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The comparison of the parasitoid complex (Hymenoptera: Pteromalidae, Eulophidae, Braconidae) of the poplar leaf miner *Phyllonorycter populifoliella* (Treitschke, 1833) (Lepidoptera: Gracillariidae) on two species of poplars in the Glazov Town

Сравнение комплекса паразитоидов (Hymenoptera: Pteromalidae, Eulophidae, Braconidae) тополевой моли-пестрянки *Phyllonorycter populifoliella* (Treitschke, 1833) (Lepidoptera: Gracillariidae) на двух видах тополей в г. Глазове

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КЛЮЧЕВЫЕ СЛОВА: Chalcidoidea, Ichneumonoidea, паразитоид, зараженность.

ABSTRACT. The assemblage of hymenopteran parasitoids associated with the poplar leaf miner, *Phyllonorycter populifoliella* (Treitschke, 1833) (Lepidoptera: Gracillariidae), developing on two poplars species (*Populus balsamifera* and *P. × jackii*) was studied in the Glazov Town of Udmurt Republic during 2023. A qualitative and quantitative comparison of miner parasitoid complexes on two poplar species revealed their significant similarity. Twenty species of *Ph. populifoliella* parasitoids were recorded, *Cirrospilus diallus*, *C. elegantissimus*, *C. elongatus*, *C. pictus*, *Elachertus fenestratus*, *Pnigalio mediterraneus*, *P. soemius*, *Sympiesis acalle*, *S. gordius*, *S. laevifrons*, *S. sericeicornis*, *Chrysocharis laomedon*, *Ch. nephereus*, *Ch. pentheus*, *Closterocerus trifasciatus*, *Pediobius saulius*, *Baryscapus pospelovi*, *Minotetrastichus frontalis* (Eulophidae), *Pteromalus semotus* (Pteromalidae), and *Pholetesor circumscriptus* (Braconidae). Four species of them, namely *C. elongatus*, *S. laevifrons*, *B. pospelovi* (Eulophidae) and *P. semotus* (Pteromalidae) were reported as the parasitoids of this miner for the first time. The rate of parasitism of the miner was negligible and ranged from 13.5 ± 2.9 (on *P. × jackii*) to 15.5 ± 2.0% (on *P. balsamifera*).

РЕЗЮМЕ. Исследован комплекс паразитоидов тополевой моли-пестрянки *Phyllonorycter populifoliella* (Treitschke, 1833) (Lepidoptera: Gracillariidae), развивающийся на двух видах тополей (*Populus balsamifera* и *P. × jackii*) в г. Глазове (Удмуртская Республика) в 2023 г. Качественное и количественное сравнение комплексов паразитоидов минера на двух видах тополей показало их значительную схожесть. Выявлено 20 ви-

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дов наездников: *Cirrospilus diallus*, *C. elegantissimus*, *C. elongatus*, *C. pictus*, *Elachertus fenestratus*, *Pnigalio mediterraneus*, *P. soemius*, *Sympiesis acalle*, *S. gordius*, *S. laevifrons*, *S. sericeicornis*, *Chrysocharis laomedon*, *Ch. nephereus*, *Ch. pentheus*, *Closterocerus trifasciatus*, *Pediobius saulius*, *Baryscapus pospelovi*, *Minotetrastichus frontalis* (Eulophidae), *Pteromalus semotus* (Pteromalidae) и *Pholetesor circumscriptus* (Braconidae). Впервые в качестве паразитоидов этого минера отмечены *Cirrospilus elongatus*, *Sympiesis laevifrons*, *Baryscapus pospelovi* (Eulophidae) и *Pteromalus semotus* (Pteromalidae). Зараженность паразитоидами гусениц и куколок *Ph. populifoliella* была незначительной и варьировала от 13,5 ± 2,9% на тополе *P.* × *jackii* до 15,5 ± 2,0% на тополе *P. balsamifera*.

Introduction

The poplar leaf miner, *Phyllonorycter populifoliella* (Treitschke, 1833), is a native Eurasian species. It is a narrow oligophage developing on 12 species of the genus *Populus* L. of the family Salicaceae [Ermolaev *et al*., 2020]. In the European part of the Russian Federation, in the Urals and Western Sibiria, the miner has a one-year generation. Due to the lack of accumulated temperatures in these areas, the larvae of the second generation do not have time to complete its development and, as a rule, die [Ermolaev, 2019].

