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An Overview of Gracillariidae Leaf Mining Moths in Slovenia with New Records for the Country

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Abstract: Gracillariidae is one of the richest families of leaf mining moths, known by its ornamental and orchard plant pests expanding their primary ranges. We here provide a revised checklist of Gracillariidae (Lepidoptera) of Slovenia that presently accounts for 123 species. Among them, four species, *Dialectica imperialella* (Zeller, 1847), *Phyllonorycter abrasella* (Duponchel, 1843), *Ph. trifoliella* (Gerasimov, 1933), and *Phyllocnistis valentinensis* M. Hering, 1936, are new records for the country. The distribution of the other three species in Slovenia, *Caloptilia honoratella* (Rebel, 1914), *Dialectica scalariella* (Zeller, 1850), and *Ph. messaniella* (Zeller, 1846), is clarified. For the above seven species, short synopses of the bionomics; a current range; and images of biotopes, adult moths, and their genitalia are provided. For *P. valentinensis* sampled at a larval stage, the DNA barcode was obtained to confirm the species identification. Given the known distribution of some of the newly recorded moths in Europe, we believe that these species do not represent a new invasion but rather are discoveries of native species, except for the newly documented *Ph. trifoliella* in Slovenia, which is likely to be an alien species for the country.

Keywords: leafminers; gracillariids; DNA barcoding; novel records; species origin; alien species; Slovenia

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1. Introduction

The interest in the fauna, geography, and trophic relationships of leaf-mining micromoths of the family Gracillariidae has increased significantly over the last decades [1–3]. This family is commonly known by its orchard or ornamental pests, which are expanding their relatively small primary ranges and colonising new countries and/or continents [4–7].

The accurate identification of family representatives found in immature stages became possible thanks to DNA barcoding [3,8,9]. Even hundred-year-old larval remnants found in historical herbaria could be identified reliably to species level [10].

The Gracillariidae fauna have been studied in Slovenia opportunistically; species were reported either through collecting leaf mines or occasionally by sampling adult moths. Summarising 30 years of research, Maček [11] published a list of leaf-mining insects of Slovenia including 88 gracillariid species. In the following years, several reports listed other gracillariids new to the Slovenian fauna [12–16]. As a result, in 2010 the first checklist of micromoths of Slovenia was compiled, containing 100 gracillariid species [17]. Additionally, *Gracillaria loriolella* Frey, 1881 was reported as new to the country [18], *Phyllonorycter issiki*i (Kumata, 1963) [19] and *Phyllocnistis citrella* Stainton, 1856 [20]. *Callisto basistrigella* Huemer, Deutsch & Triberti, 2015 described from Austria is also known to occur in Slovenia [21]. Furthermore, seven other species, *Caloptilia honoratella*, *Cupedia cupediella* (Herrich-Schäffer, 1855), *Dialectica scalariella*, *Parornix ornatella* Triberti, 1981, *Phyllonorycter aemula* Triberti, Deschka & Huemer, 1997, *Ph. monspessulanella* (Fuchs, 1897),

and *Ph. messaniella* were mentioned for Slovenia [22], however, without an indication of the localities where they were found.

Within the framework of the DNA barcoding campaign of the European Gracillariidae, 175 specimens representing 55 species from Slovenia were genetically characterized [3]. In this paper, another eight species were recorded for the Slovenian fauna: *Ph. cerasinella* (Reutti, 1852), *Ph. deschkai* Triberti, 2007, *Ph. fiumella* (Krone, 1910), *Ph. froelichiella* (Zeller, 1839), *Ph. insignitella* (Zeller, 1846), *Ph. ochreojunctella* (Klimesch, 1942), *Ph. parisiella* (Wocke, 1848), and *Sabulopteryx limosella* (Duponchel, 1844). Including the above-listed, 119 Gracillariidae species have been known for Slovenia.

We continue exploring this taxonomically rich micromoth family. Here we provide an up-to-date checklist of gracillariids, report on the findings of four species new to the Slovenian fauna, clarify the distribution of other three poorly studied species in the country, overview their bionomics, discuss the occurrence in the country, and analyse DNA barcoding data for *Phyllocnistis valentinensis*. Additionally, we illustrate adults and genitalia of the documented species in Slovenia.

2. Materials and Methods

2.1. Field Sampling

The gracillariidae adults were collected in 24 localities in Slovenia (Figure 1, Table S1) during the years 2000–2022. Most of them were attracted to UV [ultra-violet, 360 nm] light traps (the pyramidal-shape tents with two UV tube super actinic Philips TLD 05/15 W bulbs powered with lead acid batteries). The specimens were set and stored in the private collection of the first author.

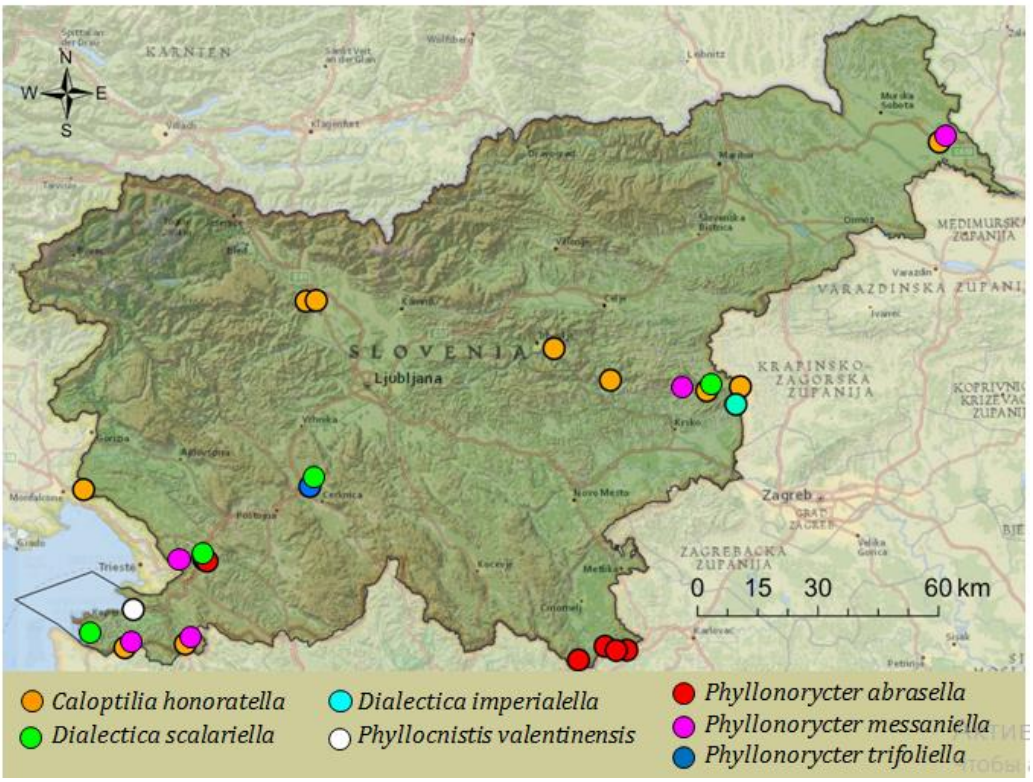

Figure 1. The localities where the gracillariid species were recorded in Slovenia (see Table S1 for geocoordinates).

White willow, *Salix alba* (Salicaceae) was surveyed for the presence of *Phyllocnistis* leaf mines in Škocjanski zatok Nature Reserve (Slovenia) at the end of October 2019 and in July and October 2021. Overall, six willow trees (3–4 m height) were examined, mostly

in the lower part of the tree crowns. Fifty mined leaves were collected in 2019; only five leaves with the mines were found in 2021 in the same locality. One larva was dissected from the leaf mine in 2019 and preserved in 95% ethanol for DNA barcoding. The leaf mines collected in 2021 were stored in the fridge (at +6 °C) for allowing the pupae to diapause. The herbarium specimens are stored in the collection of the second author at the Sukachev Institute of Forest SB RAS (Krasnoyarsk, Russia).

Detailed field sampling data including a date, time, locality, and coordinates; biotope characteristics; a host plant; etc. were collected and stored in the database of the first author. Sampled localities were mapped in WGS 1984 coordinate system using ESRI ArcGIS Pro 2.9 software. Layers of the Environmental Agency of the Republic of Slovenia, the Surveying and Mapping Authority of the Republic of Slovenia and Esri, available in the form of geographic web services, were used. The coordinates are given in decimal degrees in Table S1.

2.2. Morphological Identification

The identification of adult moths was based on forewing pattern and the characters of male and female genitalia. Genitalia dissections and permanent slide preparation were performed following Robinson [23]. Where necessary to reveal cryptic features, the genitalia were stained with eosin solution for better contrast. Genitalia were mounted on glass slides in Euparal and subsequently stored in the collection of the first author.

Gracillariidae species were identified using the key [24] and compared with the illustrations of forewing patters and male/female genitalia [25,26].

2.3. DNA Barcoding

One larva of *Phyllocnistis valentinensis* sampled from leaf mine on *Salix alba* was DNA barcoded. The mitochondrial cytochrome oxidase I gene (mtDNA COI, 658 bp) was sequenced at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) following the standard high-throughput protocol [27].

