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## Diversity and Distribution of Leaf Mining Insects on Birches (*Betula* spp.) in Siberia

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**Abstract**—The diversity and distribution of leaf mining insects developing on birches (*Betula* spp.) in Siberia were reviewed based on published records and our observations. Analysis of the literature revealed 52 species of leaf miners recorded as feeding on different *Betula* species in Siberia. Among them, three species were listed under different names and six species were erroneously recorded as birch consumers. Thus, the revised list of birch leaf miners contains 44 species. Five moth and four sawfly species are mentioned in the literature as pests of *Betula*. Four sawflies are known to be invasive in North America. Our collections comprised 25 species, including the micro-moth *Stigmella continuella* (Lepidoptera, Nepticulidae), a new species for Siberia found in Novosibirsk. Immature stages of 15 species were identified using DNA barcoding. Twenty species were recorded from several regions of Siberia for the first time. The dominant group is Lepidoptera (31 species), followed by Coleoptera (7), Hymenoptera (5), and Diptera (1). Two-thirds of all the known leaf miners develop exclusively on birches; the remaining species also colonize alders (*Alnus*, Betulaceae), some Rosaceae, Salicaceae, and Ulmaceae. In our observations, the majority of insects (96%) developed on *B. pendula*. About half of them were also observed on the East Asian birches *B. dahurica*, *B. divaricata*, *B. costata*, *B. ermanii*, and *B. gmelinii*; five species were found on the North American birches *B. occidentalis* and *B. papyrifera*. All the leaf mining species listed in our paper for Siberia also occur in Europe. The similarity between the miner faunas of these regions is discussed and it is warned about possible errors in diagnostics of the Siberian species using the keys and catalogues for the European fauna. The importance of DNA barcoding in the study of the local insect faunas of poorly explored regions is also emphasized.

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In Russia, and in particular in Siberia, birch is one of the most widespread deciduous trees (Koropachinsky and Vstovskaya, 2012). The silver birch *Betula pendula* Roth is the commonest representative of the genus. This species is the pioneer of colonization of cleared or burnt-out places and waste lands; it forms grassy secondary forests improving the soil quality (Korchagina, 1980). Birches are often used in shelter belts and urban landscaping. Due to the beautiful light green foliage, white bark, and elegant crown, not only the local but also the introduced species of *Betula* are grown for ornamental purposes in parks and public gardens (Bakulin et al., 2008).

The genus *Betula* L. is represented by a hundred species and forms in the Russian botanical gardens. The largest collection of birch species is gathered in the Main Botanical Garden of the Russian Academy of

Sciences in Moscow. The collection of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG, Novosibirsk) has over ten birch species and forms brought from various floristic regions (Vstovskaya and Koropachinsky, 2005).

Birches are damaged by many insect species (Belov, 2013). In Moscow parks and public gardens, birches serve as host plants for 163 species (Belov, 2013). In Siberia, it is estimated that about 400 arthropod species feed on birch (Barannik, 1981; Yanovsky, 2003). Among phyllophagous insects, leaf miners are regularly found on birch (Barannik, 1981; Tarasova and Sanina, 2004; Tarasova, 2005; Selikhovkin et al., 2012; Belov, 2013). This is an endophagous group of insects whose larvae live in mines in the leaf tissues (Hering, 1951). Leaf miners are known among repre-

sentatives of the orders Lepidoptera, Hymenoptera, Coleoptera, and Diptera (Hering, 1951). Mass mining of leaves reduces the surface involved in photosynthesis and disrupts water balance, leading to slower growth, premature falling of leaves, and also decreases resistance to diseases (Proctor et al., 1982; Raimondo et al., 2003; Wagner et al., 2008).

In the recent years, this ecological group of insects has attracted much attention of researchers due to the rapid expansion of many species and the extensive damage to woody plants in parks, gardens, and forests (Lopez-Vaamonde et al., 2010; Selikhovkin et al., 2012; De Prins and De Prins, 2015; Edmunds, 2015; Ellis, 2015). Invasive species are especially numerous among leaf mining moths. During the last decades, the list of invaders was supplemented, in particular, by moths of the families Gracillariidae, Yponomeutidae, Gelechiidae, and others (Šefrová and Laštůvka, 2009; Lopez-Vaamonde et al., 2010). Many of them have colonized important forest and ornamental woody plant species.

In Siberia, the group of leaf mining insects remains poorly studied. For example, the *Catalogue of the Lepidoptera of Russia* (2008) contains little data on the taxonomic diversity and distribution of leaf mining moths in Siberia as compared to western and eastern regions of the country. Data on the distribution of leaf mining beetles, sawflies, and flies in Siberia are even scarcer.

The goal of this work was to study the taxonomic diversity and distribution of leaf mining insects on birches in Siberia, based on the literary data and our records. The trophic specialization of leaf mining insects was studied, pests and invasive species were listed, and comparative analysis of the faunas of insects mining birch leaves in Siberia and Europe was performed.

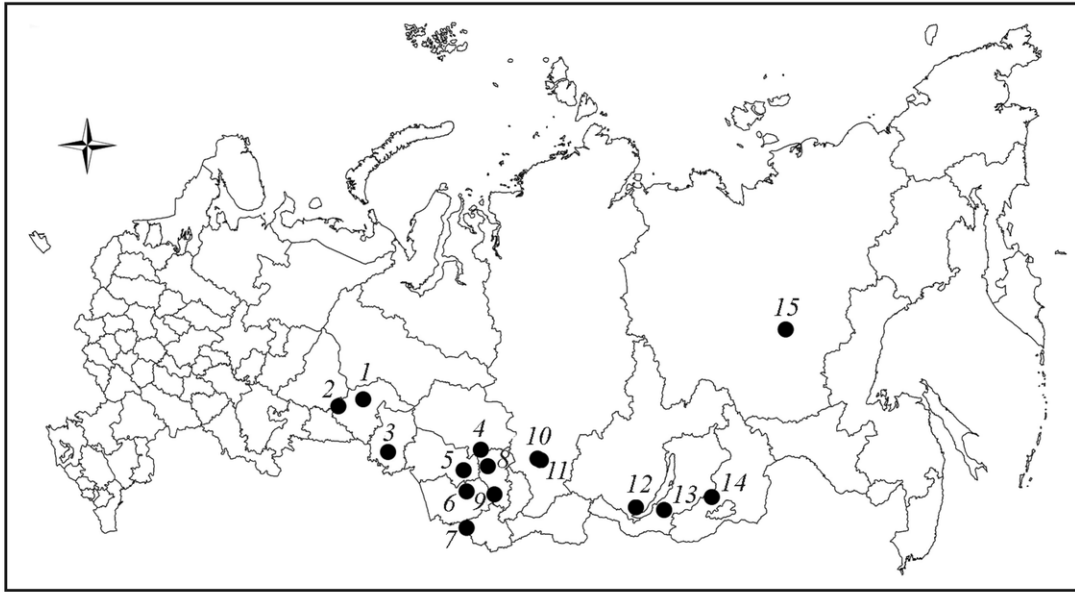
## MATERIALS AND METHODS

The taxonomic diversity of insects mining birch leaves was studied in 2008–2015 in 12 administrative regions of Siberia (Fig. 1). In Novosibirsk Province and Krasnoyarsk Territory research was carried out yearly in May–September; in other regions, once or twice in June–July 2009–2015. Collections were made in parks, arboreta, and botanical gardens in the administrative centers of the regions (Fig. 1). In addition, we examined the natural forests around Krasnoyarsk and Novosibirsk, and also the forests in Kemerovo Province and Altai Republic (see notes to Fig. 1).