This eurytopic leaf miner has a high population density in urban environments where its chronic outbreaks can last for decades. For example, such outbreaks of *Ph. populifoliella* existed for at least 36 years in Moscow in the 20th century [Ermolaev, 2019]. Chronic outbreaks of the miner are also known in the cites of Udmurt Republic, Izhevsk and Glazov. Our previous research conducted in the Izhevsk City allowed to identify 33 species of the hymenopteran parasitoid assemblage of *Ph. populifoliella* [Ermolaev *et al*., 2016].

The objectives of this research were to compere the qualitative and quantitative structure of parasitoid wasp assemblage (Hymenoptera: Pteromalidae, Eulophidae, Braconidae) developing on two poplar species (*Populus balsamifera* and *P.* × *jackii*) in Glazov Town, and also to estimate the effect of parasitoids on the host mortality.

Material and Methods

The assemblage of hymenopteran parasitoids of *Ph. populifoliella* of Glazov Town was studied from July 1 to 10, 2023 at two sample plots: No. 1 on T. Baramzina Street (58°15′ N, 52°64′ E) and No. 2 on Kalinina Street (58°14′ N, 52°69′ E) (Fig. 1). On the sample plot No. 1, ten specimens of balsam poplar (*Populus balsamifera* Linnaeus, 1753) grew; on the

Fig. 1. Research localities in the Glazov Town. Poplar *Populus balsamifera* grew on the sample plot No. 1; poplar *P.* × *jackii* grew on the sample plot No. 2. Scale: 1 : 300.

Рис. 1. Места проведения исследования в г. Глазове. На пробной площадке № 1 произрастал тополь бальзамический *Populus balsamifera*, на пробной площадке № 2 – тополь Джека *P.* × *jackii*. Масштаб: 1 : 300.

sample plot No. 2, eight specimens of Jack poplar (*P. balsamofera* × *P. deltoides* W. Bartram ex Marshall, 1785 or *P.* × *jackii*) grew. The height of trees on the sample plot No. 1 was 22.9 ± 0.6 m, for No. 2 — 18.7 ± 0.8 m. The diameter of poplar stems at the sample plot No. 1 was 77.0 ± 4.9 cm, for No. 2 — 47.8 ± 2.2 cm. The tree height was measured with a Haglöf EC II electronic clinometer, the caliper diameter, with a Haglöf Mantax Blue caliper (800 mm). All trees on the sample plots had a well-developed crown and no traces of its deformation.

During the pupation period of the first generation of *Ph. populifoliella*, 60–70 leaves with mines were collected from north-facing primary branches in the lower crown layer of each model tree. The leaves with mines were cut out with scissors and placed in plastic containers (250 ml^3) labeled with the model trees numbers. The collected material was transported to the Biological Station of Udmurt State University, Izhevsk. The mines were kept under constant conditions (temperature 22–25 °C, relative humidity 65%, photoperiod 18L:6D h).

The emergence of *Ph. populifoliella* adults and parasitoids was recorded daily. Altogether, 3032 mines were collected, 1979 specimens of *Ph. populifoliella* and 456 specimens of parasitoids were reared. The identification of *Ph. populifoliella* was confirmed by S.V. Baryshnikova (Zoological Institute of the Russian Academy of Sciences). The parasitoids were identified by O.V. Kosheleva (Eulophidae), E.V. Tselikh (Pteromalidae) and S.A. Belokobylskij (Braconidae). The species identity of poplars was confirmed by A.N. Puzyrev (Udmurt State University). Some of the miner specimens and all parasitoid specimens are deposited in the Zoological Institute of the Russian Academy of Sciences (Saint Petersburg, Russia; ZISP).

Schemes of hyperparasitism episodes between parasitoids reared from *Ph. apparella* (Herrich-Schäffer, 1855) and *Ph. populifoliella* known in literature were constructed based on materials from the Universal Chalcidoidea Database [2024]. Figure 1, showing the collection locations, was generated with a 2 GIS map.

The following parameters were calculated:

$R = M / L$, where *R* is the infestation density of an individual model tree, *M* is the total number of mines on three north-facing model branches from the lower crown layer, and *L* is the total number of leaves on these branches, i.e. number of mines per leaf.

$V = B / N \times 100$, where *V* is the pupal survival rate of *Ph. populifoliella* in an individual model tree, *B* is the number of adults reared from the mines, and *N* is the total number of the mines collected from the tree.