In addition, five DNA barcodes of *P. valentinensis* from other European countries (Austria, Bulgaria, France, Greece and The Netherlands), mined from the Barcode of Life Data Systems (BOLD), were included in the analysis, as well as nine DNA barcodes of other Salicaceae-feeding *Phyllocnistis* species from Eurasia, publicly available in BOLD: *P. asiatica* Martynova, 1955 (4 sequences), *P. canariensis* Hering, 1927 (1), *P. gracilistylella* Kobayashi, Jinbo & Hirowatari, 2011 (1), *P. ramulicola* Langmaid, Corley, 2007 (1), *P. saligna* (Zeller, 1839) (1), *Phyllocnistis* sp. (the candidate new species from the Russian Far East) (1). The DNA barcode of *Phyllonorycter populifoliella* from Russia, obtained by the second author, was used for tree-rooting. The specimen data are given in Table S2.

The DNA sequence of the Slovenian *P. valentinensis*, with the voucher data, image and trace files, and the sequences involved into the analysis, their Barcode Index Numbers (BINs) and GenBank accession numbers can be found in BOLD [8] using the link dx.doi.org/10.5883/DS-GRSLO. A Maximum Likelihood (ML) tree was constructed using the Maximum Likelihood method, the Kimura two-parameter model and a bootstrap method (1000 iterations), and intra- and interspecific genetic distances were assessed using the same approaches in MEGA X [28].

2.4. Checklist Compilation

The checklist of Gracillariidae of Slovenia was revised based on national and international publications which came out in 1763–2021 [3,11–22,29–80] and updated with the novel records provided in the present paper. The checklist includes the species names and whether or not the species are native to the country. The subfamilies and genera are presented in the list in a systematic way: species names are given in the alphabetic order following Lepiforum [81]. The subfamily classification of Gracillariidae based on molecular genetic and morphological data [1] was used. The following abbreviations of

gracillariid genera are used in the text: *Ph.—Phyllonorycter* and *P.—Phyllocnistis* to distinguish between the two names. Below, the species are listed systematically as per Lepiforum [81].

2.5. Imaging

The photographs of the moths and their genitalia, as well as leaf mines were taken using a smart phone digital camera Xiaomi 11 Lite (China, Beijing, Xiaomi Corporation) through Olympus SZ51 stereomicroscope (Japan, Tokyo, Olympus Corporation) and Bio trinocular panchromatic 2002 microscope – EUM-2000 (China, Chongqing, Chongqing Optec Instrument Co., Ltd.). The photographs of the biotopes were taken with Sony Alpha 55 and Sony Alpha 7 ii cameras (Japan, Tokyo, Sony Corporation). The images were revised using the Adobe Photoshop CS6 program.

3. Results

3.1. The Gracillariidae Checklist of Slovenia

The revised checklist of Gracillariidae of Slovenia account 123 species. Among them there are the representatives of seven subfamilies (Acrocercopinae, Gracillariinae, Ornixolinae, Parornichinae, Phyllocnistinae, Lithocolletinae, and Oecophyllembiinae) and 19 genera (*Acrocercops, Aspilapteryx, Callisto, Caloptilia, Calybites, Cameraria, Cupedia, Dialectica, Euspilapteryx, Gracillaria, Macrosaccus, Metriochroa, Micrurapteryx, Ornixola, Parectopa, Parornix, Phyllocnistis, Phyllonorycter, Sabulopteryx*) (Table S3). The majority of species are from the genus *Phyllonorycter* (73 species, i.e., 59.3% of all Gracillariidae known in Slovenia), followed by *Caloptilia* (16 species, i.e., 13%) and *Parornix* (14 species, i.e., 11.4%) (Table S3). The remaining 20 species (16.3%) are the representatives of other 15 genera. Overall, 113 species (92%) are native to Slovenia (Table S3). Ten species (i.e., 8%), namely *Caloptilia azaleella* (Brants, 1913), *Cameraria ohridella* Deschka & Dimić, 1986, *Macrosaccus robiniella* (Clemens, 1859), *Parectopa robiniella* Clemens, 1863, *Phyllocnistis citrella* Stainton, 1856, *P. vitegenella* Clemens, 1859, *Phyllonorycter issikii* (Kumata, 1963), *Ph. leucographella* (Zeller, 1850), *Ph. platani* (Staudinger, 1870), and *Ph. trifoliella*, are of alien origin.

In the period from 2000 to 2022, overall 54 adult specimens of seven species, *Caloptilia honoratella*, *Dialectica imperialella*, *D. scalariella*, *Phyllocnistis valentinensis*, *Phyllonorycter abrasella*, *Ph. messaniella*, and *Ph. trifoliella*, were collected across the country from 24 different localities (Figure 1, Table S1). Among them four species, *D. imperialella*, *P. valentinensis*, *Ph. abrasella*, and *Ph. trifoliella*, were recorded as new to the Slovenian Lepidoptera fauna. The distribution of other three species, *C. honoratella*, *D. scalariella*, *Ph. messaniella*, was intensively explored in the country. *Dialectica scalariella* and *Ph. abrasella* were sampled in warmer regions of Slovenia (in southern and submediterranean parts of the country). *Phyllonorycter trifoliella*, *D. imperialella* and *P. valentinensis* were collected in a unique locality each: the first two were sampled in an inland region and the third in a coastal area of Slovenia (Figure 1). *Caloptilia honoratella* and *Ph. messaniella* were trapped in several localities across Slovenia (Figure 1). All species were attracted to UV light traps, except *P. valentinensis*, which was sampled in the larval stage from a leaf mine and reared to adult.

3.2. The Graillariids New to Slovenia

***Dialectica imperialella* (Zeller, 1847)**

(Figure 2A–C)

Material examined. Slovenia. 1 male moth, Štajerska, Kozjansko Regional Park, Župjek, Gradišče, 46.0117 N, 15.6881 E, alt. 196 m, 11. ix. 2012, S. Gomboc leg, gen. prp. nr. GOS Euparal 514.

Bionomics. A single moth was attracted to a light trap in September in the abandoned sand pit in the ruderal area bordering mixed deciduous forest, with oak (*Quercus* spp.), beech (*Fagus sylvatica*) and willows (*Salix* spp.), and with *Pulmonaria* spp. in the ground layer (Figure 2). Young larvae live in hardly visible epidermal tunnels on the lower side

of the leaf; later they are often found in common blotch full-depth mines occupying a significant part of a leaf; pupation is external [82].

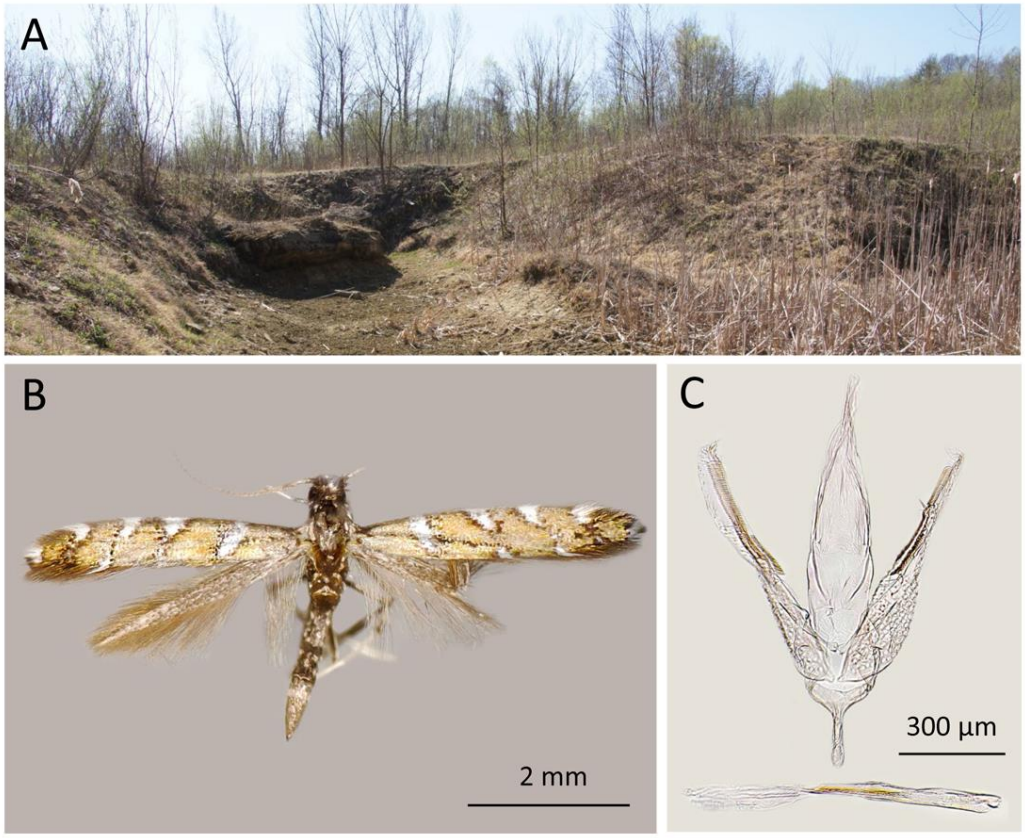

Figure 2. *Dialectica imperialella*. (**A**) habitat, Kozjansko Regional Park, Župjek, Gradišče, 31.iii.2012; (**B**) male, Kozjansko Regional Park, Župjek, Gradišče, 11.ix.2012; (**C**) male genitalia, same specimen, gen. prp. nr. GOS Euparal 514.