Overall, ten birch species were studied: the Eurasian silver birch *B. pendula* Roth and downy birch *B. pubescens* Ehrh., the Asian species *B. microphylla* Bunge, *B. dahurica* Pall., *B. divaricata* Ledeb., *B. costata* Trautv., *B. ermanii* Cham., and *B. gmelinii* Bunge, and the North American water birch *B. occidentalis* Hook. and the paper birch *B. papyrifera* Marsh. *Betula gmelinii* is a dubious species; it is regarded as a hybrid having common characters of *B. humilis* Schrank, *B. fruticosa* Pall., and *B. ermanii* (Koropachinsky and Vstovskaya, 2012, p. 199). All birch species listed above were surveyed in CSBG. In other regions, *B. pendula* was mainly studied. Altogether, about 2000 birch plants were examined, aged from 5 to 70 years and varying in height from 2.5 to 30 m.

Birch leaves were examined for damage of leaf miners (i.e., for mines). Young birch trees (up to 3 m tall) were examined along the whole height, while in older trees only the lower part of the crown was examined. The leaves with mines were collected and preserved in a herbarium (Hering, 1951; Kirichenko, 2014); the mines and their inhabitants were photographed with a Sony Nex-3 digital camera. The insect larvae and pupae found in mines were placed in 96% ethanol to preserve their DNA (Doorenweerd and Beentjes, 2012). Collecting insects directly from mines allowed us to document their host plants. In some cases, adults were reared from the larvae under laboratory conditions (Hering, 1951; Kirichenko, 2014). The miners were identified using Russian and European keys (Hering, 1957; *Key to the Insects...*, 1978, 1997; Gusev, 1984; *Insects and Acarines...*, 1999; Gregor and Patočka, 2001; Ellis, 2015). Identification of moths of the families Nepticulidae and Gracillariidae as well as weevils of the family Curculionidae was checked by experts.

Larvae and pupae of 15 insect species were used for DNA barcoding, i.e., sequencing of the barcoding fragment of the mitochondrial cytochrome oxidase I (COI) gene. DNA barcoding is widely used for reliable identification of arthropods, including leaf mining insects (Mutanen et al., 2013). A great advantage of this approach is that it allows identifying species based on their preimaginal stages, which is difficult or impossible to do using morphological characters. Genetic analysis of insects was performed in the Forest zoology laboratory of the French National Institute for Agricultural Research (INRA, Orléans). Altogether, 15 insect species were analyzed. Extraction and purification of DNA was performed using the QIAGEN



**Fig. 1.** The area of collection of birch leaf mining insects in Siberia in 2008–2015: (1, 2) Tyumen Province: (1) Tobolsk: Ermak Park, VI.2015; (2) Tyumen: Zatyumensky Park and adjoining forest zone, VI–VII.2013–2015; (3) Omsk Province, Omsk: Victory Park, VI–VII.2013–2015; (4) Tomsk Province, Tomsk: Siberian Botanical Garden of Tomsk State University, VI.2009; (5) Novosibirsk Province, Novosibirsk: CSBG and adjoining forest zone, VI–VIII.2008–2015; (6) Altai Territory, Barnaul: Izumrudny Park, VII.2015; (7) Altai Republic: Chike-Taman Pass, VII.2013; (8, 9) Kemerovo Province: (8) Kemerovo: Antoshka Park, VII.2015; (9) Kuzedeevo: lime grove, VI.2013; (10, 11) Krasnoyarsk Territory: (10) Krasnoyarsk: Arboretum of SIF SB RAS and adjoining forest zone, VI–VIII.2008–2015; (11) Trostentsovo: suburban forest, VI–VIII.2011–2015; (12) Irkutsk Province, Irkutsk: botanical garden of Irkutsk State University, VIII.2015; (13) Buryatia Republic, Ulan-Ude: plantings in Zheleznodorozhny District, VIII.2015; (14) Transbaikalia, Chita: Victory Park, VIII.2015; (15) Sakha Republic (Yakutia), Yakutsk: environs of North-Eastern Federal University, VIII.2015.

DNeasy Blood & Tissue Kit. The mitochondrial DNA fragment was amplified and sequenced using the primers LCO (5' GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO (5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3'), 658 bp long (Folmer et al., 1994). The conditions of amplification and sequencing were described in our earlier papers (Kirichenko et al., 2015, 2016). Samples of three insect species were sequenced in the Canadian Centre for DNA Barcoding (Biodiversity Institute of Ontario, University of Guelph) using the standard protocol (Ivanova et al., 2006). All sequences were edited in CodonCode Aligner V.3.7.1. and compared with references in the BOLD database (Ratnasingham and Hebert, 2007).

Data on the distribution of insects mining birch leaves in Siberia were extracted from the literature (*Forest Pests*, 1955; Ilyinsky, 1962; Tomilova, 1962, 1973; Tibatina and Stroganova, 1966; Tibatina, 1970; Dovnar-Zapolsky and Tomilova, 1978; Barannik, 1981; Gershenson, 1981; Kuznetsov, 1981; Gusev, 1984; Yanovsky, 1996, 2003; Kuznetsov and Baryshnikova, 1998, 2003; *Insects and Acarines...*, 1999; Tarasova and Sanina, 2004; Tarasova et al., 2004; Krivets, 2007; *Catalogue...*, 2008; Legalov,

2010; Baryshnikova, 2014) and supplemented with our observations. The Latin names and trophic associations of species are given according to the modern databases (De Prins and De Prins, 2015; Edmunds, 2015; Ellis, 2015; Fauna Europaea, 2015). In our paper the leaf miners are divided into mono-, oligo-, and polyphagous according to the accepted classification (Hering, 1951; Ellis, 2015): monophagous insects damage plants of one genus; oligophagous, plants of one family or, less frequently, plants of related families of the same order; polyphagous, a wide range of plant species from unrelated families and orders. Data on the richness of leaf mining insects on birches in Europe were borrowed from Hering (1951) and Ellis (2015). The leaf mining species richness in Siberia and Europe was compared using the Chekanovsky-Sørensen similarity index:  $K_S = 2C/(A + B)$ , where  $C$  is the number of species common to the lists compared, and  $A$  and  $B$  are the numbers of species in each list (Magurran, 2004). This index was calculated both for comparing the overall leaf miner richness (including representatives of all the four orders: Lepidoptera, Hymenoptera, Coleoptera, and Diptera), and for individual orders.

**Table 1.** Leaf mining insects erroneously listed as birch consumers in the Russian literature<sup>1</sup> and their actual host plants<sup>2</sup>

No.	Order, family, and species of insect	Trophic specialization <sup>3</sup>	Family and genus of actual host plants
Order LEPIDOPTERA			
Family <b>Coleophoridae</b>			
1	<i>Coleophora paripennella</i> Zeller	O	Asteraceae ( <i>Arctium</i> , <i>Arnica</i> , <i>Carduus</i> , <i>Carlina</i> , <i>Centaurea</i> , <i>Cirsium</i> , <i>Saussurea</i> , <i>Serratula</i> , <i>Solidago</i> )
Family <b>Gracillariidae</b>			
2	<i>Caloptilia elongella</i> (Linnaeus)	M	Betulaceae ( <i>Alnus</i> )
Family <b>Yponomeutidae</b>			
3	<i>Swammerdamia pyrella</i> (Villers)	O	Rosaceae ( <i>Crataegus</i> , <i>Malus</i> , <i>Prunus</i> , <i>Pyrus</i> )
Order COLEOPTERA			
Family <b>Curculionidae</b>			
4	<i>Orchestes alni</i> (Linnaeus)	M	Ulmaceae ( <i>Ulmus</i> )
Order HYMENOPTERA			
Family <b>Tenthredinidae</b>			
5	<i>Heterarthrus fasciatus</i> (Malaise)	M	Salicaceae ( <i>Populus</i> )
6	<i>H. ochropoda</i> (Klug)	M	Salicaceae ( <i>Populus</i> )

<sup>1</sup> According to: Tibatina, 1970; Dovnar-Zapolsky and Tomilova, 1978; Gershenson, 1981; Yanovsky, 2003. <sup>2</sup> According to: Hering, 1951, 1957; Edmunds, 2015; Ellis, 2015; Fauna Europaea, 2015. <sup>3</sup> Trophic specialization: M, monophagous; O, oligophagous.