$P = W / N \times 100$, where *P* is the rate of parasitism of *Ph. populifoliella* larvae and pupae, *W* is the total number of the reared parasitoids, and *N* is the total number of the collected mines.

$K = D - P$, where *K* is mortality from unknown factors (the cause of death was difficult to identify, e.g., mortality from induced response of the host tree to damage, mortality from some pathogens, mortality due to maturation feeding of parasitoids, or mortality due to intraspecific competition of the leaf miner larvae), *D* is total mortality of larvae and pupae, and *P* is mortality of larvae and pupae due to parasitoids.

The mean value and standard error were calculated in all the cases. The data were statistically processed by the standard methods [Ivanter, Korosov, 2011]. The linear correlation coefficient was calculated. The comparison of two samples was performed using the Student's T-test. When calculating the faunal similarity of parasitoid complexes of *Ph. populifoliella* from Izhevsk and Glazov, the Sørensen coefficient (K_S) was used [Sørensen, 1948]. For comparison of the means expressed as percentages, they were transformed as $\varphi = 2\arcsin\sqrt{x}$.

Results and discussion

Under the conditions of the laboratory, the appearance of the adults of *Ph. populifoliella* developing on different host plants, poplars *Populus balsamifera* and *P.* × *jackii*, was relatively asynchronous. If for the former host plant, the emergence of adults occurred from July 11 to 26 (with a peak on July 15), then for the latter — from July 13 to 28 (with peaks on July 20 and 22) (Fig. 2). Meanwhile, the emergence of *Ph. populifoliella* parasitoids from the two poplar species occurred at one period: from July 9 to July 30, with a maximum on July 15 (for *P. balsamifera*) and July 17 (for *P.* × *jackii*) (Fig. 3).

Poplars from the sample plot No. 1 (*P. balsamifera*) were closer to the center of outbreak *Ph. populifoliella* and had a significantly ($P < 0.05$) higher the pupal infestation density (*R*) than plants from the sample plot No. 2 (*P.* × *jackii*) (Table 1). At the same time, the survival rate of *Ph. populifoliella* (*V*) was also significantly higher at the sample plot No. 1. It should be noted, that at both sample plots, survival was not related to the average infestation density of the host tree by the miner (Table 2).

Total mortality of *Ph. populifoliella* caterpillars and pupae (*D*) on the sample plot No. 2 (*P.* × *jackii*) significantly exceeded this rate on the sample plot No. 1 (*P. balsamifera*) on account of the effect of mortality due to unknown factors (*K*) predominance: 48.0 ± 5.0 versus 26.0 ± 1.7% (table 1). At sample the plot No. 2, mortality associated with unknown causes significantly decreased with increasing infestation density of poplar (Table 2). The reason for this pattern remains unclear.

The rate of parasitism of *Ph. populifoliella* larvae and pupae (*P*) was relatively low and comparable between the two plots (Table 1). Moreover, only on the plot No. 2 did the parasitism level significantly increase with the density of plant infestation by the miner (Table 2). This may indicate the existence of different functional responses (Solomon, 1949; Holling, 1959) of parasitoids to *Ph. populifoliella* density inside outbreak in an urban environment. The latter circumstance may be associated both with the different levels of pollution within the town and availability of alternative hosts for parasitoids.

Our study resulted in discovery of twenty species of parasitoids of *Ph. populifoliella* in Glazov Town: *Cirrospilus diallus* Walker, 1838, *C. elegantissimus* Westwood, 1832, *C. elongatus* Bouček, 1959, *C. pictus* (Nees, 1834), *Elachertus fenestratus* Nees, 1834, *Pnigalio mediterraneus* Ferriere et Delucchi, 1957, *P. soemius* (Walker, 1839), *Sympiesis acalle* (Walker, 1848), *S. gordius* (Walker, 1839), *S. laevifrons* Kamijo, 1965, *S. sericeicornis* (Nees, 1834), *Chrysocharis laomedon* (Walker, 1839), *Ch. nephereus* (Walker, 1839), *Ch. pentheus* (Walker, 1839), *Closterocerus trifasciatus* Westwood, 1833, *Pediobius saulius* (Walker, 1839), *Baryscapus pospelovi* (Kurdjumov, 1912), *Minotetrastichus frontalis* (Nees 1834) (Eulophidae), *Pteromalus semotus* (Walker, 1834) (Pteromalidae), and *Pholetesor circumscriptus* (Nees, 1834) (Braconidae) (Table 3). The rate of parasitism of the dominant species *Sympiesis gordius*, *Chrysocharis laomedon* and *Ch. nephereus* was not correlated with the leaf miner density both on *P. balsamifera*:

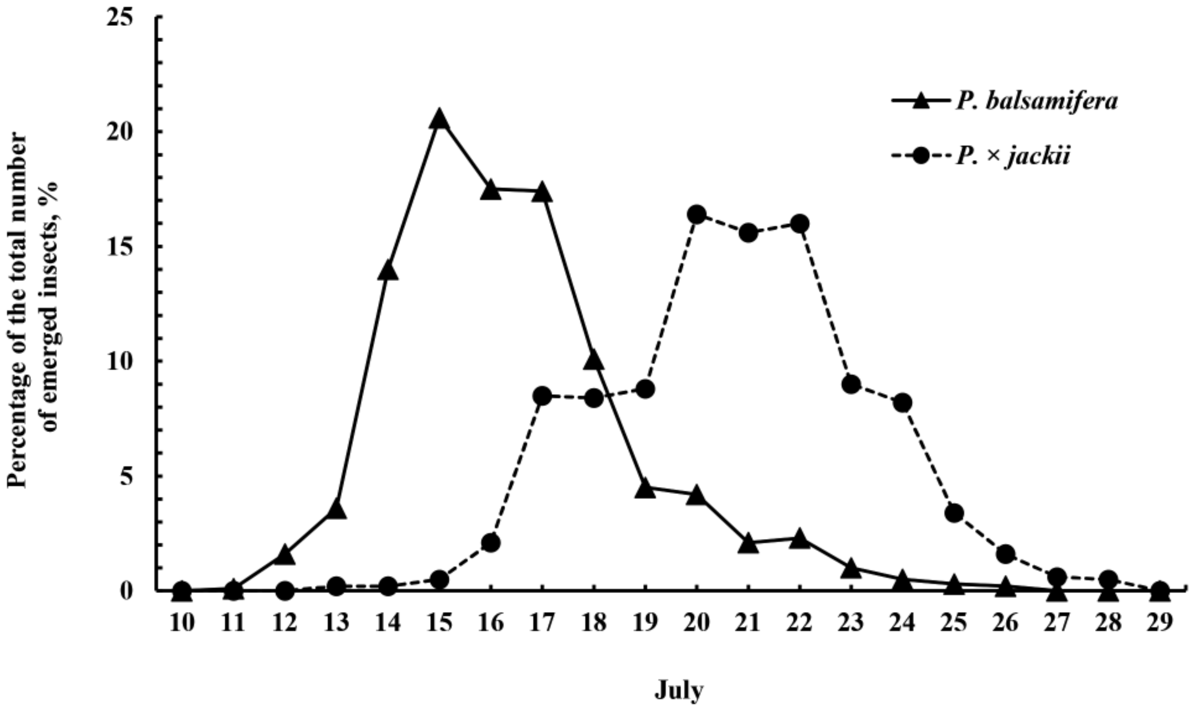

Fig. 2. Dynamics of emergence of *Ph. populifoliella* on poplars *Populus balsamifera* и *P. × jackii* in the field laboratory: material from Glazov Town, 2023.

Рис. 2. Динамика выхода молей *Ph. populifoliella* в 2023 г. на тополях *Populus balsamifera* и *P. × jackii* из г. Глазова в условиях полевой лаборатории.