Host plants. Oligophagous on *Aegonychon purpurocaeruleum*, *Echium plantagineum*, *Lithospermum officinale*, *Pentaglottis sempervirens*, *Pulmonaria angustifolia*, *P. officinalis*, *Symphytum officinale* (Boraginaceae) [82].

Distribution. Widely distributed across Europe (Austria, Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, Hungary, Latvia, Lithuania, Luxembourg, Poland, Romania, Serbia, Slovakia, Spain, Switzerland, the Netherlands, UK, Ukraine) [83,84], Estonia [85], Russia (European and Asian parts), Turkey, and Turkmenistan [24]. First record for Slovenia (present study).

Remarks. According to its wide natural range in Europe, *D. imperialella* is most likely native to Slovenia. So far, it was documented only in a single location, the Kozjansko Regional Park, despite being intensely surveyed across the country.

***Phyllonorycter abrasella* (Duponchel, 1843)**

(Figure 3A–C)

Material examined. Slovenia: 1 adult moth, Primorska, Škocjan Caves Regional Park, Naklo, meadows south of the village, 45.6587 N, 13.9946 E, alt. 397 m, 29.vii.2018, S. Gomboc leg.; 1 adult, Primorska, Škocjan Caves Regional Park, Matavun, Mali dol, Illyrian meadows and sub-Mediterranean deciduous forest along the forest road, 45.6648 N, 13.9832 E, alt. 438 m, 05.vi.2019, S. Gomboc, B. Zadravec leg.; 1 adult, Bela Krajina, Kolpa Landscape Park, Vinica, Golek pri Vinici, Žeželj, Klanec, forest road, 45.4722 N, 15.2650 E, alt. 301 m, 02.v.2021, S. Gomboc leg., gen. prp. nr. GOS Euparal 507; 6 adults, Bela Krajina, Kolpa Landscape Park, Zilje pri Vinici, church of Sv. Anton, 45.4631 N, 15.3005 E, alt. 294 m, 09.vii.2021, S. Gomboc leg.; 2 adults, Bela Krajina, Kolpa Landscape Park, Preloka,

Spodnja Preloka, dry meadows at forest edge, 45.4636 N, 15.3343 E, alt. 241 m, 15.vii.2021, S. Gomboc leg.; 3 adults, Bela Krajina, Kolpa Landscape Park, Damelj ob Kolpi, 45.4418 N, 15.1801 E, alt. 207 m, 24.vii.2021, S. Gomboc leg., gen. prp. nr. GOS Euparal 508; 1 adult, Škocjan Caves Regional Park, Matavun, sinkhole Globočak at the entrance to the cave, 45.6615 N, 13.9807 E, alt. 387 m, 25.vii.2019, S. Gomboc leg.; 4 adults, Bela Krajina, Kolpa Landscape Park, Zilje pri Vinici, church of Sv. Anton, 45.4631 N, 15.3005 E, alt. 294 m, 31.vii.2021, S. Gomboc leg.

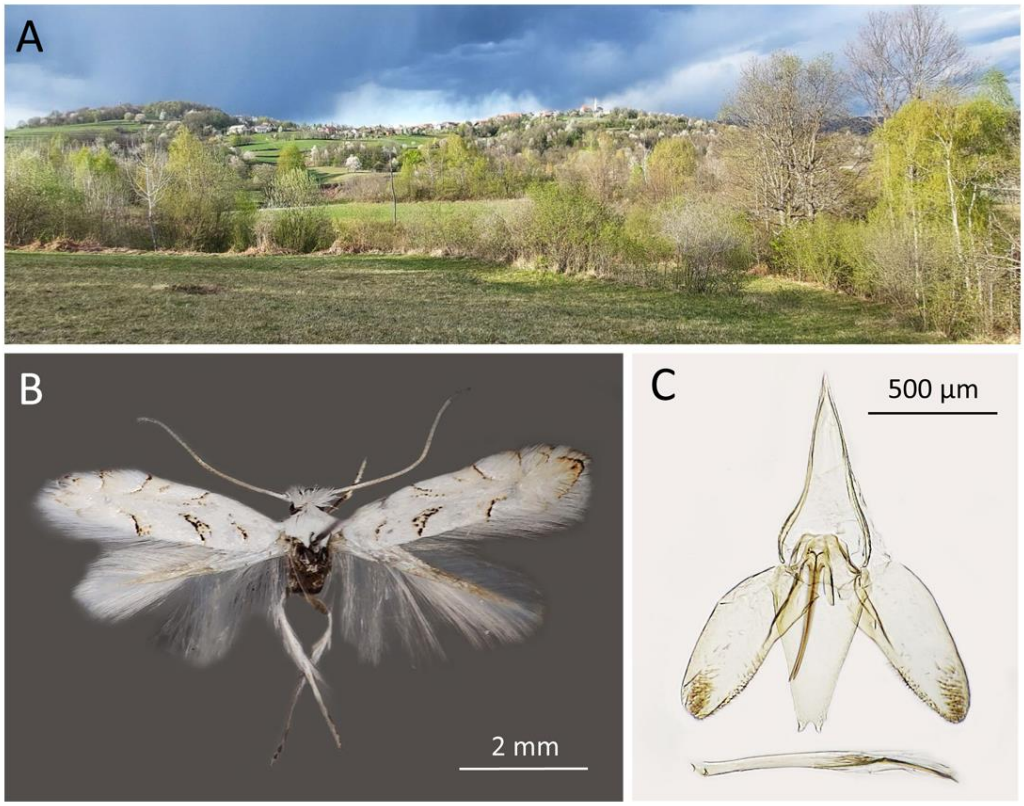

Figure 3. *Phyllonorycter abrasella.* (**A**) habitat, Bela Krajina, Kolpa Landscape Park, Preloka, Spodnja Preloka, dry meadows with forest edge, 10.iv.2022; (**B**) male moth, Bela Krajina, Kolpa Landscape Park, Vinica, Golek pri Vinici, Žeželj, Klanec, forest road, 2.v.2021; (**C**) male genitalia, same specimen, gen. prp. nr. GOS Euparal 507.

Bionomics. The moths were attracted to light traps from the beginning of May to the end of July, suggesting a prolonged flight period. All sites where specimens were collected were south-facing oak forests on limestone, predominated by *Quercus cerris* and *Q. robur* (Figure 3). Larvae live in a lower surface tentiform mine, generally in the centre of the leaf; pupation occurs in the mine [82].

Host plants. Monophagous on *Quercus robur, Q. petraea, Q. cerris, Q. pubescens, Q. ithaburensis* subsp.*macrolepis, Q. trojana* [82,84]; just on *Q. cerris* [22].

Distribution. Europe (Albania, Austria, Bulgaria, Croatia, Czech Republic, France, Hungary, Italy, North Macedonia, Romania, Slovakia, Turkey, Ukraine) [84]. Presence in European Russia [86] is not confirmed [83]. First record for Slovenia (present study).

Remarks. Considering its rather wide distribution in Europe and feeding on oaks, the species is most likely native to Slovenia, though not widely spread in the country. It was especially frequent in the Kolpa and Škocjan Caves Regional Park in our study. The species was observed in the habitats with presence of *Q. cerris*.

***Phyllonorycter trifoliella* (Gerasimow, 1933)**

(Figure 4A–D)

