect species" system, i.e., 1.37 vs. 1.03 (Table 3). This indicates that, on average, leaf miners had trophic links with slightly more than one plant species, but mostly with one plant genus. There were two exceptions: the lyonetiid *Lyonetia clerkella* (Linnaeus, 1758) and the gracillariid *Caloptilia stigmatella* (Fabricius, 1781), both of which are oligophagous species documented on plants from several genera (Figure 5). The former species was recorded on *Crataegus*, *Malus*, and *Prunus* (Rosaceae), and the latter on *Salix* and *Populus* (Salicaceae) (Figure 5).

3.5. Leaf-Mining Species New to Slovenia

Among the documented leaf miners, five species were recorded in Slovenia for the first time. Two of these species, North American *Coptodisca lucifluella* and East Asian *Cerodontha unisetiorbita*, are aliens to the country. The leaf mines of *Coptodisca lucifluella* were found in the arboretum on the alien plant Caucasian walnut *Pterocarya fraxinifolia*, while those of *Cerodontha unisetiorbita* were found on the Chinese bamboo *Phyllostachys viridiglaucescens*. The following three leaf-mining species, which we believe to be native to the country, were recorded in Slovenia for the first time: *Phyllocnistis labyrinthella, P. ramulicola*, and *P. saligna*. Their mines and larvae were collected on *Populus alba* (for *Phyllocnistis labyrinthella*) and *Salix* spp. (for *P. ramulicola* and *P. saligna*). Further information on their bionomics, trophic associations, and distribution is provided below in species essays.

Diptera: Agromyzidae

Cerodontha unisetiorbita Zlobin, 1992

(Figure 6)

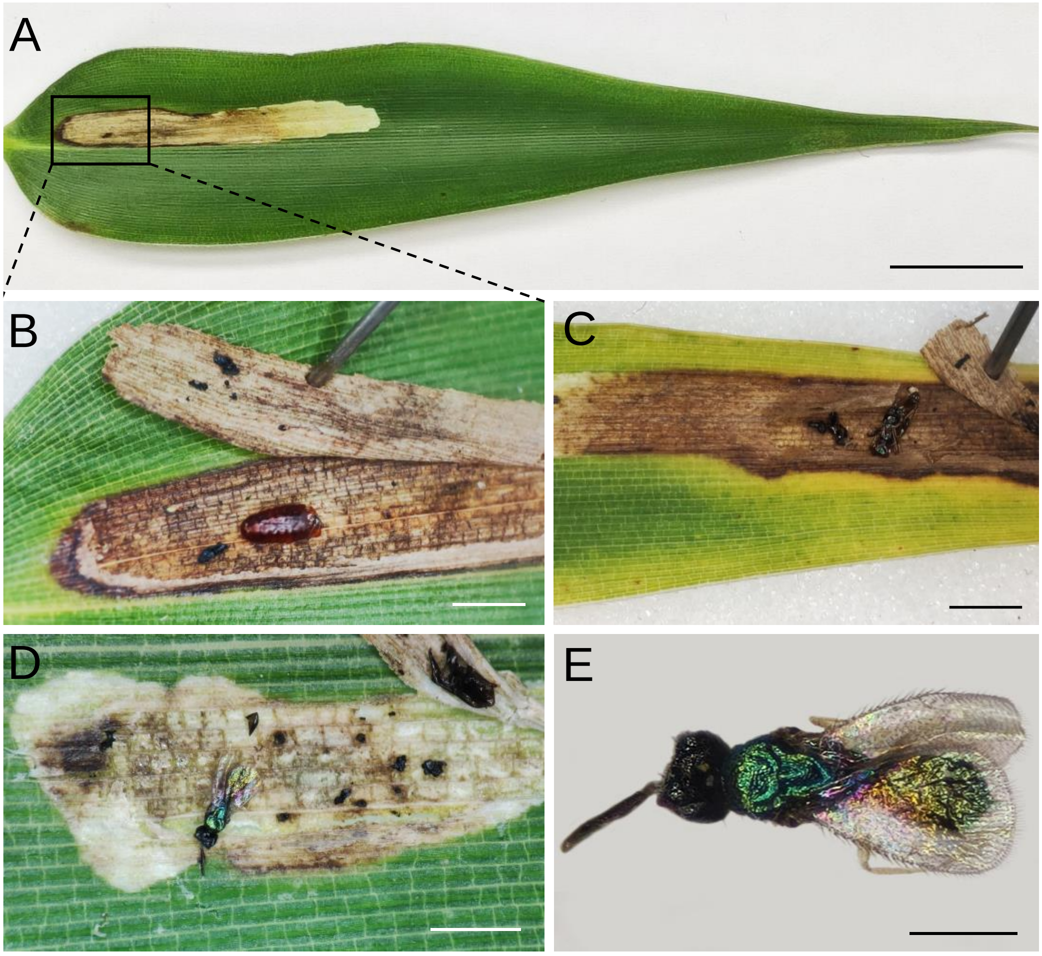

Figure 6. The mines of *Cerodontha unisetiorbita* on *Phyllostachys viridiglaucescens* found in the Volčji Potok Arboretum, Slovenia, 9. August 2022. (**A**,**B**) a mine with a dead puparium; (**C**–**E**) a mine with an adult parasitoid *Chrysocharis* sp. (Hymenoptera: Eulophidae) emerged from pupa of *C. unisetiorbita*. Scale: (**A**) 10 mm, (**B**–**D**) 2 mm, (**E**) 0.7 mm. Photo: N.I. Kirichenko, S. Gomboc.

Material examined. Slovenia, Gorenjska region, Volčji Potok Arboretum, 46.1876 N, 14.6126 E, 349 m a.s.l., 9 August 2022, eight mines (including one with dead larva and two with dead pupae), *Phyllostachys viridiglaucescens*, one parasitoid dissected from the leaf mine and DNA barcoded (process ID LPEST280-22), N.I. Kirichenko and S. Gomboc leg.

Bionomics. The mine is a broad, light-green tunnel situated on the upper side of the leaf along the midrib (Figure 6A), leaf margin, or next to the leaf tip. The abandoned mine is brown (Figure 6B), dries quickly, and the upper epidermis covers the mine cracks. Frass is scarce in the mine and appears as a few black, amorphous lumps scattered irregularly. In the Slovenian arboretum, eight leaves with mines (one mine per leaf) were found among approximately 5000 leaves examined on 20 plants. Pupation occurs inside the leaf mine in the last $\frac{1}{4}$ of the mine. The puparium is about 1.8 mm long, slightly flattened dorsoventrally, and attached to the mine bottom. The posterior spiracles are long, with 8–9 bulbs each. In the arboretum, two puparia of the fly were parasitized (Figure 6C–E). One of them had a parasitoid adult that failed to exit and died in the mine (Figure 6E). This parasitoid was identified through DNA barcoding as an eulophid, *Chrysocharis* sp.

Host plants. Monophagous on *Phyllostachys atrovaginata, Phyl. aureosulcata spectabilis, Phyl. aureosulcata, Phyl. "mitis", Phyl. nuda,* and *Phyl. parvifolia* (Poaceae), but can also develop on *Fargesia* sp. (same family) [Černý, Roháček 2015]. In Slovenia, the mines were recorded on *Phyllostachys viridiglaucescens*, which is a new host for this fly.

Distribution. Native range: East Asia—Japan [47]. Europe: first record in Italy in 2000 [48], followed by records from Switzerland in 2013 [49], Germany [49], and Czechia in

2014 [50], the UK in 2014 [51,52], France in 2014 (detected on *Phyllostachys* sp. in Parc Floral de La Source, Orleans, in September 2014) [53], and Slovenia in 2022 (present paper).

Remarks. Alien species are newly recorded in Slovenia (present paper). In the Volčji Potok Arboretum, it was found in very low abundance.