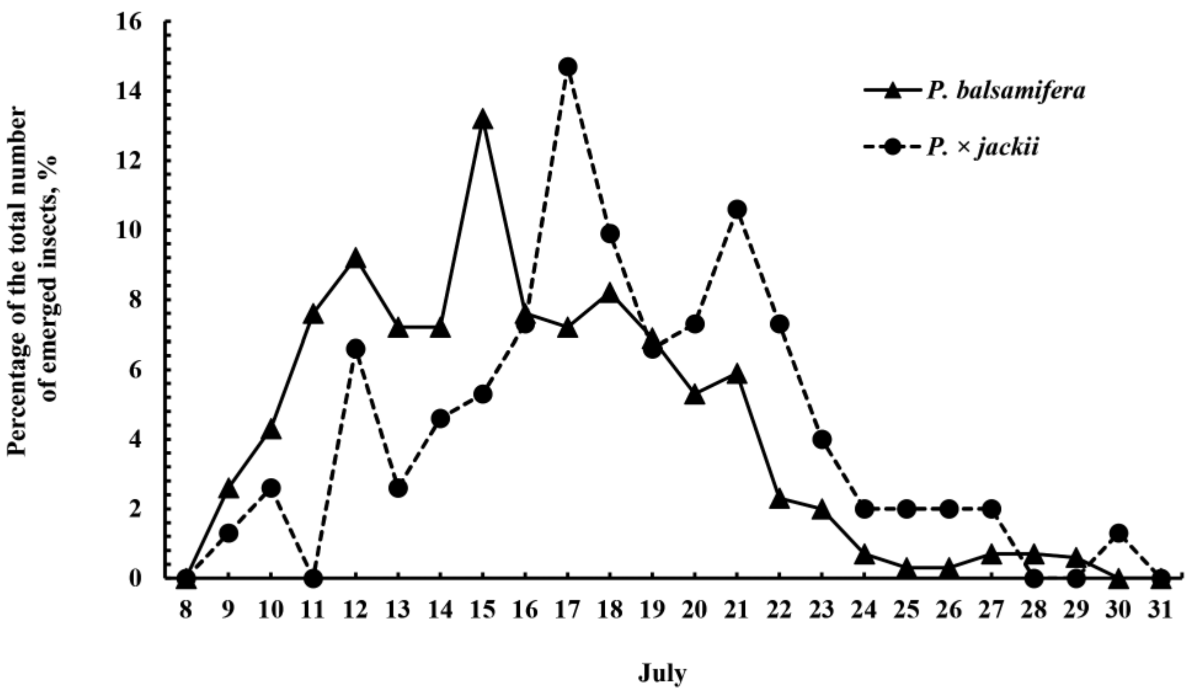

Fig. 3. Dynamics of emergence of *Ph. populifoliella* parasitoids on poplars *Populus balsamifera* и *P. × jackii* in the field laboratory: material from Glazov Town, 2023.

Рис. 3. Динамика выхода паразитоидов *Ph. populifoliella* на тополях *Populus balsamifera* и *P. × jackii* из г. Глазова в условиях полевой лаборатории.

($r = -0.11$, $n = 10$, $P > 0.05$), ($r = -0.32$, $n = 10$, $P > 0.05$), ($r = 0.26$, $n = 10$, $P > 0.05$) and on *P.* × *jackii* ($r = 0.41$, $n = 8$, $P > 0.05$), ($r = -0.17$, $n = 8$, $P > 0.05$), ($r = 0.43$, $n = 8$, $P > 0.05$), respectively.

Four species, namely *Cirrospilus elongatus*, *Sympiesis laevifrons*, *Baryscapus pospelovi* (Eulophidae) and *Pteromalus semotus* (Pteromalidae) were reported as the parasitoids of this miner for the first time.

Pteromalus semotus is a solitary, predominantly primary ectoparasitoid of various lepidopteran caterpillars [Graham, 1969]. In addition, this species is often recorded as a hyperparasitoid of primary Braconidae parasitoids [Harvey *et al*., 2012]. *P. semotus* is a widespread Palaearctic species, and it was even introduced to New Zealand for the biological control of certain Lepidoptera [Todorov *et al*., 2022]. *Cirrospilus elongatus* is a prima-

Table 1. Survival and mortality of the larvae and pupae of poplar leaf miner *Ph. populifoliella* in Glazov Town.
Таблица 1. Выживаемость и смертность гусениц и куколок тополевой моли-пестрянки *Ph. populifoliella* в г. Глазове.

Poplar	Infestation density (R) on model tree, mines per leaf	Survival rate (V) of moths, %	Mortality of moths (D), %	
			due to parasitoids (P)	due to unknown factors (K)
P. balsamifera	12.0 ± 0.8 **A**	74.0 ± 1.8 **B**	15.5 ± 2.0	10.5 ± 1.0 **C**
P. × *jackii*	7.8 ± 1.4 **A**	52.0±5.0 **B**	13.5± 2.9	34.5 ± 6.0 **C**

Statistically significant differences ($P < 0.05$) are marked with identical letters.

Table 2. Coefficients of correlation (r) between the population density of *Ph. populifoliella* and the survival and mortality rates of its larvae and pupae in Glazov Town.
Таблица 2. Значения коэффициентов корреляции между показателями плотности заселения тополя молью *Ph. populifoliella* и характеристиками выживаемости и смертности ее гусениц и куколок в г. Глазове.