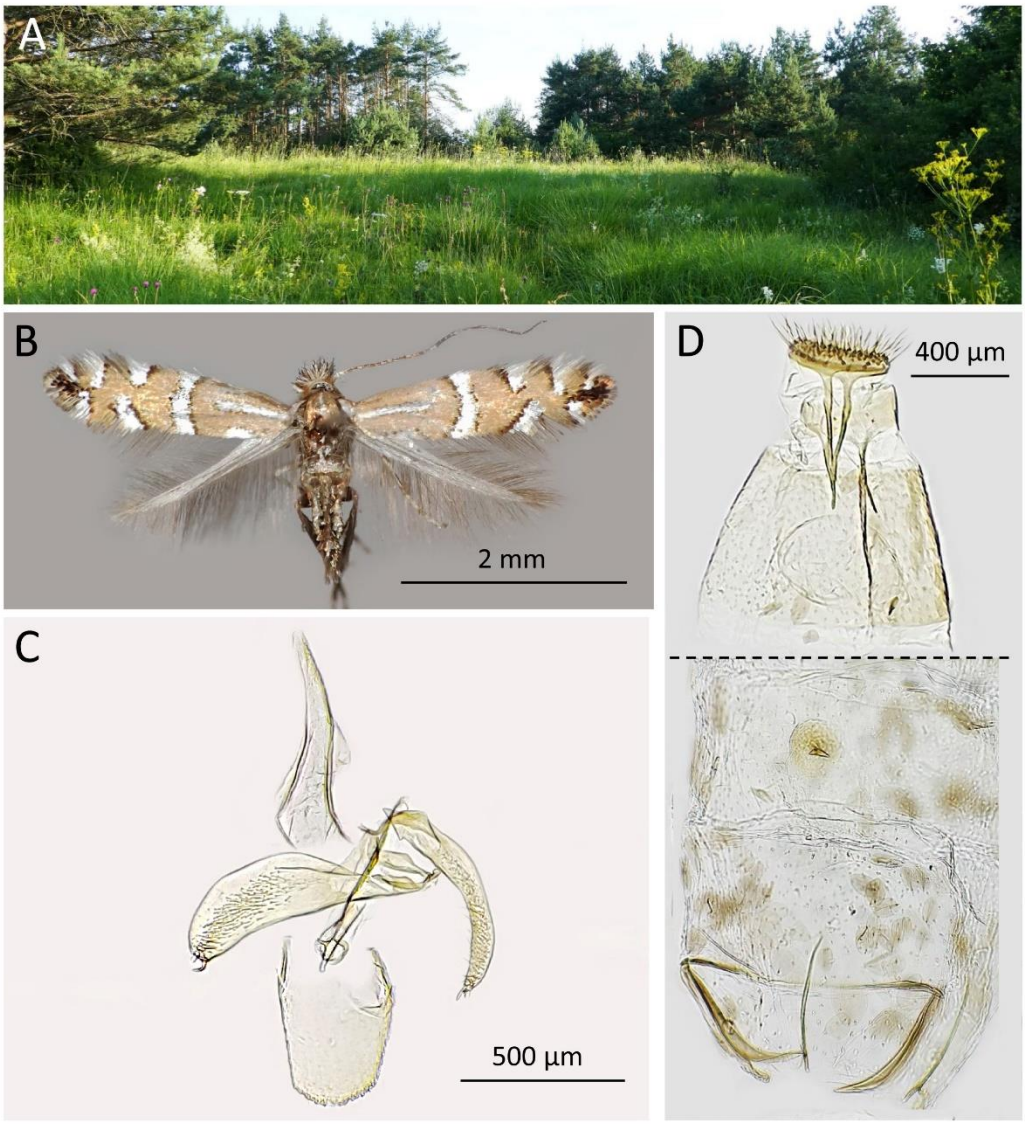

Figure 4. *Phyllonorycter trifoliella.* (**A**) habitat, Notranjska, Menišija, Rakek, Rjava luža, 5.vii.2016; (**B**) male moth, Notranjska, Menišija, Rakek, Rjava luža, 18.viii.2008; (**C**) male genitalia, same specimen, gen. prp. nr. GOS Euparal 512; (**D**) female genitalia (provided for extra information, dissected from the Croatian specimen), Croatia, Krapinsko-zagorska županija, Ivančica, top of the hill, 30.viii.2014, prp. nr. GOS Euparal 511.

Material examined. Slovenia: 1 male, Notranjska, Menišija, Rakek, Rjava luža, 45.8282 N, 14.3208 E, alt. 660 m, 18.viii.2008, S. Gomboc leg, gen. prp. nr. GOS Euparal 512.

Bionomics. A single adult moth was attracted to a light trap in a dry karstic grassland surrounded by mixed forest dominated by *Pinus sylvestris* (Figure 4). The sampled area is characterized by moderate precipitation and climate, with cold winters and hot, dry summers. As it is a karst plateau, the nights in this part are cooler than in the lowland part of Slovenia. Larvae live in a lower surface tentiform mine, with strong folds, next on the main vein; pupation is within the mine [82].

Host plants. Oligophagous on Fabaceae (*Lathyrus roseus, Trifolium*) [24].

Distribution. Estonia, Latvia, Lithuania [84,87], Finland [88], Poland [89], Russia (the Black Sea cost, Sochi, Krasnaya Polyana—the type locality), Georgia [24]. First record for Slovenia (present study). Here we also make the first record for Croatia (Figure 4D).

Remarks. For several decades, the species was known exceptionally from the southern part of European Russia. At the end of the 20th—beginning of 21st centuries, it was found in the Baltic countries [87], where its origin remains unknown. Bearing in mind long

presence of the species in Southern Russia and its recent detection in a few European countries, the species could be non-native to Europe. Thus, we suggest that the species is alien to Slovenia. So far, the only specimen was collected in the karst area of Notranjska Regional Park in the continental Slovenia.

***Phyllocnistis valentinensis* M. Hering, 1936**

(Figures 5–7)

Material examined. Slovenia: 50 leaf mines from *Salix alba*, Primorska, Slovensko primorje, Koper/Capodistria, Škocjanski zatok Nature Reserve, pathway around the freshwater swamp, 45.5485 N, 13.7617 E; alt. 0.62 m, 29.ix.2019, S. Gomboc & N. Kirichenko leg.; 2 live larvae from the mines from *S. alba*, same locality and collection data including 1 larva DNA barcoded (sample ID VZ-20-09, process ID GPRU043-21); 10 leaf mines from *S.alba*, same locality, 19.xi.2021, S. Gomboc leg.; 1 female moth, same locality and collection data, 11.ii.2022 e.p., S. Gomboc. leg., gen. prp. nr. GOS Euparal 513.

Molecular genetic data. In BOLD, the DNA barcode of the specimen from Slovenia was identified as *P. valentinensis* (99.4–100% similarity with the DNA barcodes of this species from Central Europe) and corresponded to the BIN (BOLD:AAY4829) (Figure 5).

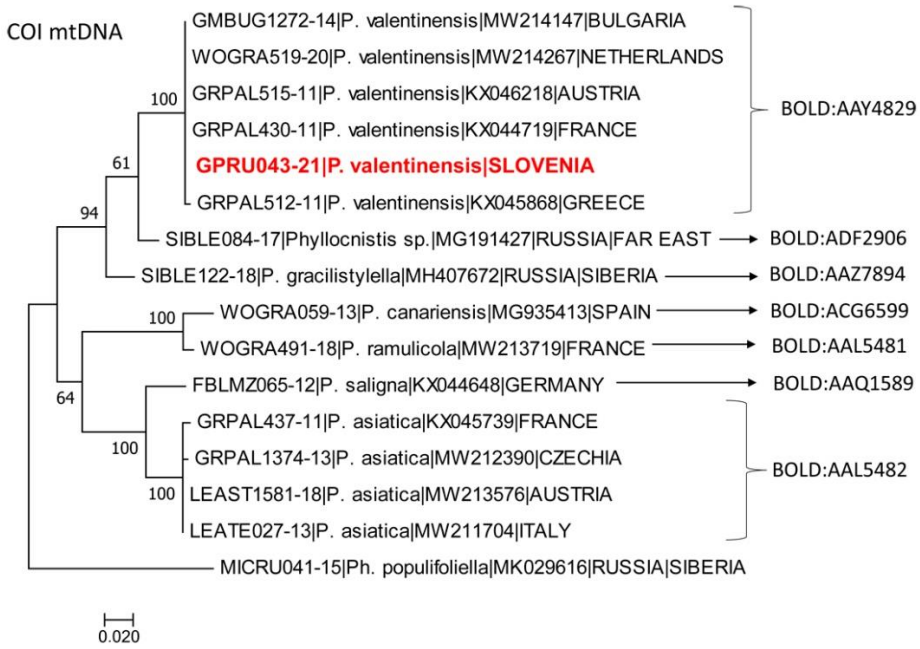

Figure 5. The maximum likelihood tree showing the relation of the Slovenian specimen of *Phyllocnistis valentinensis* to those from other parts of Europe and to other Salicaceae-feeding *Phyllocnistis* known from Eurasia, with species-specific BINs indicated. Each specimen is identified by its process ID, species name, GenBank accession number and the country. Branch lengths reflect the proportion to the number of substitutions per site. The bootstrap values are shown next to the branches.

The sequences of the other six *Salicaceae*-feeding species involved in the analysis corresponded to their specific BINs (Figure 5). In BOLD, these species, except *P. salinga*, are known by one unique BIN each, whereas *P. salinga* accounts four BINs [3]. Here we used the specimen assigned to BIN BOLD:AAQ1559, the most common in *P. saligna* populations in Central Europe.

On the maximum likelihood tree, the DNA barcode of the Slovenian *P. valentinensis* specimen formed one cluster with the sequences of this species from other five European countries (Figure 5). The sequences of other Salicaceae-feeding *Phyllocnistis*, i.e., *P. asiatica, P. canariensis, P. gracilistylella, P. ramulicola, P. saligna,* and *Phyllocnistis* sp., situated distantly from *P. valentinensis* cluster (Figure 5). In *P. valentinensis*, the maximal intraspecific

divergence reached 0.35% (Table 1). The DNA barcode of the Slovenia specimen was identical to those originating from Austria, Bulgaria, France, and the Netherlands (Figure 5).

The nearest neighbour to *P. valentinensis* was *Phyllocnistis* sp. (undescribed species from the Russian Far East) (4.7%), followed by *P. gracilistylella* (6.4 %), *P. saligna* (9.9%), *P. asiatica* (13.3%), *P. canariensis* (13.5 %), and *P. ramulicola* (13.7%) (Table 1). Among other species, the minimum interspecific distance was detected for *P. ramulicola* and *P. canariensis* (2.9%), whereas in majority of cases (71% of species pairs), the interspecific genetic distances exceeded 10% (Table 1).