## RESULTS AND DISCUSSION

**Taxonomic diversity of leaf mining insects on birches in Siberia.** In the Russian literature published in 1955–2010, a total of 52 insect species were listed as leaf miners of birches in Siberia. However, this list has some inaccuracies. In the literature sources, three insect species were simultaneously listed under the valid name and a synonym: *Eriocrania cicatricella* (Zetterstedt) was mentioned in the same publication as *E. cicatricella* and *E. rubroaurella* (Haworth); *Stigmella lapponica* (Wocke) was mentioned as *Nepticula lapponica* Wocke and *N. vossensis* Gronlien; *Swammerdamia caesiella* (Hübner) was mentioned as *S. caesiella* and *S. heroldella* Hübner (Dovnar-Zapolsky and Tomilova, 1978; Yanovsky, 2003). The micro-moth *Parornix betulae* (Stainton) was referred to as *P. devoniella* (Stainton) (*Catalogue...*, 2008), though in fact these are two different species (De Prins and De Prins, 2015; Ellis, 2015; Fauna Europaea, 2015). Birch was mistakenly mentioned as a host plant for 6 insect species, including 3 moths, 2 sawflies, and one beetle species (Table 1).

Taking into account the double names of some species and erroneous indication of host plants, the list of miner insects associated with birches in Siberia has been narrowed down to 44 species (Table 2).

Among insects mining birch leaves in Siberia, the order Lepidoptera has the highest taxonomic diversity, comprising 31 species (71% of the whole number of birch leaf miners) from 15 genera and 8 families (Fig. 2). The family Gracillariidae is the most diverse, with 8 species from 3 genera (*Caloptilia*, *Parornix*, and *Phyllonorycter*); it is followed by the families Nepticulidae with 7 species from 2 genera (*Ectoedemia*, *Stigmella*) and Eriocraniidae with 6 species from 2 genera (*Eriocrania* and *Heringocrania*) (Fig. 2).

The remaining three orders, namely Coleoptera, Hymenoptera, and Diptera, are represented by one family each (Fig. 2). The 7 beetle species inhabiting birches in Siberia belong to 4 genera: *Anoplus* Germar, *Orchestes* Ill., *Rhamphus* Clairv., and *Tachyerges* Schoenh. The identity of the weevil *Orchestes calceatus* (Germar) remains a matter of debate. Some authors consider *O. calceatus* and its close relative *O. testaceus* (Müller) to be one species, based on the absence of clear morphological differences (Legalov, 2010); others distinguish these species by the body coloration and the rostrum morphology and indicate birch as a host plant for *O. calceatus* and alder for *O. testaceus* (Dedyukhin, 2012; Košťál and Caldara, 2013). Birch leaves are also mined by 5 sawfly species from 5 genera: *Fenusia* Leach, *Fenusella* Enslin, *Heterarthrus* Steph., *Profenusia* MacGillivray, and *Sco-*

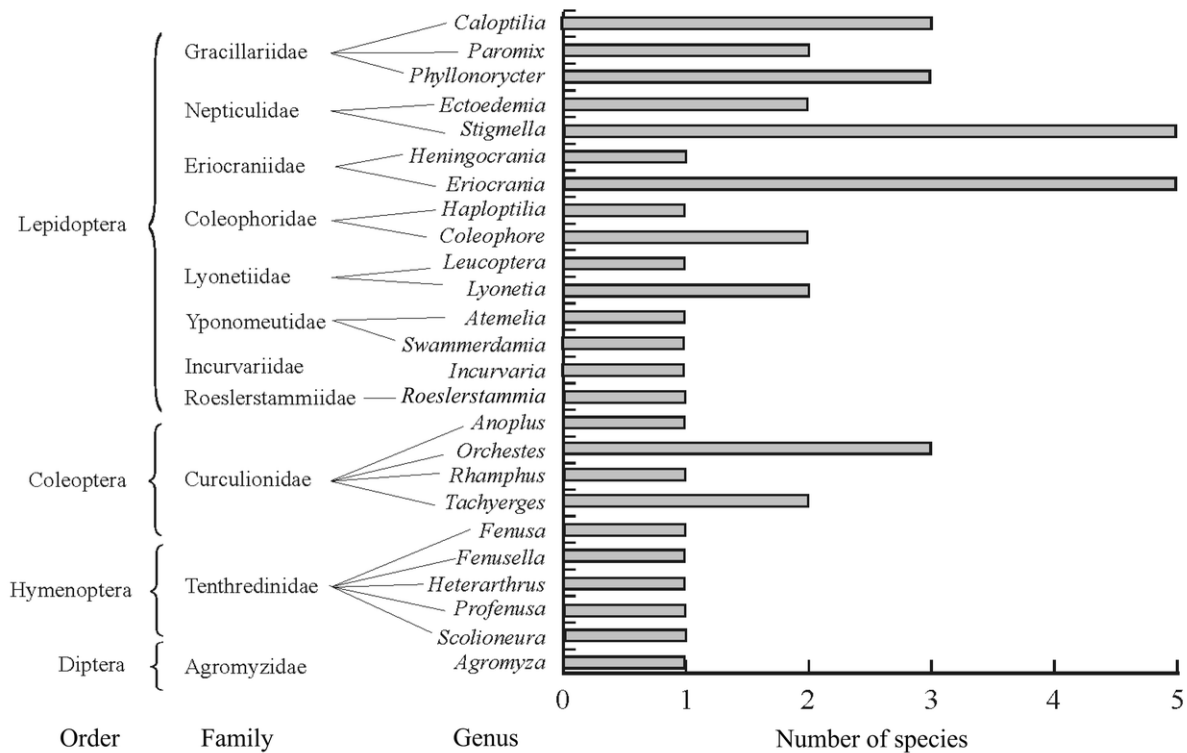


Fig. 2. Taxonomic diversity of leaf mining insects on birches in Siberia.