Poplar	Survival of moths	Mortality of moths	
		due to parasitoids	due to unknown factors
P. balsamifera	$r = 0.16$	$r = -0.22$	$r = 0.15$
P. × *jackii*	$r = 0.01$	$r = 0.96$*	$r = -0.47$*

* − correlation is significant ($P < 0.05$).

Table 3. Species structure of the parasitoid assemblage associated with the poplar leaf miner (*Ph. populifoliella*) in Glazov Town, %.
Таблица 3. Видовая структура комплекса паразитоидов тополевой моли-пестрянки *Ph. populifoliella* в г. Глазове, %.

Species	*P. balsamifera*	*P.* × *jackii*
Pteromalidae		
Pteromalus semotus (Walker, 1834)* ▲	1.0 ± 0.7	0
Eulophidae		
Cirrospilus diallus Walker, 1838*	6.8 ± 2.1	2.6 ± 1.5
C. elegantissimus Westwood, 1832*	0 **A**	2.3 ± 1.1 **A**
C. elongatus Bouček, 1959*▲	0.4 ± 0.4	0
C. pictus (Nees, 1834)*	6.7 ± 3.0	6.1 ± 2.8
Elachertus fenestratus Nees, 1834*	0.6 ± 0.6	0.6 ± 0.6
Pnigalio mediterraneus Ferriere et Delucchi, 1957*	2.7 ± 1.3	8.1 ± 3.2
P. soemius (Walker, 1839)*	2.2 ± 1.2	5.2 ± 2.3
Sympiesis acalle (Walker, 1848)*	0.7 ± 0.7	0
S. gordius (Walker, 1839)*	20.8 ± 2.6	13.7 ± 3.9
S. laevifrons Kamijo, 1965*▲	0.3 ± 0.3	0.2 ± 0.2
S. sericeicornis (Nees, 1834)*	5.7 ± 2.0	2.5 ± 2.0
Chrysocharis laomedon (Walker, 1839)	18.2 ± 3.0	18.1 ± 4.9
Ch. nephereus (Walker, 1839)	18.3 ± 3.0	26.3 ± 3.2
Ch. pentheus (Walker, 1839)	1.0 ± 0.7	1.7 ± 1.1
Closterocerus trifasciatus Westwood, 1833	5.8 ± 2.1	2.0 ± 2.0
Pediobius saulius (Walker, 1839)	0.7 ± 0.7	0
Baryscapus pospelovi (Kurdjumov, 1912) ▲	0.5 ± 0.4	0
Minotetrastichus frontalis (Nees, 1834)*	5.5 ± 1.9	4.1 ± 2.0
Braconidae		
Pholetesor circumscriptus (Nees, 1834)	2.1 ± 1.4	6.5 ± 2.2

* – ectoparasitoid. ▲ – the species is recorded for the first time as a parasitoid of *Ph. populifoliella*. Statistically significant differences ($P < 0.05$) are marked with identical letters (**A**).