Table 1. Intra- and interspecific genetic divergences in DNA barcode sequences among Salicaceae-feeding *Phyllocnistis* in Eurasia *. † Undescribed species from the Russian Far East.

№	Species of *Phyllocnistis*	1	2	3	4	5	6	7
1	*P. valentinensis*	[0.35]						
2	*Phyllocnistis* sp. †	4.7	[−]					
3	*P. gracilistylella*	6.4	5.4	[−]				
4	*P. saligna*	9.9	12.6	11.2	[−]			
5	*P. asiatica*	13.3	12.6	12.7	5.7	[0.4]		
6	*P. canariensis*	13.5	14	13.7	12.9	14.4	[−]	
7	*P. ramulicola*	13.7	13.6	12.8	12.3	14.3	2.9	[−]

*assessed based on Kimura 2-parameter (K2P) distances (%) for 15 DNA barcodes of 7 analyzed species: 6 DNA barcodes of *P. valentinensis*, 4—*P. asiatica*, and by one DNA barcodes of the remaining 4 species (*P. canariensis, P. gracilistylella, P. ramicola, P. saligna, Phyllocnistis* sp.). For each species pair, the minimal pairwise distance is shown. Values in square brackets represent maximal intraspecific distances (estimated for *P. valentinensis* and *P. asiatica* only); [−] no data because only a single specimen was sequenced.

Bionomics. Leaf mines are epidermal lower side tunnels with a thin but distinguishable central line of frass, often following the leaf margin and crossing secondary veins. The mine is associated with one leaf, and pupation occurs in the mine in the downward folded leaf margin. Up to four mines per leaf (not all fully developed) were recorded in our study. The leaf mines were abundant mostly on young willows *(Salix alba*). In 2019, the trees with the mines were present along the walkway in the freshwater swamp area surrounded by high reeds in 2019 in Škocjanski zatok Nature Reserve (Figures 6 and 7).

Interestingly, the majority of willows were infected at the time of survey (end of September 2019), with high mortality rate of larvae in mines, reaching up to 96% (the mortality factors were not studied here). However, in 2021, only five mined leaves were found in November in the same locality, suggesting that the population experienced the depression. We failed to find any leaf mines earlier in the season of 2021 (in June–July). Notably, in Belgium and the Netherlands, the mines are found on leaves from July to September [90].

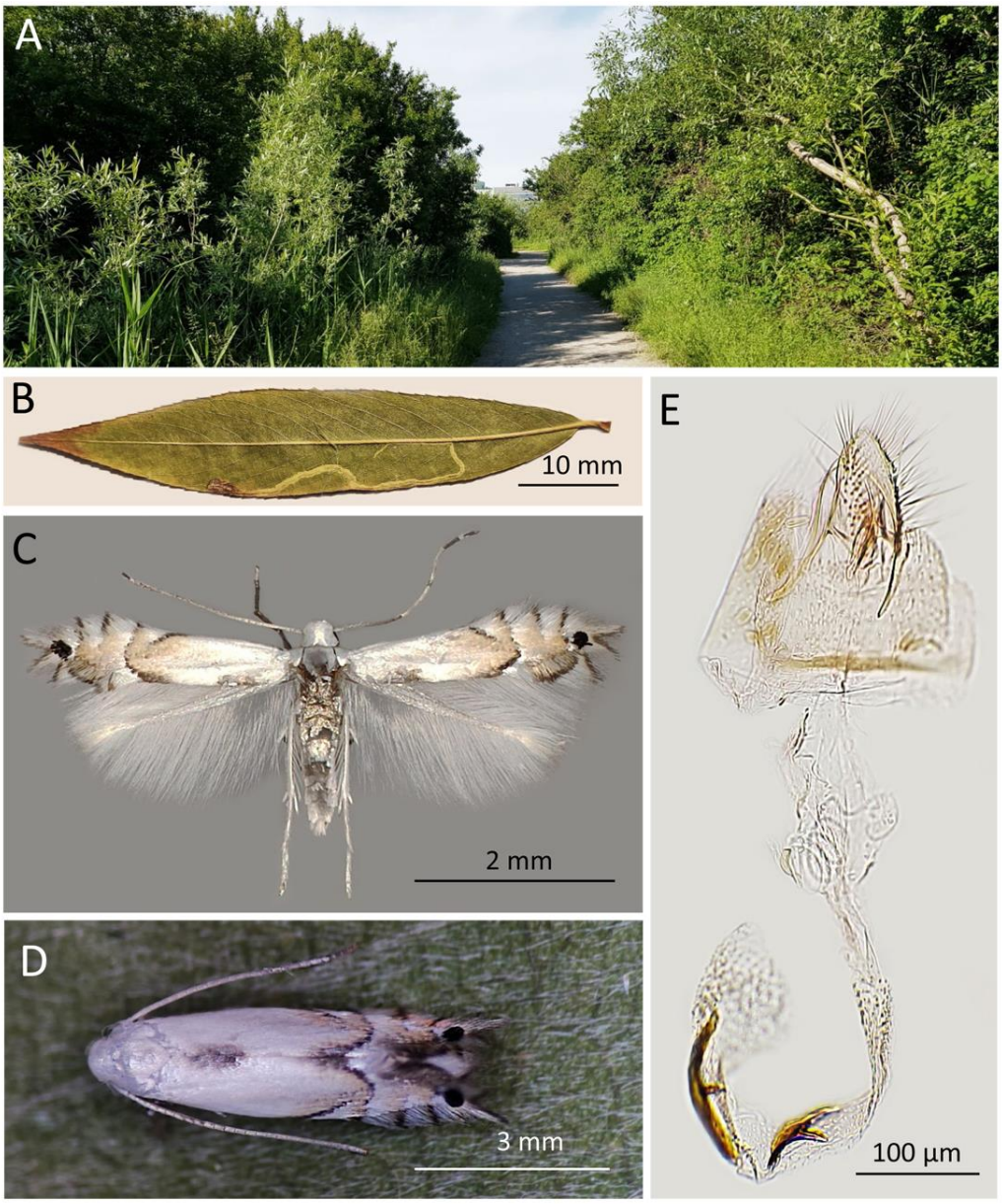

Figure 6. *Phyllocnistis valentinensis.* (**A**) habitat, Primorska, Slovensko primorje, Koper/Capodistria, Škocjanski zatok Nature Reserve, 28.v.2021; (**B**) low surface tunnel mine on *Salix alba*, same locality, 19.xi.2021; (**C**,**D**) female moth emerged from the mine, same locality, e.p. 11.ii.2022; (**E**) female genitalia, same specimen, gen. prp. nr. GOS Euparal 513.

Host plants. Monophagous on narrow leaved *Salix: S. alba, S. babylonica, S. × fragilis, S. purpurea, S. triandra* (Salicaceae) [82].

Distribution. Europe (Austria, Bulgaria, Greece, Germany, Italy, Spain) [82,91], Belgium and the Netherlands, Croatia, Slovakia, Czechia, France, Hungary [22,26,90,92–94]); Kazakhstan [85,94]; European part of Russia (questionable record) [83,90,95,96]. First record for Slovenia (present study).

Remarks. The species seems to be native to Slovenia bearing in mind that it is present in the neighbouring countries (Austria, Italy, Hungary, and Croatia). Notably, in Austria *P. valentinensis* was also found in the warmest region of the country [91]. The species seems to have a tendency to distribute northwards of Europe [90].

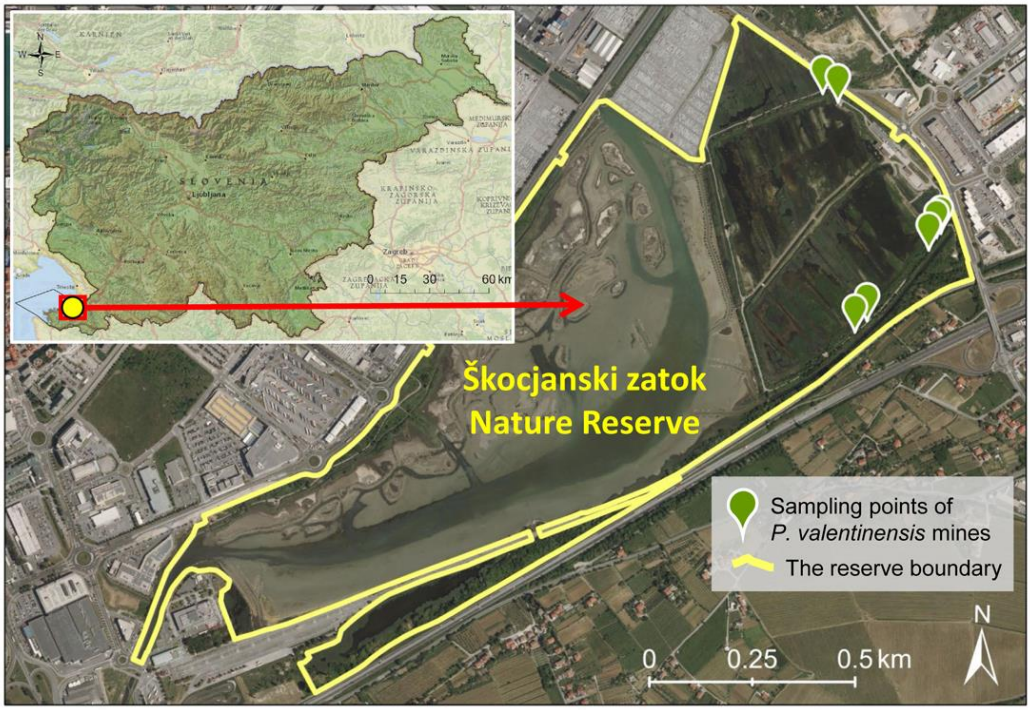

Figure 7. The collection site of *Phyllocnistis valentinensis* mines in Škocjanski zatok Nature Reserve, Koper, Slovenia.