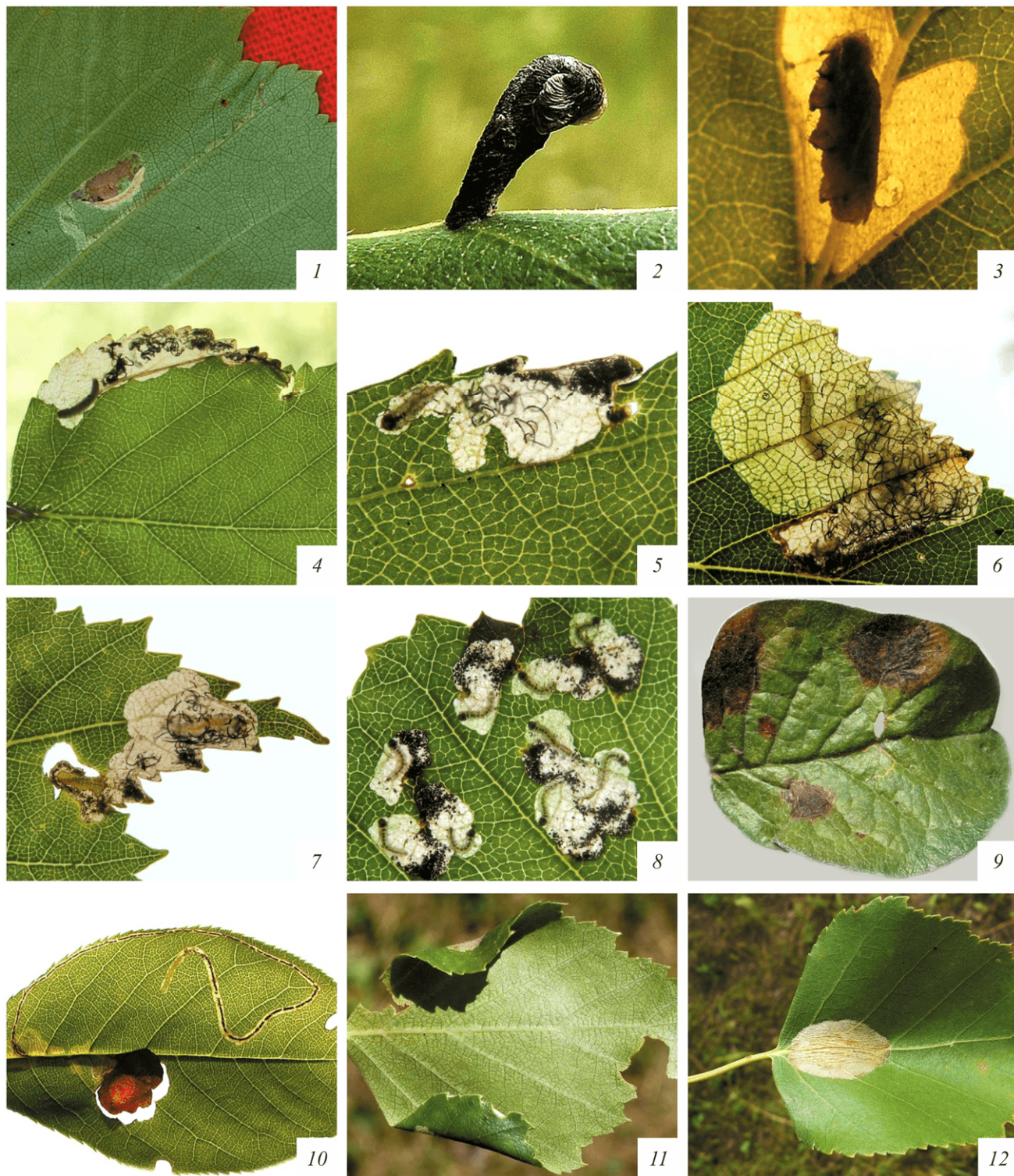
*lioneura* Konow (Fig. 2). Leaf mining flies are represented on birches in Siberia by a single species *Agromyza alnibetulae*.

Our collections included over 2500 mined leaves and 400 specimens of leaf mining larvae and pupae as well as adults reared from mines. In all, 25 leaf mining species were identified among this material, which makes up a little more than half the total number of leaf miners known on birches in Siberia according to the published data (Table 2). The species which we identified are marked with † in Table 2; the photographs of larvae and mines of most of these species, and also the cases of casebearer moths are shown in Fig. 3.

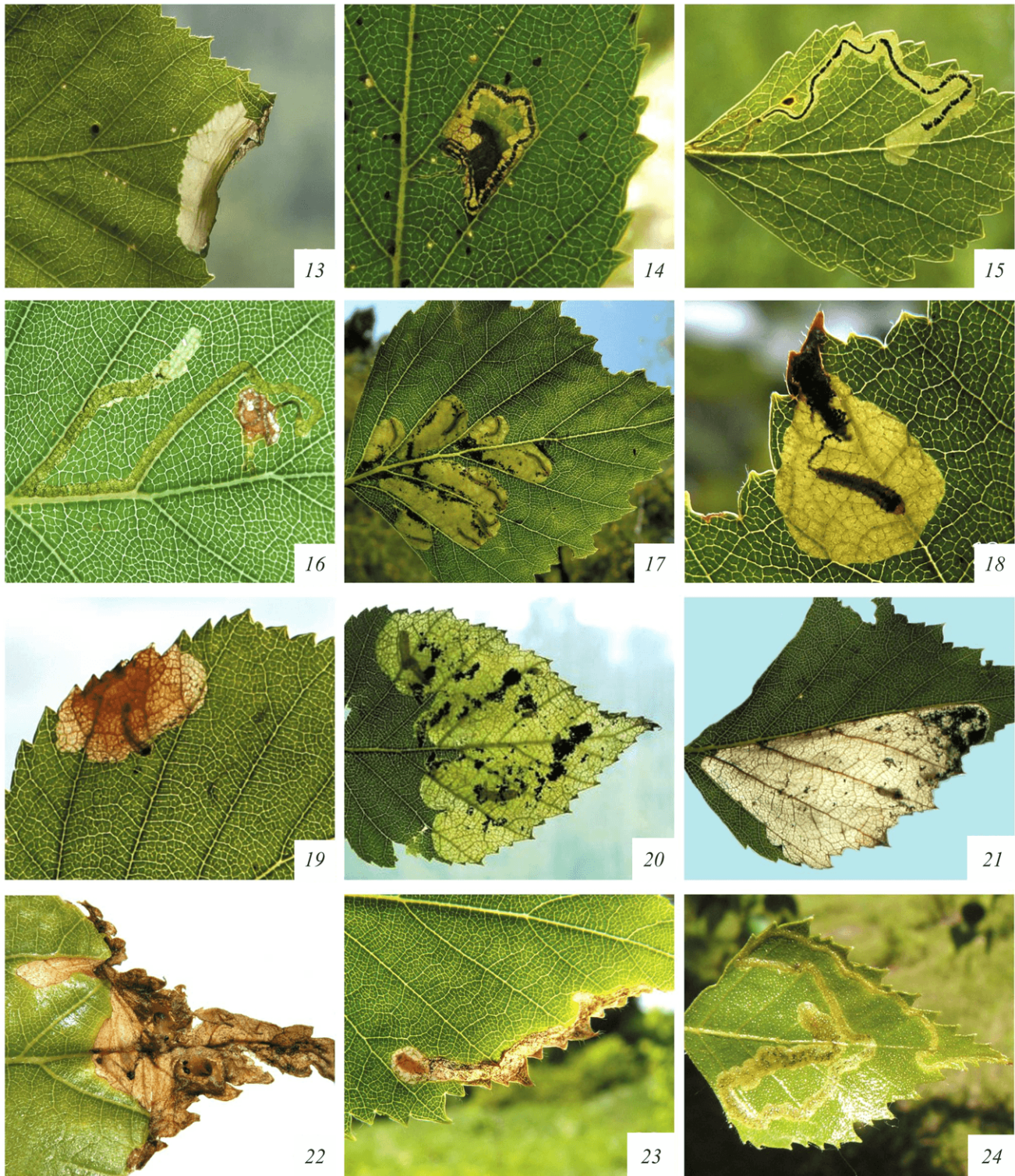
The larvae and pupae of 15 leaf mining species were identified using DNA barcoding. Their sequences were published in the BOLD database and can be accessed at [dx.doi.org/10.5883/DS-BETULEAF](https://dx.doi.org/10.5883/DS-BETULEAF). Thirteen species were identified with 99.2–100% match. The BOLD database showed a considerable similarity of the Siberian sequences to those from Europe (Finland, Germany, France, Norway, the Netherlands, Austria, and Czech Republic). In many cases, sequences of the Siberian sawflies were found to be close not only to the European but also to the Cana-

dian ones (Table 2), which could be expected keeping in mind the history of invasion of these insects into North America (see the section *Pests and invasive species* below). Two moth specimens from Siberia were identified as *Heringocrania unimaculella* and *Lyonetia clerkella* by their mitochondrial COI gene fragments with 97.46 and 98.02% match, respectively. Differentiation of the Lepidoptera species using DNA barcoding is most commonly based on a 2% threshold (Hebert et al., 2010; Rougerie et al., 2014). In our case, this threshold was slightly exceeded, which might indicate both a high level of genetic polymorphism in the geographic populations of these insects and the existence of potentially new species in Siberia. Additional studies are required to ascertain the status of these Siberian specimens.