Lepidoptera: Heliozelidae

Coptodisca lucifluella (Clemens, 1860)

(Figure 7)

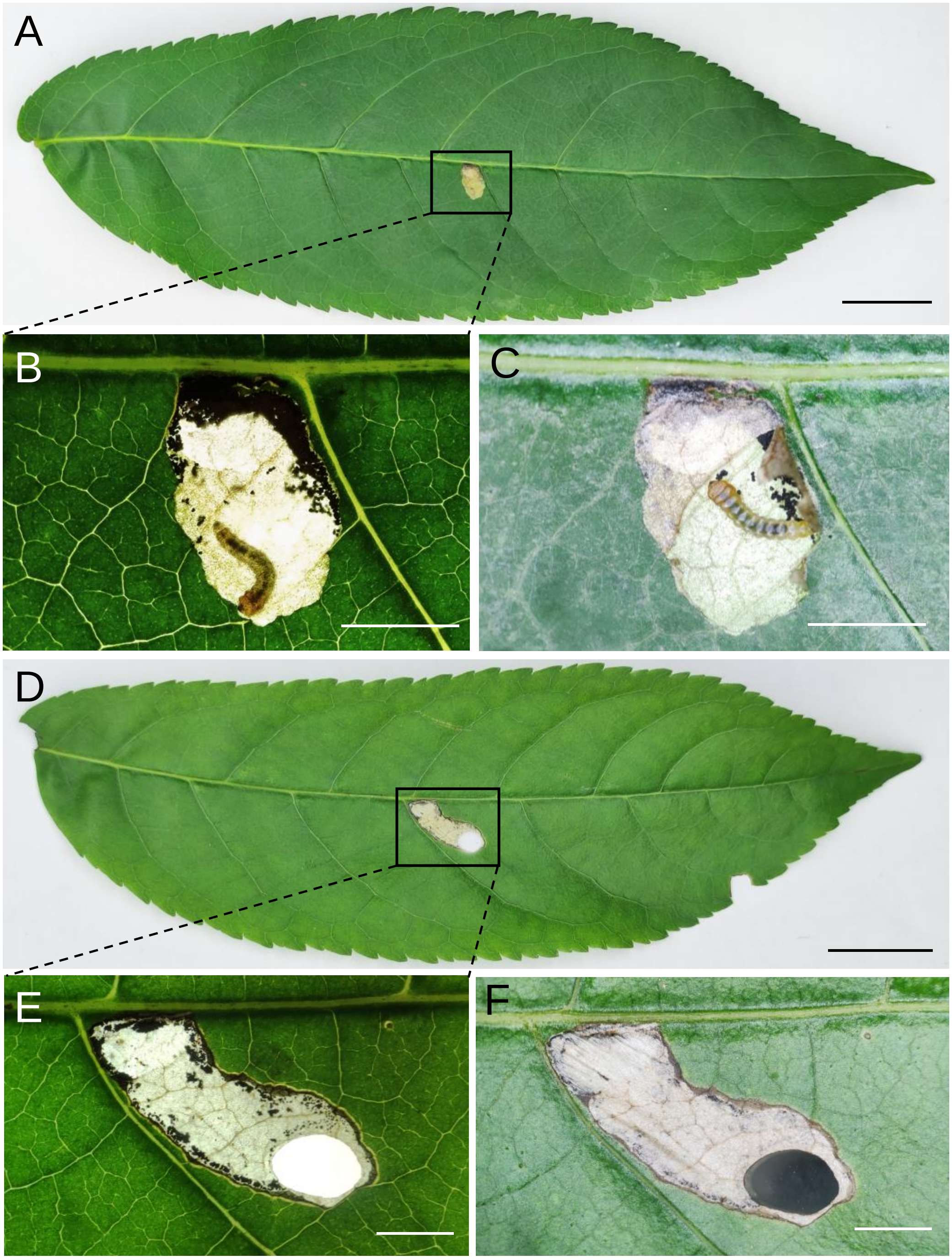

Figure 7. The mines of *Coptodisca lucifluella* on *Pterocarya fraxinifolia* found in the Volčji Potok Arboretum, Slovenia, 5–6 August 2022. (**A**–**C**) a mine with a live larva in it; (**D**–**F**) an abandoned mine with a semicircle cut made by larvae when leaving the mine; (**B**,**E**) in translucent and (**C**,**D**) in reflected light; (**B**,**E**) frass is accumulated along the mine margins. Scale: (**A**,**D**) 10 mm; (**B**,**C**,**E**,**F**) 2 mm. Photo: N.I. Kirichenko, S. Gomboc.

Material examined. Slovenia, Gorenjska region, Volčji Potok Arboretum, 46.1876 N, 14.6126 E, 349 m a.s.l., 5–6 August 2022, 22 mines, *Pterocarya fraxinifolia*, two larvae dissected from leaf mines and DNA barcoded (process IDs: LPEST271-22, LPEST272-22), N. Kirichenko and S. Gomboc leg.

Additional material examined. Slovenia, Primorska, Škocjanske jame, Matavun, 9.VIII.2022, 45.6639 N, 13.9745 E, 449 m a.s.l., 15 mines, *Juglans regia,* one larva dissected from leaf mines and DNA barcoded (process ID: LPEST284-22), N.I. Kirichenko and S. Gomboc leg.; ibidem, Notranjska, Ilirska Bistrica, Bač, 11 August 2022, 45.6348 N, 14.2709 E, 608 m a.s.l., 12 mines, *Juglans regia*, N.I. Kirichenko and S. Gomboc leg.; ibidem, Postojnska kotlina, Pivka, 11 August 2022, 45.6853 N, 14.1975 E, 545 m a.s.l., 10 mines, *Juglans regia*, N.I. Kirichenko and S. Gomboc leg.

Bionomics. The mine starts in the corner between the midrib and secondary vein or between the two secondary veins at some distance from the midrib; the mine does not cross secondary veins (Figure 7A–C). The mine is a full-depth oval blotch, up to 8 mm in length. Black grains of frass are mostly accumulated along the mine edge. Up to three mines per leaf were documented in studied arboretum. The larva is pale brown. Before pupation, it makes a case out of an oval excision at a distant part of the mine (Figure 7D–F) and leaves the mine to pupate on the tree. The abandoned mine dries, and eventually the upper or lower epidermis cracks and becomes loose.

Host plants. Oligophagous on Juglandaceae. Europe: *Carya glabra* [54], *C. cordiformis*, *Pterocarya fraxinifolia*, and *Juglans regia* [55]. In Slovenia, the mines were documented on *Pt. fraxinifolia* and *J. regia*.

Distribution. Native range: North America (Kentucky, Maryland, Missouri, North Carolina, Ohio, Oklahoma, Pennsylvania, Tennessee, Texas, and Wisconsin) [56]. Europe: recorded for the first time in Italy in 2010 [54,57], followed by records in Hungary in 2017 [55,58], Ukraine in 2019 [59], Bulgaria in 2020 [60], Romania in 2022 [61], and Slovenia in 2022 (present paper).

Remarks. C. lucifluella is an alien species that was recorded for the first time in Slovenia (present paper). A relative species, the North American *Coptodisca juglandiella* (Chambers, 1874), was recorded for the first time in Europe in Hungary in 2020, sharing the same hosts (i.e., *J. nigra, C. cordiformis,* and *Pt. fraxinifolia*) with *C. lucifluella* [55]. A comparison of adults and leaf mines of *C. lucifluella* and *C. juglandiella* is given in [55]. Likely, *C. juglandiella* will spread across Europe where its host plants are present. So far, we have not found *C. juglandiella* in Slovenia.

Lepidoptera: Gracillariidae, Phyllocnistinae

Phyllocnistis labyrinthella (Bjerkander, 1790)

(Figure 8)

Material examined. Slovenia, Gorenjska region, Volčji Potok Arboretum, 46.1876 N, 14.6126 E, 349 m a.s.l., 9 August 2022, 24 mines, *Populus alba*, one pupa was dissected from the leaf mines and DNA barcoded (process ID LPEST250-22), N.I. Kirichenko and S. Gomboc leg.

Additional material examined. Slovenia, Slovenska Bistrica, forest, 46.4025 N, 15.5652 E, alt. 270 m, 11.VIII.2022, two mines, *Populus tremula*, one dead larva dissected from leaf mines, N.I Kirichenko and M. de Groot leg.