3.3. Novel Distribution Data

The findings of *Caloptilia honoratella*, *Dialectica scalariella* and *Phyllonorycter messaniella* in Slovenia were mentioned by Laštůvka et al. [22]. However, the authors did not provide any specific data on sampled regions/localities, mentioning only the country (Slovenia). Below we provide the localities, where the species can be found in Slovenia and clarify the species bionomics.

***Caloptilia honoratella* (Rebel, 1914)**

(Figure 8A–D)

Material examined. Slovenia: 1 adult moth, Posavsko hribovje, Podkovk, Hrastnik, 46.1386 N, 15.1037 E, alt. 400 m, 29.ix.2000, S. Gomboc leg.; 1 adult moth, Prekmurje, Mala Polana, Črni log, 46.5922 N, 16.3565 E, alt. 166 m, 03.iv.2004, S. Gomboc leg.; 2 adult moths, Primorska, Podgorski Kras, Movraž pri Hrastovljah, Kraški rob, 45.4731 N, 13.9291 E, alt. 284 m, 28.x.2006, S. Gomboc leg.; 1 adult moth, Gorenjska, Ljubljanska kotlina, Šmarjetna gora, the top of the hill, 46.2438 N, 14.3363 E, alt. 643 m, 16.i.2011, S. Gomboc leg.; 1 adult moth, Gorenjska, Kranj, Sveti Jošt above Kranj, 46.2425 N, 14.3034 E, alt. 840 m, 08.ix.2011, S. Gomboc leg.; 2 adult moths, Sežanski Kras, Brestovica pri Komnu, 45.8146 N, 13.5967 E, alt. 26.7 m, 26.ix.2013, S. Gomboc, H. Deutsch, B. Zadravec, E. Benedikt leg.; 2 adult moths, Štajerska, Kozjansko, Kozjansko Regional Park, Podsreda, Stara sveta gora, the way of the cross, 46.0427 N, 15.5943 E, alt. 349 m, 28.x.2013, S. Gomboc, D. Klenovšek leg.; 1 adult moth, Štajerska, Zasavje, Lisca, around Tončkov dom and downhill, 46.0672 N, 15.2850 E, alt. 921 m, 23.ix.2017, S. Gomboc, D. Klenovšek leg.; 4 adult moths, Krško hribovje, Orešje, Kozja Peč, 46.0508 N, 15.7030 E, alt. 360 m, 08.x.2017, S. Gomboc leg.; 1 adult moth, same locality, 16.x.2017, S. Gomboc leg., gen. prp. nr. GOS Euparal 518; 1 adult moth, Primorska, Istra, Brič above Dragonja, 45.4630 N, 13.7361 E, alt. 230 m, 26.x.2019, S. Gomboc, C. Wieser leg, gen. prp. nr. GOS Euparal 517.

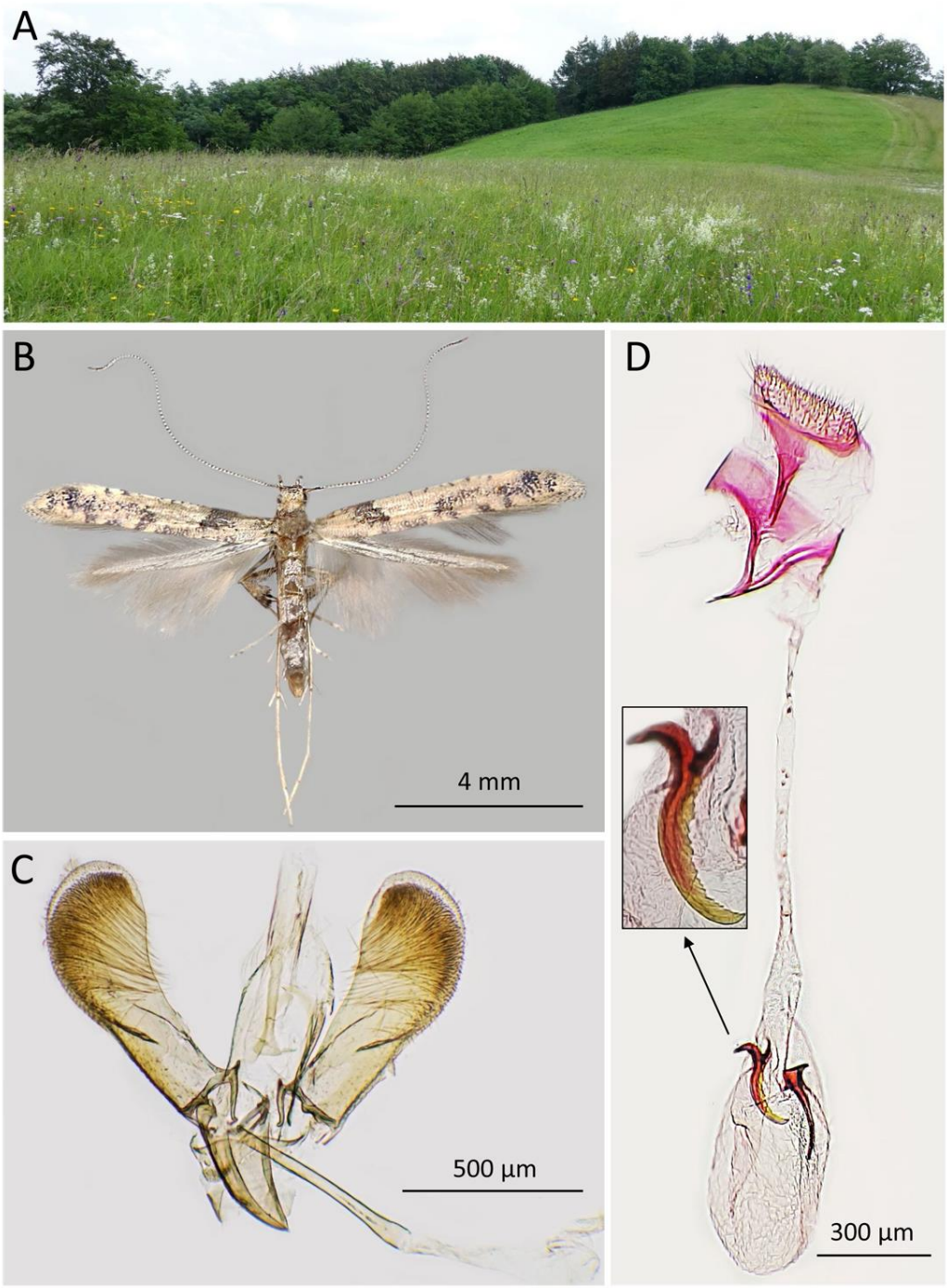

Figure 8. *Caloptilia honoratella.* (**A**) habitat, Štajerska, Zasavje, Lisca, around Tončkov dom and downhill, 24.vi.2016; (**B**) male, Primorska, Istra, Brič above Dragonja, 26.x.2019; (**C**) male genitalia, Primorska, Istra, Brič above Dragonja, 26.x.2010, gen. prp. nr. GOS Euparal 517; (**D**) female genitalia with characteristic spines in the ductus and signa on the bursa copulatrix (insert shows enlarged signum), Krško hribovje, Orešje, Kozja Peč, 16.x.2017, gen. prp. nr. GOS Euparal 518.

Bionomics. The moths were attracted to light traps in September–October and January, confirming that it is a late summer species overwintering as adult. In Austria, it is also known as a late summer species [97]. In Slovenia, all specimens were sampled in forested areas where *Acer* spp. are present or in abandoned grassland successions surrounded by forest and forest roads, or on warm slopes of mosaic landscape (Figure 8). Young larvae live in a flat blotch mine situated in the angle between main and secondary veins on the

lower surface of leaf; older larvae live freely in leaves rolled into a cone. Pupation occurs on the lowerside of the leaf in a cocoon [82].

Host plants. Monophagous on *Acer monspessulanum, A. pseudoplatanus* (Sapindaceae) [82,84].

Distribution. Europe (Austria, Belgium, Croatia, Denmark, Germany, Hungary, Italy, North Macedonia, Slovakia, Spain, Switzerland, the Netherlands, UK) [84,97,98], Czechia [99], European part Russia (questionable records) [83]. Slovenia (without indicating the localities) [22], species distribution in Slovenia (present paper).