**Trophic specialization.** Almost two-thirds of the insects mining birch leaves in Siberia are monophagous, developing only on *Betula* spp. (Table 2). About 5% of the leaf mining species are oligophagous and occur not only on birches but also on other representatives of the family Betulaceae, most often alder (*Alnus*). These are the micro-moths *Incurvaria pectinea* and *Atemelia torquatella*, the weevils *Anoplus plantaris* and *Orchestes testaceus*, and the sawfly



**Fig. 3.** Insect mines found on birch leaves in Siberia in 2008–2015. Lepidoptera: (1) *Caloptilia betulicola* on *Betula pendula*, Krasnoyarsk, Akademgorodok, Yenisei river bank, 19.VI.2013; (2) a case of *Coleophora betulella* on *B. pendula*, Novosibirsk, CSBG, 17.VI.2010; (3) a case of *Haploptilia serratella* on *B. pendula*, Krasnoyarsk, arboretum of Sukachev Institute of Forest (SIF), 31.V.2009; (4) *Eriocrania sangii* on *B. pendula*, Krasnoyarsk, Akademgorodok, Yenisei river bank, 13.VI.2013; (5) *E. semipurpurella* on *B. pendula*, same locality, 13.VI.2013; (6) *E. sparmanella* on *B. pendula*, Novosibirsk, CSBG, 17.VI.2010; (7) *Heringocrania unimaculella* (?) on *B. pendula*, Krasnoyarsk, Akademgorodok, Yenisei river bank, 19.VI.2013; (8) *Incurvaria pectinea* on *B. pendula*, same locality, 19.VI.2013; (9) *Leucoptera malifoliella* on *Cotoneaster melanocarpus*, Altai Republic, Chuisky highway, Chike-Taman Pass, 15.VII.2012; (10) *Lyonetia clerkella* (?) on *Prunus padus*, Novosibirsk, CSBG, 13.VI.2011; (11) *Parornix betulae* on *B. costata*,



Novosibirsk, CSBG, 14.VI.2012; (12) *Phyllonorycter corylifoliella* on *B. pendula*, same locality, 10.VII.2010; (13) *Ph. ulmifoliella* on *B. pendula*, Krasnoyarsk, SIF arboretum, 10.VII.2011; (14) *Stigmella betulicola* on *B. occidentalis*, same locality, 7.VII.2009; (15) *S. lapponica* on *B. microphylla*, same locality, 12.VI.2011; (16) *S. continuella* on *B. pendula*, same locality, 11.VII.2012. Hymenoptera: (17) *Fenusa pumila* on *B. pendula*, Novosibirsk, CSBG, 17.VI.2010; (18) *Fenusella nana* on *B. pendula*, same locality, 12.VI.2009; (19) *Heterarthrus nemoratus* on *B. pendula*, Krasnoyarsk, Akademgorodok, Yenisei river bank, 11.VII.2011; (20) *Profenusa thomsoni* on *B. pendula*, same locality, 11.VII.2011; (21) *Scolioneura betuleti* on *B. pendula*, same locality, 11.VII.2011. Coleoptera: (22) *Orchestes jota* on *B. pendula*, Novosibirsk, CSBG, 11.VII.2012; (23) *O. rusci* on *B. pendula*, same locality, 16.VI.2010. Diptera: (24) *Agromyza alnibetulae* on *B. pendula*, same locality, 16.VI.2010. Photos by N.I. Kirichenko.



**Table 2.** Birch leaf mining insects in Siberia: accuracy of DNA diagnostics and a list of their host plants<sup>1</sup>

No.	Order, family, and species of insect	Specimen ID and accuracy of DNA diagnostics <sup>2</sup>	Trophic specialization <sup>3</sup>	Host plants in Siberia <sup>4</sup>
<b>Order LEPIDOPTERA</b>				
<b>Family Coleophoridae</b>				
1	<i>Coleophora anatipenella</i> (Hübner, 1796)	–	P	<i>Betula</i> , many Rosaceae
2	<i>C. betulella</i> Heinemann et Wocke, 1876 †	–	M	<i>Betula</i> , [ <i>B. pendula</i> ]
3	<i>Haploptilia serratella</i> (Linnaeus, 1761) †	–	P	<i>Betula</i> , ( <i>Acer</i> ), [ <i>Betula dahurica</i> *, <i>B. divaricata</i> *, <i>B. pendula</i> , <i>B. ermanii</i> *]
<b>Family Eriocraniidae</b>				
4	<i>Eriocrania cicatricella</i> (Zetterstedt, 1839) †	–	M	<i>Betula</i> , [ <i>B. pendula</i> ]
5	<i>E. salopiella</i> (Stainton, 1854)	–	M	<i>Betula</i>
6	<i>E. sangii</i> (Wood, 1891) †	SIBLE009-15 99.48%; FI	M	<i>Betula</i> , [ <i>B. pendula</i> ]
7	<i>E. semipurpurella</i> Stephens, 1835 †	–	M	<i>Betula</i> , [ <i>B. pendula</i> ]
8	<i>E. sparrmannella</i> (Bosc, 1791) †	SIBLE006-15 100%; FI	M	<i>Betula</i> , [ <i>B. dahurica</i> *, <i>B. pendula</i> , <i>B. microphylla</i> , <i>B. gmelinii</i> *]
9	<i>Heringocrania unimaculella</i> (Zetterstedt, 1839) • †	SIBLE008-15 97.46%; AT	M	<i>Betula</i> , [ <i>B. pendula</i> ]
<b>Family Gracillariidae</b>				
10	<i>Caloptilia betulicola</i> (Hering, 1928) †	GRAS002-13 99.83%; FI, NL	M	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. pubescens</i> ]
11	<i>C. populetorum</i> (Zeller, 1839)	–	M	<i>Betula</i> , ( <i>Populus</i> , <i>Alnus</i> )
12	<i>C. suberinella</i> (Tengström, 1848)	–	M	<i>Betula</i>
13	<i>Parornix betulae</i> (Stainton, 1854) †	GRPAL1104-13 100%; DE, FI, NL	M	<i>Betula</i> , [ <i>B. costata</i> *, <i>B. micro-</i> <i>phylla</i> , <i>B. occidentalis</i> *, <i>B. pen-</i> <i>dula</i> ]
14	<i>P. traugotti</i> Svensson, 1976	–	M	<i>Betula pubescens</i>
15	<i>Phyllonorycter cavella</i> (Zeller, 1846)	–	M	<i>Betula</i> ?
16	<i>Ph. corylifoliella</i> (Hübner, 1796) †	GRPAL1112-13 99.82%; NL, DE, CZ	P	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. ermanii</i> *], in Europe Rosaceae
17	<i>Ph. ulmifoliella</i> (Hübner, 1817) †	GRPAL1127-13 100%; AT, DE, FI, FR	M	<i>Betula</i> , [ <i>B. pendula</i> ]
<b>Family Incurvariidae</b>				
18	<i>Incurvaria pectinea</i> Haworth, 1828 †	SIBLE003-15 99.24%; DE	P	<i>Alnus</i> , <i>Betula</i> , <i>Malus</i> , <i>Rhododen-</i> <i>dron</i> , [ <i>Betula pendula</i> ]
<b>Family Lyonetiidae</b>				
19	<i>Leucoptera malifoliella</i> (O. Costa, 1836) †	SIBLE001-15 99.83%; FI	P	<i>Betula</i> , many Rosaceae [ <i>Cotone-</i> <i>aster melanocarpus</i> , <i>C. uniflorus</i> , <i>Crataegus</i> sp., <i>Betula pendula</i> ]
20	<i>Lyonetia clerkella</i> (Linnaeus, 1758) • †	SIBLE004-15 98.02%; NO	P	<i>Betula</i> , <i>Cotoneaster</i> , <i>Prunus</i> , <i>Malus</i> [ <i>Prunus padus</i> , <i>P. virgin-</i> <i>iana</i> , <i>Cotoneaster melanocarpus</i> ]