Bionomics. The mine is a long, relatively broad epidermal tunnel mostly situated on the upper side of the leaf and rarely on the lower side of the leaf, with a greenish or darker central line of frass (Figure 8A). The mine starts near the leaf margin or next to the secondary veins in the central part of the leaf, covers most of the surface of the leaf lamina (crossing the main vein closer to the leaf tip), slightly widens during larval growth, and finishes at the leaf margin where pupation takes place. At the pupation site, the leaf margin (6–9 mm in width) is folded upwards (Figure 8A–B). The pupa is brown (Figure 8C). The characteristics of the pupal cremaster are given in [62]. In Slovenia, in early August, pupae were mostly found in the mines.

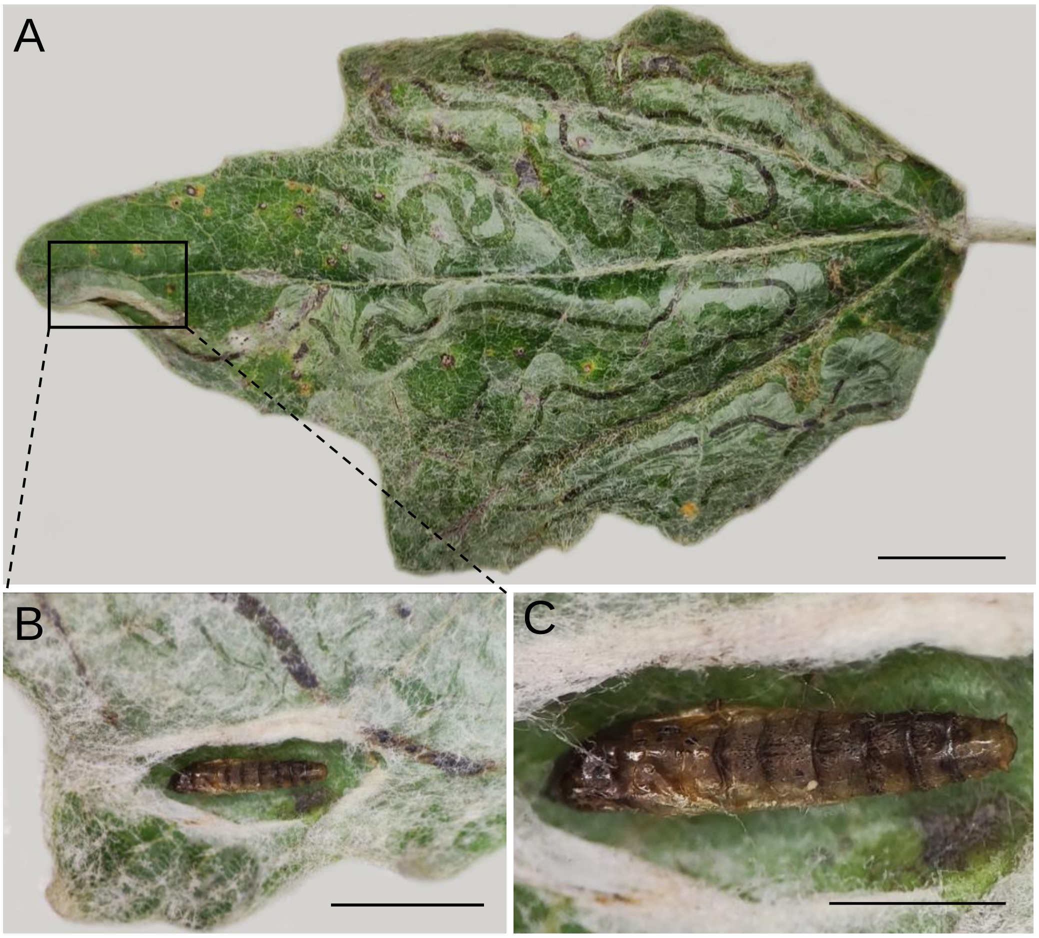

Figure 8. The mines of *Phyllocnistis labyrinthella* on *Populus alba* found in the Volčji Potok Arboretum, Slovenia, 9 August 2022. (**A**–**C**) a mine with a live pupa in it. Scale: (**A**) 10 mm, (**B**) 5 mm, (**C**) 2 mm. Photo: N.I. Kirichenko, S. Gomboc.

Host plants. Monophagous on *Populus tremula* (Salicaceae) [36,63], *Pop. alba* [22]. In Slovenia, the leaf mines were found on *Pop. alba* and *Pop. tremula.*

Distribution. Native species to Eurasia. It is distributed across most of Western and Central Europe [43], Russia [64], Kazakhstan, and Uzbekistan [65] and was found in Slovenia in 2022 (present paper).

Remarks. Native species was recorded for the first time in Slovenia (present paper). A closely related species, *Phyllocnistis xenia* M. Hering, 1936, is known to occur sympatrically on *Populus alba* [36,63]. Some authors also list *Pop. tremula* [22] and the hybrid of *Pop. tremula* and *Pop. alba*, i.e., *Populus* × *canescens*, as host plants of *P. xenia* [66,67]. The mine of *P. labyrinthella* can be distinguished from that of *P. xenia* by its narrower dark area [68]. The adult morphology is highly similar [68]. Furthermore, the DNA barcoding data of these two species is also similar [36] (Figure 3; Table 1). The species status of *P. xenia* remains a topic of debate. Some authors consider it a junior synonym of *P. labyrinthella* [69,70]. However, in recent studies, they have been listed as separate species [36,63]. Further research is needed to study the intra- and interspecific variability of morphological and molecular genetic characteristics in *P. xenia* and *P. labyrinthella* across their ranges.

Phyllocnistis ramulicola Langmaid & Corley, 2007
(Figure 9)

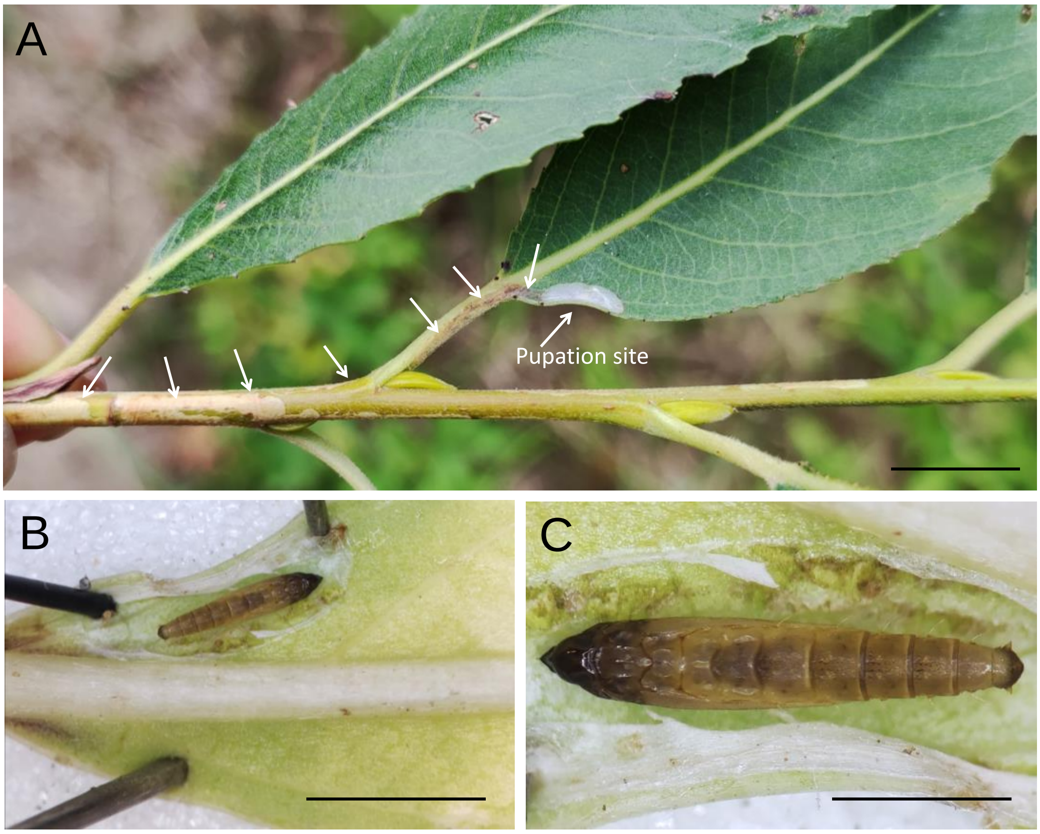

Figure 9. The mines of *Phyllocnistis ramulicola* on *Salix cinerea* (**A**) and *S. alba* 'Aurea' (**B**,**C**) found in the Volčji Potok Arboretum, Slovenia, 9 August 2022. (**A**) a long tunnel (shown by arrows) in the twig and the pupation site at the leaf base (indicated); (**B**,**C**) a pupation site on the lower side of the leaf near the leaf base with the pupa. Scale: (**A**) 20 mm; (**B**) 5 mm; (**C**) 2 mm. Photo: N.I. Kirichenko, S. Gomboc.