Remarks. One specimen collected by H. Deutsch and S. Gomboc and photographed by H. Deutsh (female, Slovenia, Primorska, Brestovica, 26.x.2013), determined by O. Rist, H. Deutsch and P. Buchner, was posted in Lepiforum under the erroneous name, *Caloptilia falconipennella* (Hübner, 1813) and later corrected to *Caloptilia honoratella* [100]. In our paper, we confirm the presence of *C. honoratella* in Slovenia and refer to its first detection in the country back to 2000. It is a native and widely distributed species in the country that prefers warmer places. The species might have originated from the Balkan and Italian peninsulas [101]; presently it seems to expand its range across Western Europe [102].

***Dialectica scalariella* (Zeller, 1850)**

(Figure 9A–C)

Material examined. Slovenia: 1 adult moth, Notranjska, Menišija, Rakek, Rjava luža, 45.8282 N, 14.320 E, alt. 660 m, 28.v.2008, S. Gomboc leg.; 2 adult moths, Primorska, Slovensko primorje, Sečovlje Salina Nature Park, old coal mine, freshwater reeds, 45.4791 N, 13.6193 E, alt. 0.6 m, 05.x.2010, S. Gomboc leg., gen. prp. nr. GOS Euparal 515; 3 adult moths, Štajerska, Kozjansko, Kozjansko Regional Park, Podsreda, Stara sveta gora, the way of the cross, 46.0427 N, 15.5943 E, alt. 349 m, 28.x.2013, S. Gomboc, D. Klenovšek leg.; 1 adult moth, same locality, 05.vi.2016, S. Gomboc, D. Klenovšek leg.; 1 adult moth, Primorska, Škocjan Caves Regional Park, Matavun, sinkhole Globočak at the entrance to the cave, 45.6615 N, 13.9807 E, alt. 387 m, 20.viii.2019, S. Gomboc leg.

Bionomics. The moths were collected at light traps in May–June and August–October suggesting that the species develops two generations in Slovenia. All were trapped on warm, southern slopes or ruderal areas (Figure 9) with *Echium vulgare* and/or *Borago* sp. present. Young larvae make narrow tunnels in epidermis on the lower side of the leaf; later they live in blotch mines occupying significant part of leaf lamina between the main vein and leaf margin; several larvae may occur in one blotch; pupation takes place in the mine [82].

Host plants. Oligophagous species on *Anchusa strigosa, Borago, Cynoglossum creticum, Echium aculeatum, E. giganteum, E. plantagineum, E. vulgare, Myosotis latifolia, Symphytum officinale* (Boraginaceae) [82,84].

Distribution. Most of Europe (Austria, Bulgaria, Croatia, France, Germany, Greece, Italy, Portugal, Spain, Switzerland, Ukraine), Russia (Caucasus), Crimea, Algeria, Israel, Jordan, Madagascar, Malta, Morocco, Turkey, Turkmenistan [25,84]; alien to Australia and New Zealand [103,104]. Slovenia (without indicating the localities) [22], species distribution in Slovenia (present paper).

Remarks. A wide natural range in Europe suggests that the species is native to Slovenia, where it seems to be locally distributed, also in ruderal areas with *E. vulgare*. According to our observations, it is also commonly found in Croatia (across the coastland and on the islands).

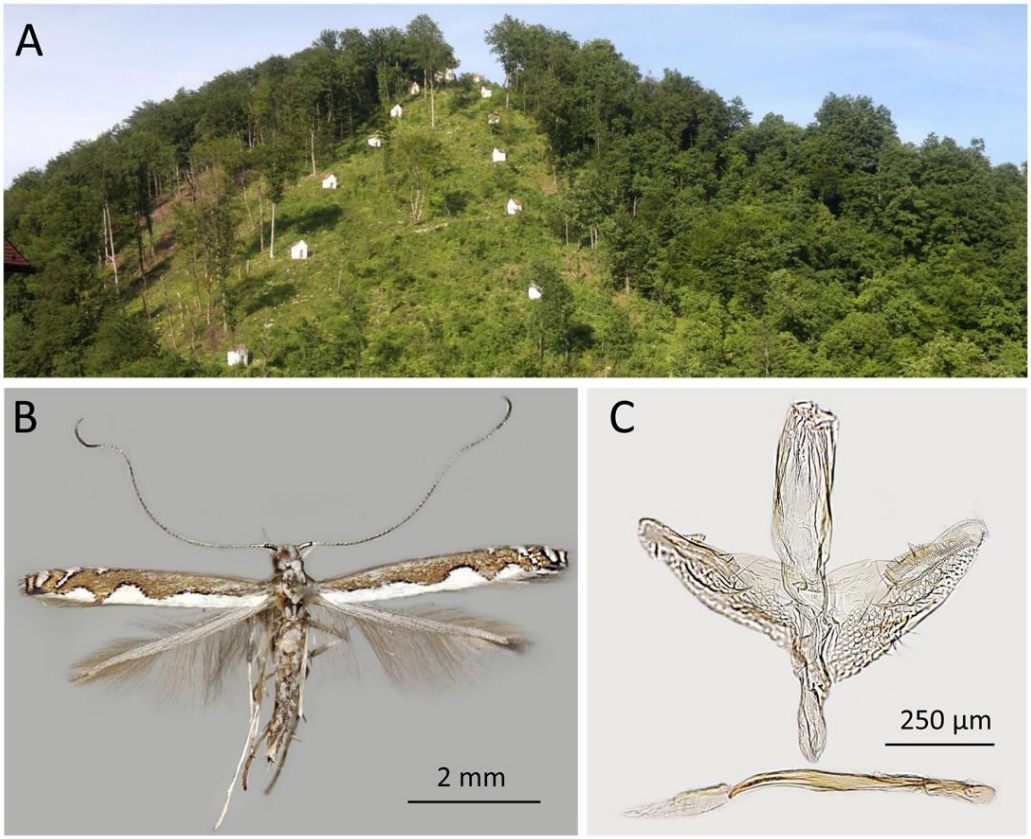

Figure 9. *Dialectica scalariella.* (**A**) habitat, Kozjansko, Kozjansko Regional Park, Podsreda, Stara sveta gora, the way of the cross, 29.v.2011; (**B**) male, Primorska, sinkhole Globočak, 20.viii.2019; (**C**) male genitalia, Slovensko primorje, Sečovlje Salina Nature Park, old coal mine, freshwater reeds, 5.x.2010, gen. prp. nr. GOS Euparal 515.

***Phyllonorycter messaniella* (Zeller, 1846)**

(Figure 10A–D)

Material examined. Slovenia: 2 adults, Primorska, Podgorski Kras, Movraž pri Hrastovljah, Kraški rob, 45.4731 N, 13.9291 E, alt. 284 m; 28.x.2006, S. Gomboc, B. Porenta leg.; 2 adults, Štajerska, Kozjansko, Kozjansko Regional Park, Vetrnik, lower part with deciduous forest, 46.0585 N, 15.5466 E, alt. 610 m, 09.x.2014, S. Gomboc leg., gen. prp. nr. GOS Euparal 509; 1 adult, Prekmurje, Črni log, Banuta, at the forest chapel, 46.5984 N, 16.3953 E, alt. 163 m, 11.x.2014, S. Gomboc leg.; 1 adult, Primorska, Sežanski Kras, Lipica, Kokoš hill, 45.6439 N, 13.9001 E, alt. 664 m, 08.v.2015, S. Gomboc, B. Zadravec, R. Štanta, M. Zadrgal leg.; 1 adult, Primorska, Istra, Brič ob Dragonji, 45.4630 N, 13.7361 E, alt. 230 m, 26.x.2019, S. Gomboc, C. Wieser leg.

Bionomics. The moths were attracted to light in May and October suggesting the presence of two generations in Slovenia. In Great Britain, a third generation may occur in December–March [105] (Colin W Plant, personal communication 2022); a third generation is not ruled out for the warmer regions of Slovenia. The majority of specimens originated from warm part of Slovenia (i.e., the regions with hot summers and mild winters). The sampled localities are mainly sunny, exposed slopes in oak forest on the karst hills (Figure 10). In eastern Slovenia, these localities were on the Pannonian Plain—a typical flood plain with the oak hornbeam forest inside a boggy area. Larvae live in tentiform mines on the lower side of leaves, often between two secondary veins; pupation occurs in the mine [82].