Table 2. (Contd.)

No.	Order, family, and species of insect	Specimen ID and accuracy of DNA diagnostics <sup>2</sup>	Trophic specialization <sup>3</sup>	Host plants in Siberia <sup>4</sup>
21	<i>L. prunifoliella</i> (Hübner, 1796)	–	P	<i>Betula</i> , <i>Cotoneaster</i> , <i>Prunus</i>
Family <b>Nepticulidae</b>				
22	<i>Ectoedemia minimella</i> (Zetterstedt, 1839)	–	M	<i>Betula</i>
23	<i>E. occultella</i> (Linnaeus, 1767)	–	M	<i>Betula</i>
24	<i>Stigmella betulicola</i> (Stainton, 1856) †	–	M	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. dahurica</i> *, <i>B. divaricata</i> *, <i>B. occidentalis</i> *]
25	<i>S. confusella</i> (Wood et Walsingham, 1894)	–	M	<i>Betula</i>
26	<i>S. lapponica</i> (Wocke, 1862) †	–	M	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. dahurica</i> *, <i>B. divaricata</i> *]
27	<i>S. continuella</i> (Stainton, 1862) †	–	M	[ <i>B. pendula</i> ]
28	<i>S. luteella</i> (Stainton, 1857)	–	M	<i>Betula</i>
Family <b>Roeslerstammiidae</b>				
29	<i>Roeslerstammia erxebella</i> (Fabricius, 1787)	–	P	<i>Betula</i> , in Europe also <i>Tilia</i> , rarely <i>Acer</i>
Family <b>Yponomeutidae</b>				
30	<i>Atemelia torquatella</i> (Lienig et Zeller, 1846)	–	P	<i>Betula</i> , <i>Alnus</i> , <i>Ulmus</i>
31	<i>Swammerdamia caesiella</i> (Hübner, 1796)	–	M	<i>Betula</i>
Order COLEOPTERA				
Family <b>Curculionidae</b>				
32	<i>Anoplus plantaris</i> (Naezén, 1794)	–	O	<i>Betula</i> , in other parts of Russia also <i>Alnus</i>
33	<i>Orchestes jota</i> (Fabricius, 1787) †	–	P	<i>Betula</i> , <i>Alnus</i> , <i>Myrica gale</i> , ( <i>Salix</i> , <i>Populus</i> ), [ <i>Betula microphylla</i> , <i>B. occidentalis</i> *, <i>B. pendula</i> ]
34	<i>O. rusci</i> (Herbst, 1795) †	ISSIK118-14 99.85%; FI	M	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. pubescens</i> , <i>B. microphylla</i> , <i>B. occidentalis</i> *]
35	<i>O. calceatus</i> (Germar, 1821) #	–	O	<i>Alnus</i> , <i>Betula</i>
36	<i>Rhamphus pulicarius</i> (Herbst, 1795)	–	P	<i>Betula</i>
37	<i>Tachyerges pseudostigma</i> (Tempère, 1982)	–	P	<i>Alnus</i> , <i>Betula</i> , <i>Corylus</i> , <i>Salix</i>
38	<i>T. stigma</i> (Germar, 1821)	–	P	<i>Alnus</i> , <i>Betula</i> , <i>Salix</i> , ( <i>Populus</i> )
Order HYMENOPTERA				
Family <b>Tenthredinidae</b>				
38	<i>Fenusa pumila</i> Leach, 1817 †	ISSIK109-14 99.54%; NL	O	<i>Betula</i> , <i>Alnus</i> , [ <i>Betula costata</i> *, <i>B. dahurica</i> *, <i>B. gmelinii</i> *, <i>B. pendula</i> ]
40	<i>Fenusella nana</i> (Klug, 1816) †	ISSIK135-14 100%; CA, DE, FI, NO	M	<i>Betula</i> , [ <i>B. pendula</i> , <i>B. dahurica</i> *, <i>B. microphylla</i> , <i>B. papyrifera</i> *]
41	<i>Heterarthrus nemoratus</i> (Fallén, 1808) †	ISSIK119-14 100%; CA, DE, NL	M	<i>Betula</i> , [ <i>B. pendula</i> ]
42	<i>Profenusa thomsoni</i> (Konow, 1886) †	ISSIK122-14 100%; CA, FI, FR	M	<i>Betula</i> , [ <i>B. pendula</i> ]

**Table 2.** (Contd.)

No.	Order, family, and species of insect	Specimen ID and accuracy of DNA diagnostics <sup>2</sup>	Trophic specialization <sup>3</sup>	Host plants in Siberia <sup>4</sup>
43	<i>Scolioneura betuleti</i> (Klug, 1816) †	–	M	<i>Betula</i> , ( <i>Populus</i> ), [ <i>Betula pendula</i> ]
Order DIPTERA Family <b>Agromyzidae</b>				
44	<i>Agromyza alnibetulae</i> Hendel, 1931 †	–	M	<i>Betula</i> , ( <i>Alnus</i> ), [ <i>Betula pendula</i> , <i>B. ermanii</i> *]

<sup>1</sup> Based on the literature data (see *Materials and Methods*) and our records (in the latter case the species are marked with †).

<sup>2</sup> This column contains identification number (ID) of Siberian samples in the BOLD database, the reliability (%) of molecular diagnostics, and the origin of the reference specimens: AT, Austria; CA, Canada; CZ, Czech Republic; DE, Germany; FI, Finland; FR, France; NL, the Netherlands; NO, Norway. <sup>3</sup> Trophic specialization: M, monophagous on *Betula*; O, oligophagous; P, polyphagous (see *Materials and Methods*). <sup>4</sup> Host plants are listed based on the literature data (see *Materials and Methods*); doubtful indications of host plants in Russian literature (*Forest Pests*, 1955; Tomilova, 1962, 1972; Dovnar-Zapolsky and Tomilova, 1978; Barannik, 1981; Kuznetsov, 1981; Gusev, 1984; Yanovsky, 2003) which need to be verified are given in parentheses; our data are given in square brackets; \* introduced plant species; # for notes on the identity of *O. testaceus* and *O. calceatus*, see *Results and Discussion*; • Additional studies are required to confirm the status of the Siberian specimens (see *Results and Discussion*).

*Fenusa pumila* (Table 2). About 30% of the leaf mining species are polyphagous; this group includes only micro-moths and beetles (Table 2). Besides birch and alder, they may feed on plants of the family Rosaceae and, less frequently, on Salicaceae and Ulmaceae.