Material examined. Slovenia, Gorenjska region, Volčji Potok Arboretum, 46.1876 N, 14.6126 E, 349 m a.s.l., 6 August 2022, 15 leaf and twig mines, *Salix alba* 'Aurea', one live pupa dissected from the leaf mines and DNA barcoded (process ID: LPEST279-22), N.I. Kirichenko and S. Gomboc leg.; ibidem, 6 August 2022, ten leaf and twig mines, *Salix alba*, N.I. Kirichenko and S. Gomboc leg.; ibidem, 9 August 2022, seven leaf and twig mines, *Salix acutifolia*, N.I. Kirichenko and S. Gomboc leg.; ibidem, 9 August 2022, three leaf and twig mines, *Salix alba* '*Fantasia*'), N.I. Kirichenko and S. Gomboc leg.

Bionomics. The mine begins on a young twig as a long, slightly widening tunnel in the bark (Figure 9A), often running up and down, reaching 35 cm in length. As the mine progresses, it moves toward the leaf, entering the leaf base, where the larva pupates (Figure 9B,C). The pupation site is characterized by a slightly bent upward leaf margin (Figure 9A). In early August, mostly dead larvae, pupae, or pupal exuviae were found in mines in the arboretum.

Host plants. Monophagous on *Salix* (Salicaceae), it can be found on many willows: *Salix alba* [36], *Salix aurita, S. cinerea, S. caprea, S. euxina, S.* × *fragilis, S. melanopsis, S. pentandra, S. purpurea, S. triandra,* and *S. viminalis* [22]. In Slovenia, we recorded leaf mines on *S. cinerea*, *S.* × *fargilis*, *S. alba* and its cultivars, and on a new host, *S. acutifolia* (Table S2).

Distribution. Native to Europe. England, Portugal [71], Slovakia [72], France [73,74], Spain, Switzerland, Italy, the Czech Republic [22,36], and Slovenia (present paper).

Remarks. Native species recorded for the first time in Slovenia (present paper); it was abundant on several willow species in the Volčji Potok Arboretum in early August 2022.

Phyllocnistis saligna (Zeller, 1839)

Figure 10

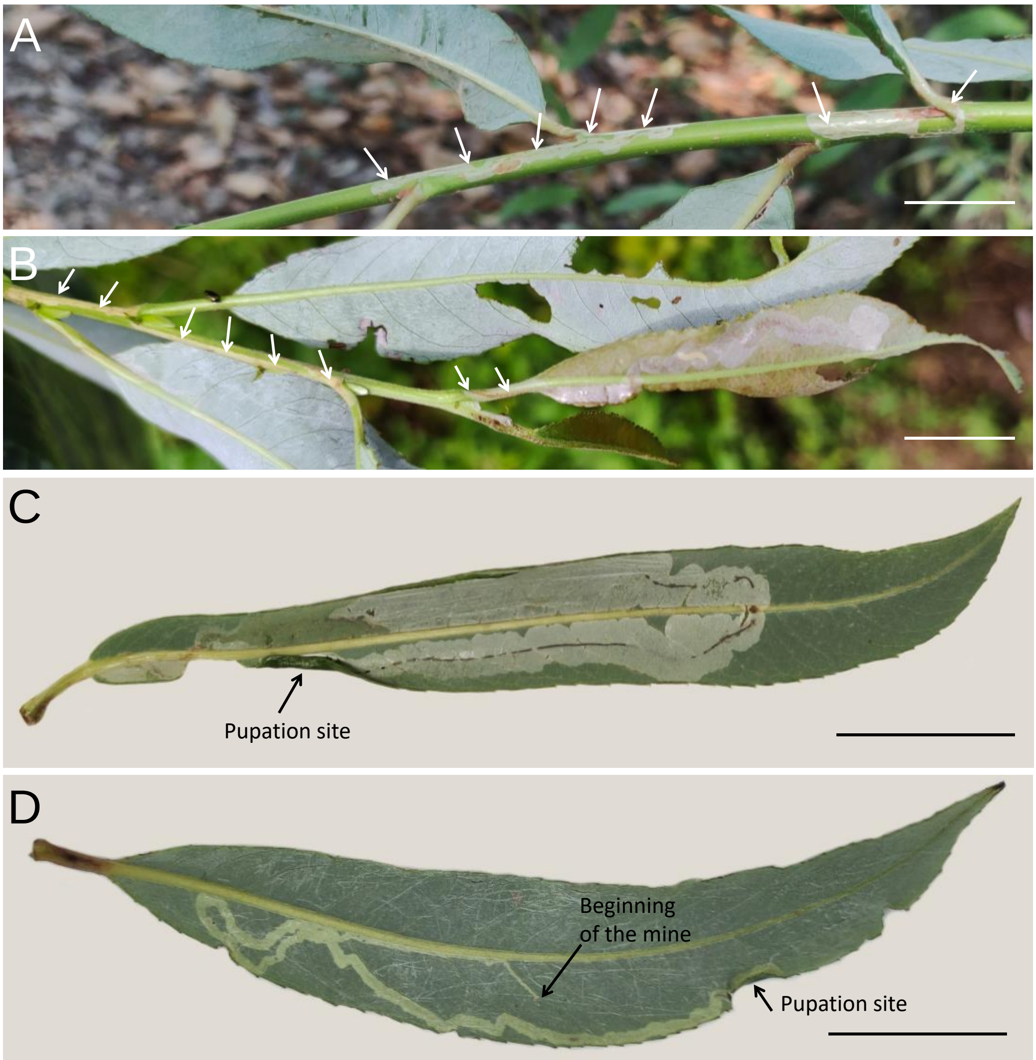

Figure 10. The mines of *Phyllocnistis saligna* (**A**–**C**) and *P. valentinensis* (**D**) on *Salix alba* in the Volčji Potok Arboretum, Slovenia, 9 August 2022. (**A**,**B**) a tunnel (shown by arrows) in the twig and the leaf mine with feeding larva (**B**); (**C**) a relatively broad leaf mine of *P. salinga* occupying most of the leaf lamina and the pupation site at the leaf margin; (**D**) a narrow tunnel of *P. valentinensis* wholly located on the leaf lamina. Scale: (**A**,**B**) 10 mm; (**C**,**D**) 20 mm. Photo: N.I. Kirichenko, S. Gomboc.

Material examined. Slovenia, Gorenjska region, Volčji Potok Arboretum, 46.1876 N, 14.6126 E, 349 m a.s.l., 9.VIII.2022, 11 leaf and shoot mines, *Salix alba 'Fantasia'*, N.I. Kirichenko and S. Gomboc leg.

Additional material examined. Slovenia, Gorenjska Region, Julian Alps, Radovna Valley, Radovna, Zatrep, 46.3905 N, 14.0184 E, 659 m a.s.l., 24 July 2022, eight leaf and shoot mines, *Salix alba*, one larva dissected from the leaf mine and DNA barcoded (process ID LPEST161-22), N.I. Kirichenko and S. Gomboc leg.; ibidem, Radovna, Žagarjev rovt, 46.3965 N, 14.0006 E, 670 m a.s.l., 24 July 2022, 30 leaf and shoot mines, *Salix alba*, N.I. Kirichenko and S. Gomboc leg.; ibidem, Pokljuka, Planina Konjščica, 46.3484 N, 13.8991 E, 1463 m a.s.l., 25 July 2022, five leaf and shoot mines, *Salix* sp., N.I. Kirichenko and S. Gomboc leg.; ibidem, Pokljuka, Planina Uskovnica, Jele, 46.3249 N, 13.9082 E, 1212 m a.s.l., 25 July 2022, six leaf and shoot mines, *Salix* sp., two larvae dissected from leaf mines and DNA barcoded (process IDs: LPEST184-22, LPEST185-22), N.I. Kirichenko and S. Gomboc leg.