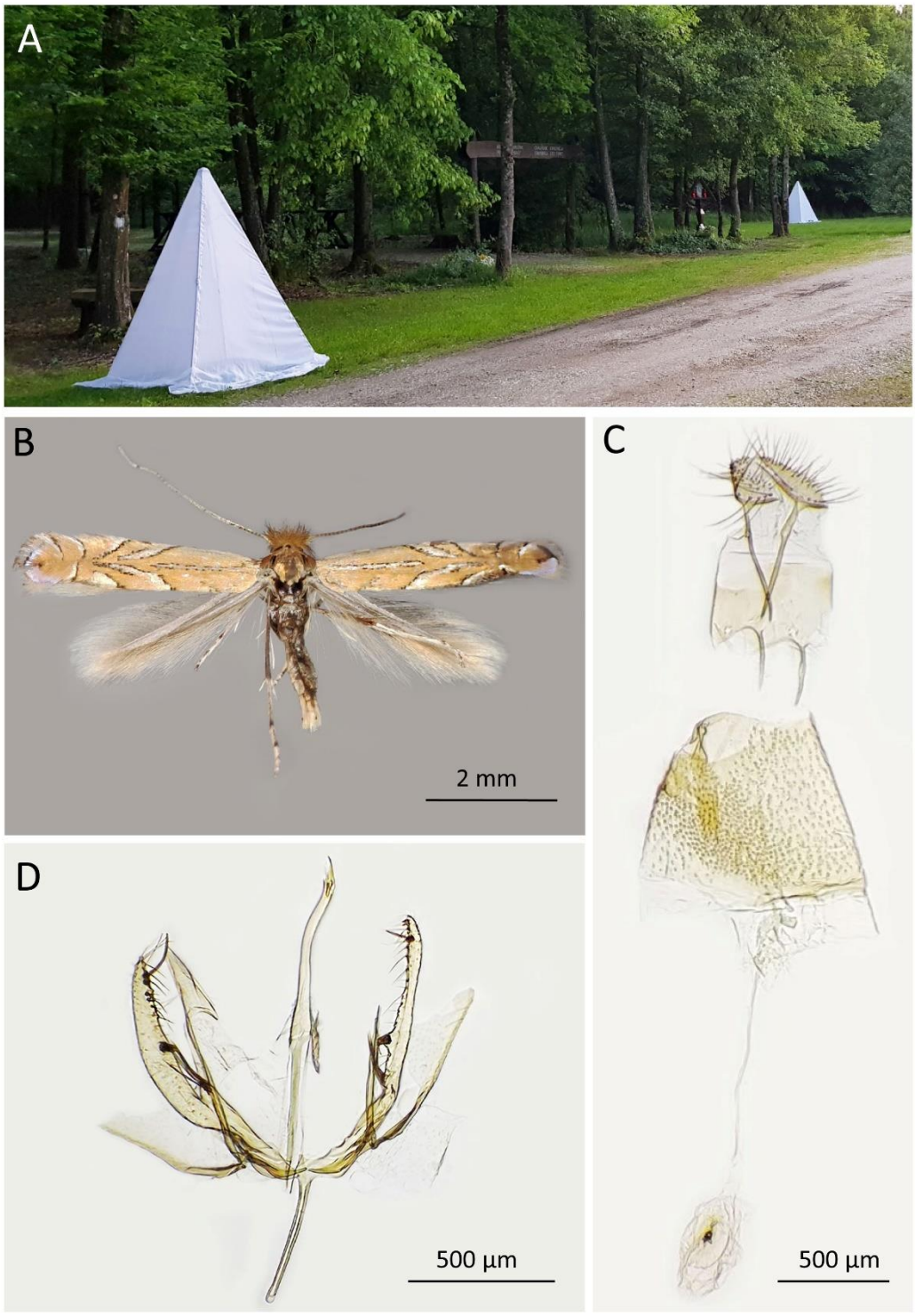

Figure 10. *Phyllonorycter messaniella.* (**A**) habitat, Prekmurje, Črni log, Banuta, at the forest chapel, 21.v.2021; (**B**) female moth, Štajerska, Kozjansko, Kozjansko Regional Park, Vetrnik, 09.x.2014; (**C**) female genitalia, Štajerska, Kozjansko, Kozjansko Regional Park, Vetrnik, deciduous forest, 9.x.2014, gen. prp. nr. GOS Euparal 509; (**D**) male genitalia, Croatia, Lošinj Island, Artatore, Airport, grasslands with makqui, 19.x.2014, gen. prp. nr. GOS Euparal 510.

Host plants. Polyphagous species with a natural range across Europe and European part of Russia [84,85], developing on *Quercus* (main host), *Fagus, Castanea* (Fagaceae), occasionally on *Tilia* (Malvaceae), *Betula, Carpinus* (Betulaceae), *Malus, Prunus* (Rosaceae)

[82,84,106]. In Australia, it also feeds on local *Nothofagus* (Fabaceae) and *Acca sellowiana* (Myrtaceae) [104,107].

Distribution. In Europe from Great Britain to the European part of Russia (predominantly in warm regions) inclusively [24,83,84]. An invasive species in Australia and New Zealand [104]. Slovenia (without indicating the localities) [22], species distribution in Slovenia (present paper).

Remarks. The species occurs naturally in Europe, including countries bordering with Slovenia; thus, it must be native to Slovenia too. The distribution of the species seems to be much localized as we found it only in a few places, despite long-term monitoring of the moth fauna throughout Slovenia.

4. Discussion

In Europe, between 263 and 273 Gracillariidae species have been reported [3,25]. In the countries neighbouring Slovenia, the gracillariid fauna varies from 106 species in Croatia and 127 species in Hungary to 146 species in Austria and 160 in Italy [25,108,109]. Our contribution increases the list of gracillariids of Slovenia from 119 to 123 species, suggesting that the diversity of this group is relatively well studied here. At present, the Slovenian fauna accounts around 46% of all Gracillariidae species known in Europe.

Among the species we newly recorded in Slovenia, only *Phyllonorycter trifoliella* seems to be an alien species to the country (see remarks in the species essay above). The other species discussed in the paper, *Caloptilia honoratella, Dialectica scalariella, D. imperialella*, *Phyllonorycter abrasella, Ph. messaniella,* and *Phyllocnistis valentinensis* must be native as they are present in some of the countries bordering with Slovenia (see the species essays above). Besides formally documenting the gracillariids, our study contributed to the knowledge of their distribution in Slovenia. We obtained detailed data on the occurrence of *D. scalariella, Ph. messaniella, Ph. abrasella,* and *C. honoratella* by catching the moths in up to 12 geographically distant localities in Slovenia over different periods of years 2000–2022. Apart from geographical data, such surveys provided an insight to the species phenology.

As our study showed, even opportunistic samplings may result in novel regional findings. Ongoing global changes in the environment (most notably climate change and habitat degradation), progressing globalization and international trade of live plants and plant materials can significantly facilitate species distribution from their native range [110]. We may expect that some alien species of gracillariids could arrive in Slovenia through different means (including natural spreading or found here as already established species. Furthermore, other species present in Europe might be found in Slovenia, in particular, *Phyllonorycter medicaginella* (Gerasimov, 1930), *Ph. gerasimowi* (M. Hering, 1930), *Ph. malella* (Gerasimov 1931), *Micrurapteryx caraganella* (Hering, 1957), *Phyllocnistis asiatica, P. saligna*, and *P. ramulicola*. Some of them (e.g., *Ph. medicaginella, Ph. malella, M. caraganella*), originating from the Eastern Palaearctic, have been already found in some parts of Europe [4,11,13,111–113], but not yet in Slovenia. Thus, we believe that the checklist of Gracillariidae of the country could be extended to 133 species, if not more.

Gracillariidae moths are known to be moderately attracted to light traps; they come to the light occasionally if they are abundant in a research area (Gomboc, Kirichenko: pers. observation). Complementary searching for leaf mines may provide additional valuable information on the leaf mining insects present in the country. Importantly, leaf mines can be found during the whole season, often carrying larvae or pupae inside the mines [114]. Adult moths may be reared indoor from sampled mines for morphological identification [114]. Furthermore, freshly sampled leaves with the mines, as well as those preserved in ancient herbaria and storing remnants of insects in the mines, can be effectively used for species identification through DNA barcoding [112]. Even empty, freshly abandoned mines can sometimes still be utilized for molecular genetic study and species identification [115]. The use of different sampling approaches as well as involvement of DNA barcoding open great prospects to exploring deeply the regional faunas of leaf mining moths.

In our study, the use of DNA barcoding confirmed the identity of *Phyllocnistis valentinensis* found at the larval stage in a leaf mine. Curiously, *P. valentinensis* showed a higher genetic similarity to an undescribed *Salix*-feeding *Phyllocnistis* sp. which we discovered in the Russian Far East (4.7% interspecific genetic divergence) than to *Phyllocnistis saligna, P. asiatica, P. ramulicola* known in Europe on *Salix alba*, the host of *P. valentinensis.*

Further studies, including sampling of leaf mines on various host plants, are needed for improving knowledge on the moths' distribution and trophic relations and for building a complete molecular genetic library of gracillariids of Slovenia.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/d14100811/s1. Table S1. The list of sampled localities in Slovenia with the coordinates. Table S2. The specimen data of *Phyllocnistis valentinensis* collected in Slovenia and the sequences of the related *Salix*-feeding species publicly available in BOLD used for comparison in the present study. Table S3: The checklist of Gracillariidae of Slovenia.

Author Contributions: Conceptualization and methodology, S.G. and N.I.K.; software, S.G. and N.I.K.; validation, S.G. and N.I.K.; formal analysis and investigation, S.G. and N.I.K.; resources, N.I.K.; data curation, S.G. and N.I.K.; writing—original draft preparation, review and editing, visualization, S.G. and N.I.K.; supervision, project administration, funding acquisition, N.I.K. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Genetic data used in the study are publicly accessible in BOLD using the link dx.doi.org/10.5883/DS-GRSLO.

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

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