For six insect species mining birch leaves in Siberia, the Russian publications listed some host plants which are not typical of these insects according to the modern databases (De Prins and De Prins, 2015; Edmunds, 2015; Ellis, 2015; Fauna Europaea, 2015). In particular, maple (*Acer*) was mentioned as a host plant for the casebearer *Haploptilia serratella* (Barannik, 1981), alder (*Alnus*) and poplar (*Populus*), for the moth *Caloptilia populetorum* (Tomilova, 1962, 1973; Kuznetsov, 1981), poplar and willow (*Salix*), for the weevils *Orchestes jota* and *Tachyerges stigma* (Yanovsky, 2003), poplar for the sawfly *Scolioneura betuleti* (Tomilova, 1962), and, finally, alder for the leafminer fly *Agromyza alnibetulae* (*Forest Pests*, 1955; Dovnar-Zapolsky and Tomilova, 1978; Gusev, 1984). The ability of these insects to develop on the indicated host plants needs to be confirmed.

In our survey, 23 out of 25 species of leaf mining insects were recorded on *B. pendula* and one, on *B. pubescens* (Table 2). About half of these species were also found on the introduced birches, mostly on the Eastern Asian species *B. costata*, *B. dahurica*, *B. divaricata*, *B. ermanii*, and *B. gmelinii* (Table 2). Five species (the micro-moths *Parornix betulae* and *Stigmella betulicola*, the beetles *Orchestes jota* and

*O. rusci*, and the sawfly *Fenusella nana*) occurred on the North American birches *B. occidentalis* and *B. papyrifera* (Table 2).

The apple leaf miner *Lyonetia clerkella* is a polyphagous species occasionally feeding on birch. We recorded this moth in Siberia on different plants of the family Rosaceae but never on birch (Table 2). Another polyphagous species, the pear leaf blister moth *Leucoptera malifoliella*, usually occurs on Rosaceae in Siberia. In August 2015, we found numerous mines of this species on leaves of young birches in Yakutsk.

**Pests and invasive species.** Nine insect species were listed in the literature as birch pests in the Palaearctic. This group includes 5 species of leaf mining micro-moths: *Caloptilia betulicola*, *C. populetorum*, *C. suberinella*, *Coleophora serratella*, and *Phyllonorycter ulmifoliella* (Barannik, 1981; *Key to the Insects...*, 1981) and 4 species of sawflies: *Fenusa pumila*, *Fenusella nana*, *Heterarthrus nemoratus*, and *Profenusa thomsoni* (*Forest Pests*, 1955; Tibatina and Stroganova, 1966; Stroganova, 1973). The sawfly *H. nemoratus* damages young birches in nurseries and shelter belts in Europe and European Russia (*Forest Pests*, 1955).

The four remaining species of polyphagous micro-moths, namely *Coleophora anatipenella*, *Leucoptera malifoliella*, *Lyonetia clerkella*, and *Phyllonorycter corylifoliella*, cause considerable harm not to birches (although they can develop on them) but to trees of the family Rosaceae in gardens and parks; they are pests

of apple, pear, hawthorn, rowan, plum, cherry, and other plants (*Forest Pests*, 1955; Tomilova, 1973; *Insects and Acarines...*, 1999). No pests are known among the leaf mining beetles and flies occurring on birches.

At the beginning of the XX century the leaf mining sawflies *Fenusa pumila*, *Fenusella nana*, *Heterarthrus nemoratus*, and *Profenusa thomsoni*, whose native ranges lie in the Palaearctic (including Siberia), invaded North America (Digweed et al., 2009). There they considerably damage the North American birch species, especially *B. papyrifera*. There are no invasive species among representatives of the orders Lepidoptera, Coleoptera, and Diptera occurring on birches in Siberia.

**Distribution in Siberia.** The distribution of leaf mining insects on birches in Siberia remains poorly known. In this respect, Novosibirsk and Irkutsk Provinces, where over a half of the known birch leaf miners have been recorded (Table 3), are the best explored regions of Siberia.

Our surveys provided new data about the distribution of leaf mining insects trophically associated with birches in Siberia. New distribution records were made for 20 species in Siberia (Table 3). Most of these species were for the first time recorded in Krasnoyarsk Territory (Krasnoyarsk) and Novosibirsk Province (Novosibirsk), which could be expected since material was collected repeatedly in these regions. For instance, some species of leaf mining micro-moths of the families Gracillariidae, Eriocraniidae, and Incurvariidae, and also sawflies earlier known from other Siberian regions were recorded in Krasnoyarsk for the first time. Some species of the genus *Stigmella* were for the first time found in several regions, and one of them, *S. continuella*, was discovered in Siberia for the first time (Fig. 3, 16). In Russia, this micro-moth species had been earlier known only from the European part and from Primorskii Territory (*Catalogue...*, 2008). We recorded *S. continuella* in Western Siberia (Novosibirsk Province: Novosibirsk, CSBG, *Betula pendula*, mines with larvae, 11.VI.2012, coll. N.I. Kirichenko, det. E. van Nieukerken, 3 specimens). The following leaf mining insect species were for the first time recorded in three regions: *Phyllonorycter coryli-foliella* in Omsk and Novosibirsk Provinces and Krasnoyarsk Territory; *Stigmella lapponica* in Tomsk and Kemerovo Provinces and the Buryatia Republic; *Agromyza alnibetulae* in Tomsk Province and in Altai

and Krasnoyarsk Territories (Table 3). The pear leaf blister moth *Leucoptera malifoliella* was for the first time recorded in the Altai and Sakha (Yakutia) Republics (Table 3).

Findings of leaf mining insect species in new regions do not necessarily indicate an extension of their ranges but rather reflect insufficient knowledge of these insects in Siberia.

**Richness of birch leaf miners in Siberia and Europe.** The fauna of insects mining birch leaves in Siberia is surprisingly similar to that of Europe, as indicated by the relatively high value of the Chekanovsky-Sørensen index (0.77). The group of birch leaf miners includes 44 species in Siberia (Tables 2, 3) and 70 species in Europe (Hering, 1951; Ellis, 2015). All the species listed herein for Siberia can also be found on birches in Europe (Hering, 1951; De Prins and De Prins, 2015; Ellis, 2015). Comparison of the birch leaf miner faunas of Siberia and Europe yields the following values of the Chekanovsky-Sørensen index: 0.68, 0.91, 0.93, and 1 for Lepidoptera, Hymenoptera, Coleoptera, and Diptera, respectively. The number of leaf mining beetles and sawflies is one species greater in Europe than in Siberia. The polyphagous jewel beetle *Trachys minutus* (Linnaeus) can be found on *Betula* in Europe, although in Siberia it develops on other woody plants but not on birches. The leaf mining sawfly *Scolioneura vicina* Konow feeds on birches in Europe but has never been recorded in Siberia. It is noteworthy that both regions are inhabited by the closely related species *S. betuleti*, which is, however, indistinguishable from *S. vicina* according to the recent molecular genetic research (Leppänen et al., 2012).

The difference between the faunas of Siberia and Europe is mainly due to leaf mining micro-moths. There are 10 species of Coleophoridae, 4 species of Nepticulidae, 3 species of Gracillariidae, and 1 species of Yponomeutidae that occur on birches in Europe but have never been recorded in Siberia (Hering, 1951; De Prins and De Prins, 2015; Ellis, 2015). Leaf mining micro-moths of the families Heliozelidae and Bucculatricidae were recorded on birches only in Europe (Ellis, 2015).