Bionomics. The mine begins on a young twig with a whitish, glossy tunnel that widens slightly (Figure 10A,B), often running up and down, and reaching up to 20 cm in length. At first, the tunnel in the twig is transparent or whitish but later turns brown. The tunnel then enters the petiole and continues to the leaf lamina (upper or lower side of the leaf), where it widens slightly and zigzags, occupying a significant part of the leaf lamina. The mine may pass through several leaves. The frass is a relatively broad, obscure line, but in some cases, it can be a more visible, dark line. Pupation takes place near the leaf margin. In early August, mostly pupal exuviae or dead larvae were found in mines in the arboretum.

Host plants. Monophagous on *Salix* (Salicaceae), it can be found on many willows: *Salix alba*, *S. babylonica*, *S. daphnoides*, *S.* × *fragilis*, *S. lanata*, *S. purpurea*, *S.* × *salamonii*, *S. triandra*, *S. viminalis*, and *S. euxina* [22,36]. In Slovenia, we recorded the mines on *Salix alba* and *Salix alba 'Fantasia'* (Table S2).

Distribution. Native to Europe and distributed across most of the continent [22,36], Slovenia (present paper).

Remarks. Native species were recorded for the first time in Slovenia (present paper). It was relatively abundant in the Volčji Potok Arboretum (Table S2). In the arboretum and beyond, we noted that *P. saligna* occurs sympatrically with *P. ramulicola* and *P. valentinensis*. Their mines were found on the same trees, on the same branches, and sometimes they were even restricted to the same leaves. The mine of *P. saligna* is distinguishable from that of the other two species for several reasons: (1) its tunnel on the twig is shorter than compared to that of *P. ramulicola*; (2) the mine can pass several leaves, occupying a significant area of the leaf lamina (unlike *P. ramulicola*, which only enters the leaf base) (Figures 9A and 10B,C); (3) the leaf mine is relatively broad and has a whitish, silvery appearance; and (4) pupation occurs at the leaf margin at any distance from the leaf base (restricted to the leaf base in the case of *P. ramulicola*) (Figures 9A and 10C). In contrast to *P. saligna* and *P. ramulicola*, the mine of *P. valentinensis* is wholly situated on the leaf lamina (most often on the lower side of the leaf) and differs from that of *P. saligna* by its narrower size, deeper location inside the leaf tissue, a whitish (not glossy) mine color, and a barely visible or invisible, narrow, blackish frass line (Figure 10D).

4. Discussion

Our case study demonstrated the effectiveness of using an arboretum for detecting and identifying endophagous folivores, such as leaf miners, and exploring their trophic relationships with alien woody plants. Even a brief survey conducted over a few days in early August and January was highly fruitful in several ways.

Firstly, the survey resulted in the discovery of 62 species of leaf-mining insects and the identification of five new species for Slovenia, two of which were alien: the heliozelid *Coptodisca lucifluella* and the agromyzid *Cerodontha unisetiorbita*. This highlights the usefulness of arboreta for conducting regional faunal inventories and intercepting alien species. A growing body of research supports the use of arboreta and botanical gardens for alien pests [75–78]. For example, a recent study conducted in a Siberian arboretum detected 14 species of true bugs, representing novel geographical records, and one alien species from East Asia [79].

Furthermore, the analysis of trophic relations led to the identification of 23 new trophic associations between leaf miners and woody plants, out of 106 recorded cases. Nearly one quarter of the trophic associations documented in our pilot study were previously unknown to science. Of these new associations, the majority involved native leaf miners colonizing alien woody plants, primarily originating from Asia and, to a lesser extent, from North America. This underscores the role of the arboreta plantings as sentinels for detecting native insects capable of switching to alien hosts and, if they are favorable, benefiting from the new relationships and increasing their population densities. Indeed, botanical gardens and arboreta are attracting increasing attention from researchers and practitioners for their potential to identify emerging pests among native insects and determine the potential threat these insects pose to their novel hosts [11,77,78].

Another important point to consider is that our brief survey found a high concentration of alien leaf miner species in one location. For instance, in the arboretum, we recorded six out of nine species (i.e., 67%) of invasive alien gracillariids previously documented in Slovenia (see the actual gracillariid checklist in [38]), including *Caloptilia azaleella*, *Cameraria ohridella*, *Phyllonorycter issikii*, *Ph. Platani*, *Macrosaccus robiniella*, and *Parectopa robiniella*. Curiously, the Asian moth *Caloptilia azaleella* was known to be present in Slovenia by only one record, which was an intercepted specimen on a potted *Azalea* (*Rhododendron*) imported from Germany in 2001 to a nursery in a suburb of Ljubljana [80]. In January 2023, we detected noticeable damage caused by this moth on alien *Rhododendron* bushes planted in the Slovenian arboretum, which is the second record for the country and highlights the importance of arboreta and botanical gardens as live targets for non-native pests. These types of man-made plantings often contain a wide variety of non-native plants from various floristic and biogeographic regions, providing a favorable environment for alien pests to find common hosts or establish novel trophic links with other plants [11]. Furthermore, pests can be unintentionally introduced with their host plants in ornamental plantings [81,82].

Three alien gracillariid species previously documented in Slovenia, i.e., *Phyllocnistis vitegenella*, *P. citrella*, and *Phyllonorycter leucographella* (see checklist in [38]), were not found in the studied arboretum. This is because their main host plants (*Vitis, Parthenocissus*, the host of the first moth species) were not present in the arboretum or were removed from the collections due to the renovation of some parts of the arboretum (*Pyracantha*, the host of the second moth species), or were used rarely and only as potted plants for temporary compositions in the warm period or for feeding exotic butterflies in a butterfly house, and were thus replaced by new plants on a regular basis (such as *Citrus* spp., the host of the third moth species) [83].

The use of an integrative approach involving DNA barcoding allowed us to identify or confirm the species of leaf-mining insects that were collected at their immature (larval or pupal) stage. This approach also provided additional genetic data to update the DNA barcoding library that we are currently developing for the leaf miners of Slovenia. DNA barcoding is a valuable addition to classical morphological identification methods because it allows insect taxa to be identified at any life stage using only a small piece of tissue for analysis [21,23,24]. Even fresh insect remnants (molting larval or pupal exuvia) can still be used for DNA barcoding [84]. Furthermore, a recent study showed that freshly abandoned leaf mines containing insect frass can also be utilized for sequencing [85].

However, our study has highlighted a few existing taxonomic problems. In particular, we encountered difficulties in identifying *Phyllocnistis labyrinthella*, a species recorded for the first time in Slovenia, based on its mine morphology and DNA barcoding. This species has a small genetic divergence from the related *Phyllocnistis xenia*, and both are assigned to a single BIN in the genetic database BOLD [24]. Therefore, it is still unclear whether they are two separate species or one species. In contrast, *Phyllocnistis unipunctella*, which was detected in the Slovenian arboretum, is known for having a high level of genetic divergence across its native range in Eurasia [86], with different geographical populations (Europe and Asia) assigned to three different clusters in BOLD. The specimen from Slovenia was highly similar to the representatives from Europe. It is unclear whether or not it is one species across Eurasia with exceptionally high interspecific divergence (>5%). Further studies are required, including a more comprehensive exploration of species ranges and their morphological and genetic features, to address these questions.

A few leaf miners remained unidentified at the species level in our study, such as *Stigmella* and *Caloptilia* on North American *Aesculus glabra* and *Acer saccharinum*, respectively. Before our study, there was no known data on Nepticulidae mining the leaves of *Aesculus* [22,36]. This is the first record of *Stigmella* on North American *Aesculus glabra* in Europe. We were unable to identify this insect at the species level based only on the morphology of the leaf mine, and no larvae were present in the mine for DNA barcoding. Therefore, it remains unknown whether this was a host shift of a native nepticulid or an alien species. Another interesting case was recorded on North American *Acer saccharinum*, on which the leaf mine and the leaf cone of *Caloptilia* sp. 3 were found. It also remains unclear if the damage was caused by a native *Caloptilia*, as several species from this genus are known to affect native maples in Slovenia [38], or by an alien species that arrived in the arboretum with its host plant.

5. Conclusions

The study conducted in Slovenia demonstrated the effectiveness of arboreta and botanical gardens as research sites for investigating folivore insect complexes, detecting both alien and overlooked native insect species, and exploring the trophic associations between insects and native and non-native woody plants. The study produced many interesting results, emphasized the importance of using integrative taxonomy, including DNA barcoding, for species identification, and highlighted problematic cases. The novel trophic associations between native leaf-mining insects and alien woody plants from Asia and North America documented in the Slovenian arboretum are significant findings that warrant further observation to better understand the benefits these interactions may bring to native insects in terms of increasing population density and potential spread beyond their native range.

This study reminds researchers and practitioners to focus more attention on arboreta and botanical gardens, tree nurseries, parks, and other man-made plantings, bearing in mind that such sites may provide great potential to detect new alien species and identify emerging pests among native species that have the ability to build new trophic associations with alien woody plants [11,14,87]. Such data will be invaluable for early warning and early action systems and, therefore, for effective plant protection against tree pests [11,13,19,87].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f14030641/s1, Table S1: Origin of woody plant species involved in the study in the Volčji Potok Arboretum (Gorenjska region, Slovenia) in the period 2022–2023, Table S2: Diversity, abundance, and trophic associations of leaf-mining insects documented on various woody plants in the Volčji Potok Arboretum (Gorenjska region, Slovenia) in the period 2022–2023, Table S3: Specimen data of leaf-mining insects collected in the Volčji Potok Arboretum (Gorenjska region, Slovenia) in the period 2022–2023 and DNA barcodes of the same species publicly available in BOLD, which were utilized for comparison in the present study, Figure S1: The maximum likelihood tree illustrating the relationship between the parasitoid *Chrysocharis* sp. (Hymenoptera: Eulophidae) obtained from the leaf mine of *Cerodontha unisetiorbita* Zlobin, 1992 (Diptera: Agromyzidae) in the Volčji Potok Arboretum (Gorenjska region, Slovenia) and representatives of the parasitoid species from Italy.

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Data Availability Statement: The genetic data used in the study are publicly accessible in BOLD using the link https://dx.doi.org/10.5883/DS-ARBOLM.

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References

1. Diagne, C.; Leroy, B.; Vaissière, A.C.; Gozlan, R.E.; Roiz, D.; Jarić, I.; Salles, J.-M.; Corey, J.; Bradshaw, A.; Courchamp, F. High and rising economic costs of biological invasions worldwide. *Nature* **2021**, *592*, 571–576. [CrossRef] [PubMed]
2. Meyer, S.E.; Callaham, M.A.; Stewart, J.E.; Warren, S.D. Invasive species response to natural and anthropogenic disturbance. In *Invasive Species in Forests and Rangelands of the United States*; Poland, T.M., Patel-Weynand, T., Finch, D.M., Miniat, C.F., Hayes, D.C., Lopez, V.M., Eds.; Springer: Cham, Switzerland, 2021; pp. 85–110. [CrossRef]
3. MacLachlan, M.J.; Liebhold, A.M.; Yamanaka, T.; Springborn, M.R. Hidden patterns of insect establishment risk revealed from two centuries of alien species discoveries. *Sci. Adv.* **2021**, *7*, eabj1012. [CrossRef] [PubMed]
4. de Groot, M.; O'Hanlon, R.; Bullas-Appleton, E.; Csóka, G.; Csiszár, Á.; Faccoli, M.; Gervasini, E.; Kirichenko, N.; Korda, M.; Marinšek, A.; et al. Challenges and solutions in early detection, rapid response and communication about potential invasive alien species in forests. *Manag. Biol. Invasions* **2020**, *11*, 637–660. [CrossRef]
5. Bennett, B. Learning in paradise: The role of botanic gardens in university education. In *Innovative Strategies for Teaching in the Plant Sciences*; Quave, C., Ed.; Springer: New York, NY, USA, 2014; pp. 213–229. [CrossRef]
6. Heywood, V.H. The future of plant conservation and the role of botanic gardens. *Plant Divers.* **2017**, *39*, 309–313. [CrossRef]
7. Chen, G.; Sun, W. The role of botanical gardens in scientific research, conservation, and citizen science. *Plant Divers.* **2018**, *40*, 181–188. [CrossRef]
8. Hulme, P.E. Addressing the threat to biodiversity from botanic gardens. *Trends Ecol. Evol.* **2011**, *26*, 168–174. [CrossRef]
9. Ferus, P.; Hoťka, P.; Košútová, D.; Konôpková, J. Invasions of alien woody plant taxa across a cluster of villages neighbouring the Mlyňany Arboretum (SW Slovakia). *Folia Oecol.* **2020**, *47*, 121–130. [CrossRef]
10. Wondafrash, M.; Wingfield, M.J.; Wilson, J.R.U.; Hurley, B.P.; Slippers, B.; Paap, T. Botanical gardens as key resources and hazards for biosecurity. *Biodiv. Conserv.* **2021**, *30*, 1929–1946. [CrossRef]
11. Eschen, R.; O'Hanlon, R.; Santini, A.; Vannini, A.; Roques, A.; Kirichenko, N.; Kenis, M. Safeguarding global plant health: The rise of sentinels. *J. Pest. Sci.* **2019**, *92*, 29–36. [CrossRef]
12. Morales-Rodríguez, C.; Bastianelli, G.; Aleandri, M.; Doğmuş-Lehtijärvi, T.; Oskay, F.; Vannini, A. Revealing novel interactions between oak and *Tubakia* species: Evidence of the efficacy of the sentinel arboreta strategy. *Biol. Invasions* **2021**, *23*, 3749–3765. [CrossRef]
13. Roques, A.; Fan, J.; Courtial, B.; Zhang, Y.; Yart, A.; Auger-Rozenberg, M.-A.; Denux, O.; Kenis, M.; Baker, R.; Sun, J.-H. Planting sentinel European trees in Eastern Asia as a novel method to identify potential insect pest invaders. *PLoS ONE* **2017**, *10*, e0120864. [CrossRef] [PubMed]
14. Culley, T.M.; Dreisilker, K.; Clair Ryan, M.; Schuler, J.A.; Cavallin, N.; Gettig, R.; Havens, K.; Landel, H.; Shultz, B. The potential role of public gardens as sentinels of plant invasion. *Biodivers. Conserv.* **2022**, *31*, 1829–1844. [CrossRef]
15. Tomoshevich, M.; Kirichenko, N.; Holmes, K.; Kenis, M. Foliar fungal pathogens of European woody plants in Siberia: An early warning of potential threats? *For. Pathol.* **2013**, *43*, 345–359. [CrossRef]
16. Kirichenko, N.; Kenis, M. Using a botanical garden to assess factors influencing the colonization of exotic woody plants by phyllophagous insects. *Oecologia* **2016**, *182*, 243–252. [CrossRef] [PubMed]
17. Franić, I.; Prospero, S.; Adamson, K.C.; Allan, E.; Attorre, F.; Auger-Rozenberg, M.-A.; Augustin, S.; Avtzis, D.; Baert, W.; Barta, M.; et al. Worldwide diversity of endophytic fungi and insects associated with dormant tree twigs. *Sci. Data* **2022**, *9*, 1–9. [CrossRef]
18. Barham, E. The unique role of sentinel trees, botanic gardens and arboreta in safeguarding global plant health. *Plant Biosyst. Int. J. Deal. Asp. Plant Biol.* **2016**, *150*, 377–380. [CrossRef]
19. Kenis, M.; Li, H.; Fan, J.; Courtial, B.; Auger-Rozenberg, M.-A.; Yart, A.; Eschen, R.; Roques, A. Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. *Sci. Rep.* **2018**, *8*, 11217. [CrossRef]
20. Roques, A.; Cleary, M.; Matsiakh, I.; Eschen, R. (Eds.) *Field Guide for the Identification of Damage on Woody Sentinel Plants*; CAB International: Wallingford, UK, 2017; p. 263. [CrossRef]
21. Morales-Rodríguez, C.; Anslan, S.; Auger-Rozenberg, M.-A.; Augustin, S.; Baranchikov, Y.; Bellahirech, A.; Burokienė, D.; Čepukoit, D.; Çota, E.; Davydenko, K.; et al. Forewarned is forearmed: Harmonized approaches for early detection of potentially invasive pests and pathogens in sentinel plantings. *NeoBiota* **2019**, *47*, 95–123. [CrossRef]
22. Ellis, W. Plant Parasites of Europe: Leafminers, Galls and Fungi. Available online: http://bladmineerders.nl/ (accessed on 24 January 2023).
23. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* **2003**, *270*, 313–321. [CrossRef]
24. Lopez-Vaamonde, C.; Kirichenko, N.; Cama, A.; Doorenweerd, C.; Godfray, H.C.J.; Guiguet, A.; Gomboc, S.; Huemer, P.; Landry, J.-F.; Laštůvka, A.; et al. Evaluating DNA barcoding for species identification and discovery in European gracillariid moths. *Front. Ecol. Evol.* **2021**, *9*, 626752. [CrossRef]
25. Hering, E.M. *Biology of the Leaf Miners*; Junk, W., Ed.; Gravenhage: The Hague, The Netherlands, 1951; p. 490.
26. Connor, E.F.; Taverner, M.P. The evolution and adaptive significance of the leaf-mining habit. *Oikos* **1997**, *79*, 6–25. [CrossRef]
27. Kirichenko, N.; Augustin, S.; Kenis, M. Invasive leafminers on woody plants: A global review of pathways, impact and management. *J. Pest Sci.* **2019**, *92*, 93–106. [CrossRef]
28. Mastnak, M. *A Short Guide to the Volčji Potok Arboretum*; Arboretum: Volčji Potok, Slovenia, 2005; p. 50.