The Ural Mountains separating the European and the Asian parts of Russia do not seem to be an obstacle for dispersal of leaf miners and mixing of the regional faunas. However, this suggestion should be treated with some caution. Since the leaf mining insects of

**Table 3.** Distribution of insect species mining birch leaves in the regions of Siberia\*

No.	Insect species	Regions of Siberia													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
<b>Order LEPIDOPTERA</b>															
<b>Family Coleophoridae</b>															
1	<i>Coleophora anatipenella</i>						+					+			
2	<i>C. betulella</i> †				×										
3	<i>Haploptilia serratella</i> †	+			+			+	○			+			
<b>Family Eriocraniidae</b>															
4	<i>Eriocrania cicatricella</i> †	+	+		+	+			○			+			
5	<i>E. salopiella</i>											?			
6	<i>E. sangii</i> †	+	+		+	+			○			?			
7	<i>E. semipurpurella</i> †	+	+	+	+	+			○			+		+	+
8	<i>E. sparrmannella</i> †	+	+	+	○	+						+		+	
9	<i>Heringocrania unimaculella</i> †	+	+		×	+									
<b>Family Gracillariidae</b>															
10	<i>Caloptilia betulicola</i> †				+		?	×	○			+		+	
11	<i>C. populetorum</i>											+		+	
12	<i>C. suberinella</i>										+			+	
13	<i>Parornix betulae</i> †*	+	+		×	+			×			×		×	○
14	<i>P. traugotti</i>											?	?		
15	<i>Phyllonorycter cavella</i> #														
16	<i>Ph. corylifoliella</i> †		○		○				○			+			
17	<i>Ph. ulmifoliella</i> †		+		×	+		○	×	+		×	○	×	+
<b>Family Incurvariidae</b>															
18	<i>Incurvaria pectinea</i> †	+	+		×	+			○			+		+	
<b>Family Lyonetiidae</b>															
19	<i>Leucoptera malifoliella</i> †	+	+		+	+	○	○				+		+	○
20	<i>Lyonetia clerkella</i> †				○		×	○				+		+	
21	<i>L. prunifoliella</i>											+	+		
<b>Family Nepticulidae</b>															
22	<i>Ectoedemia minimella</i>													?	
23	<i>E. occultella</i>											?			
24	<i>Stigmella betulicola</i> †				○							?			
25	<i>S. confusella</i>											+		+	
26	<i>S. lapponica</i> †	+	+	○	×	+		○	×			×	○	×	+
27	<i>S. continuella</i> †				○										
28	<i>S. luteella</i>											+			
<b>Family Roeslerstammiidae</b>															
29	<i>Roeslerstammia erxlebelli</i>		+		+	+			+	+		+		+	
<b>Family Yponomeutidae</b>															
30	<i>Atemelia torquatella</i>											+			
31	<i>Swammerdamia caesiella</i>		+		+	+								+	+

Table 3. (Contd.)

No.	Insect species	Regions of Siberia													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Order COLEOPTERA															
Family <b>Curculionidae</b>															
32	<i>Anoplus plantaris</i>				+			+	+			+			
33	<i>Orchestes jota</i> †			+	×	×		+				+		+	+
34	<i>O. rusci</i> †	+		+	×	+	+	×	×			+		+	+
35	<i>O. calceatus</i>	+	+	+	+	+	+	+	○		+	+	+	+	+
36	<i>Rhamphus pulicarius</i>				+						+	+	+	+	+
37	<i>Tachyerges pseudostigma</i>			+	+			+				+	+	+	
38	<i>T. stigma</i>							+		+		+	+		
Order HYMENOPTERA															
Family <b>Tenthredinidae</b>															
38	<i>Fenusa pumila</i> †				×			+				+			
40	<i>Fenusella nana</i> †		○		+				○			×			
41	<i>Heterarthrus nemoratus</i> †				+			+	○			×			
42	<i>Profenusa thomsoni</i> †								○			+		+	
43	<i>Scolioneura betuleti</i> †				×		+		×			+			
Order DIPTERA															
Family <b>Agromyzidae</b>															
44	<i>Agromyza alnibetulae</i> †			○	×		○		○			×			

\* Based on the literature data (see *Materials and Methods*) and our records (in the latter case the species are marked with †). Regions of Siberia: 1, Tyumen Province; 2, Omsk Province; 3, Tomsk Province; 4, Novosibirsk Province; 5, Altai Territory; 6, Altai Republic; 7, Kemerovo Province; 8, Krasnoyarsk Territory (south); 9, Republic of Khakassia; 10, Republic of Tuva; 11, Irkutsk Province; 12, Republic of Buryatia; 13, Transbaikalia; 14, Sakha Republic (Yakutia). + regions for which the presence of species is recorded in the literature (see references in *Materials and Methods*); × regions for which the presence of species was confirmed by our data corresponding to the records in the literature; ○ regions indicated for the first time based on our records; ? (question mark) presence of the species in the region needs to be verified (*Catalogue of the Lepidoptera of Russia*, 2008); \* the species is listed under the name *Parornix devoniella* (Stainton) in the *Catalogue...* (2008); # *Phyllonorycter cavella* was recorded in Siberia without indication of regions (Kuznetsov and Baryshnikova, 1998).

Siberia are commonly identified using the keys for Europe and European Russia, some diagnostics errors may occur. New and still undescribed Siberian leaf mining species may be quite similar to the European ones, differing only in small, barely discernible morphological details, so that such species can be quite easily confused with European ones when identified with European keys. A good example is our recent finding of the leaf mining micro-moth from the genus *Micrurapteryx* (Gracillariidae) on the peashrub *Caragana arborescens* Lam. in Siberia. For decades, this insect has been confused with *M. gradatella* (Herrich-Schäffer, 1855) in the Russian literature (Dovnar-Zapolsky and Tomilova, 1978; Kuznetsov and Baryshnikova, 1998; *Insects and Acarines...*, 1999). However, the latter is distributed in Europe on some

other legumes. Based on DNA barcoding of the Siberian specimens and subsequent examination of the adult genitalia, we differentiated the two species and redescribed the Siberian species as *M. caraganella* (Hering, 1957) (Kirichenko et al., 2016).

Careful revision of the leaf mining insect fauna in Siberia with the use of not only morphological but also modern molecular genetic tools would allow the researchers to reliably assess the taxonomic richness of this group in Siberia and to reveal new leaf miner species which for a long time remained undiscovered or confused with European species. Development of DNA barcoding library of leaf mining insects of Siberia (Kirichenko et al., 2014) would increase the efficiency and reliability of their identification in this poorly studied region of the Palaearctic.

## CONCLUSIONS

Analysis of the Russian literature and our data allowed us to revise the list of birch leaf miners, to study their distribution, and also to clarify their trophic relations with the native and introduced *Betula* species in Siberia. Altogether, 44 leaf mining insect species were recorded on birches in Siberia. Two-thirds of them are micro-moths; some of them are known as pests of *Betula* and cultivated Rosaceae in the Palaearctic. Four out of five sawfly species damage birches not only in the Palaearctic but also in North America where they invaded several decades ago.

As the result of our study, new distribution records were made for 20 leaf mining species; besides, the micro-moth *Stigmella continuella* (Stainton, 1856) from the family Nepticulidae, previously known only from the extreme western and eastern regions of Russia, was found for the first time in Central Siberia. The majority of leaf-mining insect species from Siberia was identified using DNA barcoding. The efficiency and accuracy of this modern molecular genetic approach and its applicability to diagnostics of insects by larvae and pupae offer new source of data to researchers. This method will significantly improve our knowledge not only about taxonomic diversity, distribution, and trophic relations of leaf mining insects but also about their phylogeny and phylogeography.

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