29. ESRI ArcGIS Pro Software. Available online: https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview (accessed on 30 January 2022).
30. ESRI ArcGIS Online Countries and River Layers. Available online: https://www.arcgis.com/home/index.html (accessed on 30 January 2022).
31. Ministry of Public Administration of the Republic of Slovenia: GIS Portal. Map Services. Available online: https://gis.gov.si/arcgis/services (accessed on 30 January 2022).
32. Ogrin, D.; Vysoudil, M.; Ogrin, M.; Koželj, T. Topoklimatske razmere. In *Kamniška Bistrica—Geografska Podoba Gorske Doline*; Ogrin, D., Ed.; GeograFF 22; Znanstvena Založba Filozofske Fakultete Univerze v Ljubljani: Ljubljana, Slovenia, 2018; pp. 45–70. [CrossRef]
33. Mastnak, M.; Arboretum Volčji Potok, Gorenjska region, Slovenia. On insecticide treatment in the Arboretum Volčji Potok. Personal communication, 2023.
34. Martinčič, A. (Ed.) *Mala Flora Slovenije*, 4th ed.; Tehniška Založba Slovenije: Ljubljana, Slovenia, 2007. (In Slovenian)
35. GBIF.org, GBIF Home Page. 2023. Available online: https://www.gbif.org (accessed on 24 January 2023).
36. Laštůvka, A.; Laštůvka, Z.; Liska, J.; Šumpich, J. *Motyli a Housenky Stredni Evropy, V. Drobni Motyli I [Moths and Caterpillars of Central Europe V—Micromoths*; Atlas, I., Ed.; Academia: Praha, Czech Republic, 2018; p. 532. (In Czech)
37. Maček, J. Hiponomološka Favna Slovenije/Hyponomologische Fauna Sloweniens [Leaf Mining Fauna of Slovenia]. Slovenska akademija znanosti in umetnosti, Razred za naravoslovne vede, Classis IV. *Hist. Nat.* **1999**, *37*, 385. (In Slovenian)
38. Gomboc, S.; Kirichenko, N.I. An Overview of Gracillariidae leaf mining moths in Slovenia with new records for the country. *Diversity* **2022**, *14*, 811. [CrossRef]
39. de Waard, J.; Ivanova, N.; Hajibabaei, M.; Hebert, P.D.N. Assembling DNA barcodes: Analytical methods. In *Methods in Molecular Biology: Environmental Genetic*; Cristofre, M., Ed.; Humana Press: Totowa, NJ, USA, 2008; pp. 275–293.
40. Ratnasingham, S.; Hebert, P.D.N. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS ONE* **2013**, *8*, e66213. [CrossRef] [PubMed]
41. Jeanmougin, F.; Thompson, J.D.; Gouy, M.; Higgins, D.G.; Gibson, T.J. Multiple sequence alignment with Clustal, X. *Trends Biochem. Sci.* **1998**, *23*, 403–405. [CrossRef] [PubMed]
42. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molec. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
43. De Prins, J.; De Prins, W. Global Taxonomic Database of Gracillariidae. Available online: http://www.gracillariidae.net/ (accessed on 27 January 2023).
44. Dormann, C.F.; Fruend, J.; Gruber, B.; Beckett, S.; Devoto, M.; Felix, M.F.; Gabriel, M.F.; Iriondo, J.M.; Opsahl, T.; Pinheiro, R.B.P.; et al. Visualising Bipartite Networks and Calculating Some (Ecological) Indices. Version 2.18. Available online: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://cran.r-project.org/web/packages/bipartite/bipartite.pdf (accessed on 3 February 2023).
45. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/ (accessed on 24 January 2023).
46. Dormann, C.F.; Gruber, B.; Fruend, J. Introducing the bipartite Package: Analysing Ecological Networks. *R News* **2008**, *8*, 8–11.
47. Zlobin, V.V. Review of mining flies of the genus Cerodontha. IV. Subgenus Poemyza (Diptera: Agromyzidae). *Zoosyst. Rossica* **1993**, *1*, 117–141.
48. Süss, L. *Cerodontha (Poemyza) unisetiorbita* Zlobin (Diptera Agromyzidae) nuova per l´Europa. *Boll. Zool. Agrar. Bachic. Ser. II* **2001**, *33*, 73–77.
49. Hannover, R. Available online: http://www.diptera.info/forum/viewthread.php?forum_id=4&thread_id=56490 (accessed on 27 January 2023).
50. Černý, M.; Roháček, J. *Cerodontha (Poemyza) unisetiorbita* Zlobin, 1993 (Diptera: Agromyzidae), a leaf-miner on bamboo: First records from Central Europe. *Acta Musei Sil. Sci. Nat.* **2015**, *64*, 91–96.
51. British Leafminers. Cerodontha Unisetiorbita Zlobin. 1993. Available online: http://www.leafmines.co.uk/html/Diptera/C.unisetiorbita.htm (accessed on 27 January 2023).
52. Heckford, R.J. *Cerodontha (Poemyza) unisetiorbita* Zlobin (Diptera, Agromyzidae), an adventive species new to the British Isles. *Dipterists. Digest.* **2017**, *24*, 49–51.
53. Kirichenko, N.; Sukachev Institute of Forest, Siberian Branch of the Russian Academy of Sciences, Federal Research Center "Krasnoyarsk Science Center SB RAS", Krasnoyarsk, Russia. Detection of leaf mines of Cerodontha unisetiorbita on bamboo in the Parc Floral de La Source, Orleans. Unpublished work. 2014.
54. Bernardo, U.; Sasso, R.; Gebiola, M.; Viggiani, G. First record of a walnut shield bearer *Coptodisca* (Lepidoptera: Heliozelidae) in Europe. *J. Appl. Entomol.* **2011**, *136*, 638–640. [CrossRef]
55. Takács, A.; Szabóky, C.; Tóth, B.; Bozsó, M.; Kutas, J.; Molnár, S.; Richter, I. Nearctic walnut leafminers invade Europe: First *Coptodisca lucifluella* (Clemens, 1860) and now *Coptodisca juglandiella* (Chambers, 1874) (Lepidoptera, Heliozelidae). *Nota Lepidopterol.* **2020**, *43*, 77–93. [CrossRef]
56. North American Moth Photographers Group. *Coptodisca Lucifluella (Clemens, 1860)*; Mississippi State University: Starkville, MI, USA, 2013. Available online: http://mothphotographersgroup.msstate.edu/large_map.php?hodges=247 (accessed on 27 January 2023).

57. Bernardo, U.; van Nieukerken, E.J.; Sasso, R.; Gebiola, M.; Gualtieri, L.; Viggiani, G. Characterization, distribution, biology and impact on Italian walnut orchards of the invasive North-American leafminer *Coptodisca lucifluella* (Lepidoptera: Heliozelidae). *Bull. Entomol. Res.* **2015**, *105*, 210–224. [CrossRef]
58. Takács, A.; Szabóky, C.; Kutas, J. A dióaknázó fényesmoly (*Coptodisca lucifluella* Clemens, 1860 Lepidoptera—Heliozelidae) magyarországi megjelenése. [The appearance of the walnut leafminer (*Coptodisca lucifluella* Clemens, 1860 Lepidoptera—Heliozelidae) in Hungary]. *Növényvédelem* **2017**, *78*, 539–541. (In Hungarian)
59. Pályi, B.; Takács, A.; Szabóky, C.S. Új diókártevő Kárpátalján. [New pest of walnut in Subcarpathia]. *Kárpátaljai Vállalkozók Lapja* **2019**, *4*, 16. (In Hungarian)
60. Tomov, R. First Records of the Walnut Shield Bearer *Coptodisca lucifluella* (Clemens, 1860) (Lepidoptera: Heliozelidae) in Bulgaria. *Acta Zool. Bulg.* **2020**, *72*, 697–700.
61. Chireceanu, C.; Mustățea, R.-V.; Teodoru, A. The walnut shield bearer *Coptodisca lucifluella* (Clemens, 1860) (Lepidoptera: Heliozelidae)—The first record in Romania. *Rom. J. Plant Prot.* **2022**, *XV*, 15–23. [CrossRef]
62. Gregor, F.; Patočka, J. Die Puppen der mitteleuropischen Lithocolletinae. Mitteilungen des internationalen entomologischen Vereins. *Supplement* **2001**, *8*, 186. (In German)
63. Huemer, P. *Die Schmetterlinge Österreichs (Lepidoptera). Systematische und Faunistische Checkliste*; Studiohefte 12; Tiroler Landesmuseen: Innsbruck, Austria, 2013; p. 304.
64. Baryshnikova, S.V. Gracillariidae. In *Catalogue of the Lepidoptera of Russia*, 2nd ed.; Sinev, S.Y., Ed.; Zoological Institute RAS: St. Petersburg, Russia, 2019; pp. 36–43. (In Russian)
65. Kuznetzov, V.I. The family Gracillariidae (Lithocolletidae). In *Insects and Mites—The Pests of Agricultural Plants. III (2). Lepidoptera*; Kuznetsov, V.I., Ed.; Nauka: St. Petersburg, Russia, 1999; pp. 9–46. (In Russian)
66. Leraut, P. Contribution à l'étude des Lépidoptéres de la Brie occidentale. *Alexanor* **2003**, *22*, 3–237.
67. Huisman, K.J.; Koster, J.C.; van Nieukerken, E.J.; Ulenberg, S.A. Microlepidoptera in Nederland in 2003. *Entomol. Ber.* **2005**, *65*, 30–42.
68. Aarvik, L.; Bengtsson, B.Å.; Elven, H.; Ivinskis, P.; Jürivete, U.; Karsholt, O.; Mutanen, M.; Savenkov, N. Nordic-Baltic Checklist of Lepidoptera. *Nor. J. Entomol.* **2017**, *3*, 1–236.
69. Emmet, A.M. Phyllocnistidae. In *The Moths and Butterflies of Great Britain and Ireland*; Heath, J., Emmet, A.M., Eds.; Harley Books: Devon, UK, 1985; Volume 2, pp. 363–368.
70. Huemer, P.; Tarmann, G. Die Schmetterlinge Österreichs. Systematisches Verzeichnis mit Verbreitungsangaben für die einzelnen Bundesländer. Veröff. tirol. *Landesmus Ferdinandeum Innsbr.* **1993**, *5*, 1–224. (In German)
71. Langmaid, J.; Corley, M. *Phyllocnistis ramulicola* sp.nov (Lepidoptera: Gracillariidae) in England and Portugal. *Entomol. Gazette* **2007**, *58*, 227–237.
72. Tokár, Z.; Laštůvka, A.; Pastorális, G.; Šumpich, J.; Štefanovič, R.; Elsner, G. Nové druhy drobných motýl'ov (Microlepidoptera) pre faunu Slovenska. *Entomofauna Carpathica* **2021**, *33*, 1–20. (In Slovenian)
73. Mutanen, M.; Kivelä, S.M.; Vos, R.A.; Doorenweerd, C.; Ratnasingham, S.; Hausmann, A.; Huemer, P.; Dincă, V.; van Nieukerken, E.J.; Lopez-Vaamonde, C.; et al. Species-level para- and polyphyly in dna barcode gene trees: Strong operational bias in European Lepidoptera. *Syst. Biol.* **2016**, *65*, 1024–1040. [CrossRef] [PubMed]
74. Thomas, E. *Phyllocnistis ramulicola* Langmaid & Corley. 2007. Available online: https://oreina.org/artemisiae/observatoire/index.php?module=fiche&action=fiche&d=micro&id=814417 (accessed on 27 December 2022).
75. Beránek, J. First records of *Leptoglossus occidentalis* Heidemann, 1910 (Heteroptera: Pentatomorpha: Coreidae) in the Czech Republic. *Plant Protect. Sci.* **2007**, *43*, 165–168. [CrossRef]
76. Matošević, D. Box Tree Moth (*Cydalima perspectalis*, Lepidoptera; Crambidae), new invasive insect pest in Croatia. *South-East Eur. For.* **2013**, *4*, 89–94. [CrossRef]
77. Baranchikov, Y.N.; Seraya, L.G.; Grinash, M.N. All European ash species are susceptible to Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)—A Far Eastern Invader. *Sib. For. J.* **2014**, *6*, 80–85. (In Russian)
78. Kerchev, I.A.; Krivets, S.A. An attack of *Ips amitinus* (Coleoptera: Curculionidae: Scolytinae) on arboretum in West Siberia: New host of invasive bark beetle among exotic conifers. *J. Asia-Pac. Entomol.* **2021**, *24*, 148–152. [CrossRef]
79. Rudoi, V.V.; Vinokurov, N.N.; Korshunov, A.V.; Efimov, D.A.; Kirichenko, N.I. New records of native and alien true bugs (Heteroptera) from Kemerovo Region, Western Siberia, Russia. *Acta Biol. Sibirica* **2022**, *8*, 483–506. [CrossRef]
80. Gomboc, S. Novi vrsti listnih zavrtačev v Sloveniji [New records of leaf miners in Slovenia]. In Proceedings of the Zbornik Predavanj in Referatov 6, Slovenskega Posvetovanja o Varstvu Rastlin, Zreče, Slovenia, 4 June 2003; Društvo za Varstvo Rastlin Slovenije: Zreče, Slovenia, 2003; pp. 423–429. (In Slovenian)
81. Seljak, G. The dynamics of introduction of alien phytophagous insects and mites into Slovenia. *Acta Entomol. Slov.* **2013**, *21*, 85–122. (In Slovenian)
82. Seljak, G. Six new alien phytophagous insect species recorded in Slovenia in 2011. *Acta Entomol. Slov.* **2012**, *20*, 31–44. (In Slovenian)
83. Mastnak, M.; Arboretum Volčji Potok, Gorenjska region, Slovenia. About absence of some woody plants from live collection of the Arboretum Volčji Potok. Personal communication, 2023.
84. Kranzfelder, P.; Ekrem, T.; Stur, E. Trace DNA from insect skins: A comparison of five extraction protocols and direct PCR on chironomid pupal exuviae. *Mol. Ecol. Resour.* **2016**, *16*, 353–363. [CrossRef]

85. Mlynarek, J.J.; Kim, J.-H.; Heard, S.B. Identification of leaf-mining insects via DNA recovered from empty mines. *FACETS* **2016**, *1*, 217–224. [CrossRef]
86. Jordan, M.P.; Langmaid, J.R.; Doorenweerd, C. Morphological difference between upperside and underside leaf-mining larvae of *Phyllocnistis unipunctella* (Stephens, 1834) (Lep.: Gracillariidae) and its changing phenology. *Entomol. Rec. J. Var.* **2016**, *128*, 121–127.
87. PM 3/91(1) Sentinel woody plants: Concepts and application. *Bull. OEPP/EPPO Bull.* **2020**, *50*, 429–436. [CrossRef]