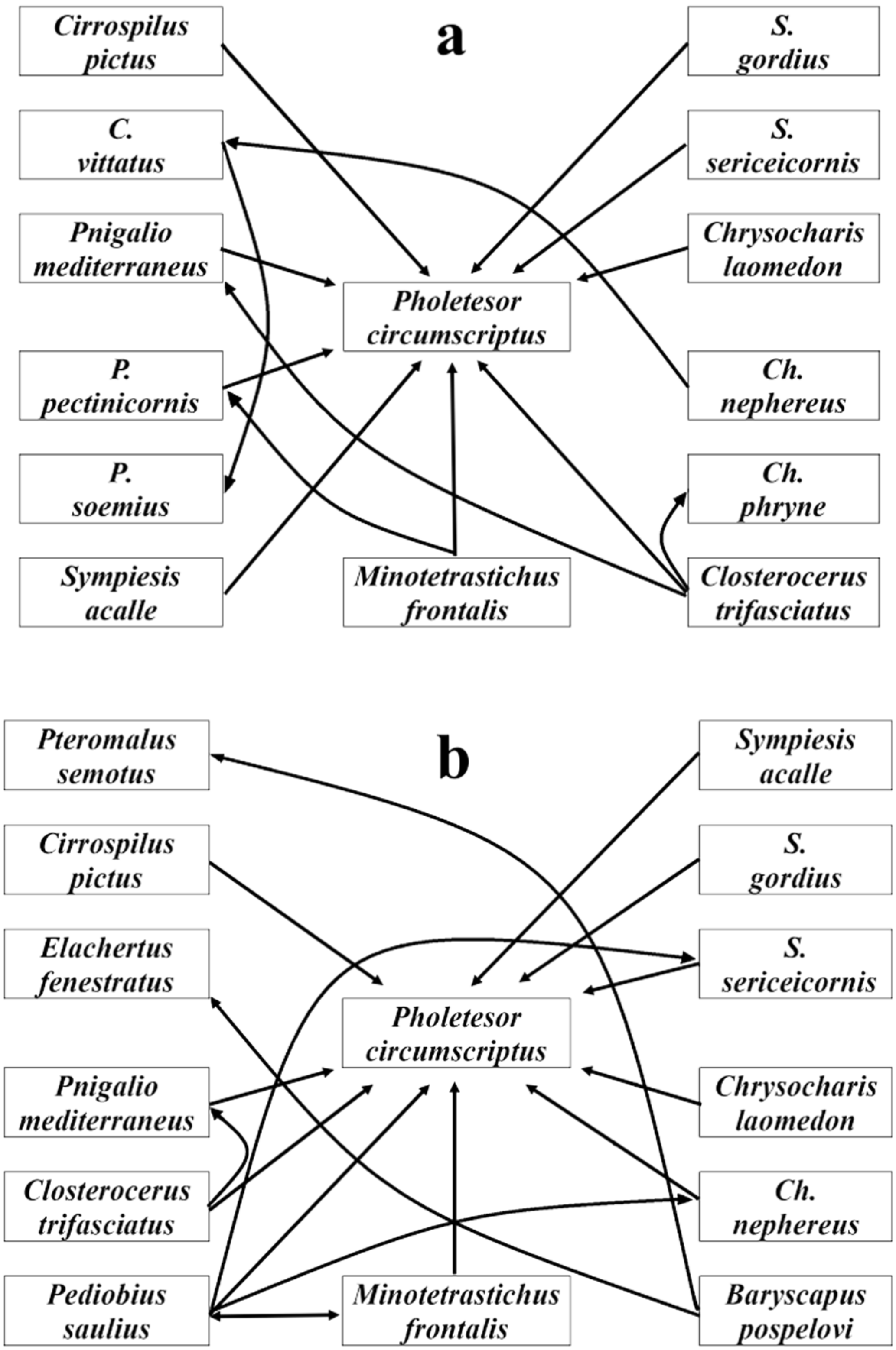

Fig. 4. Scheme of known cases of hyperparasitism between parasitoids reared from aspen leaf miner *Ph. apparella* in Izhevsk City [Ermolaev *et al*., 2022] (a) and poplar leaf miner *Ph. populifoliella* in Glazov Town (b). The scheme is based on the material from the Universal Chalcidoidea Database [2024].

Рис. 4. Схема известных случаев гиперпаразитизма между паразитоидами, выведенными из осиновой моли-пестрянки *Ph. apparella* в Ижевске [Ermolaev *et al*., 2022] (а) и тополевой моли-пестрянки *Ph. populifoliella* в г. Глазове (б). Схема построена на основании материалов базы данных Universal Chalcidoidea Database [2024].

ry ectoparasitoid of the members of some Lepidoptera [Belokobylskij *et al*., 2019], distributed in several countries of Europe, but in Russia, it was previously known only from the Republic of Crimea [Kosheleva, 2019]. *Sympiesis laevifrons* is a primary ectoparasitoid of caterpillars of the family Gracillariidae [Belokobylskij *et al*., 2019]. In the Far East, the species successfully attacks *Phyllonorycter issikii* (Kumata, 1963) [Kamijo, 1965]. However, *S. laevifrons* has never previously been recorded as a parasitoid of *Ph. issikii* throughout the entire secondary areal of the miner. *Baryscapus pospelovi* is an endoparasitoid that infests caterpillars of a number of the lepidopteran families [Graham, 1991]. In addition, the species has been noted as a secondary parasitoid of representatives of the families Pteromalidae (including *Pteromalus semotus*), Eulophidae and Braconidae [Graham, 1991]. *B. pospelovi* is distributed in the Western Europe and Russia [Belokobylskij *et al*., 2019].

In the previous study in Izhevsk, Udmurtia, 33 species were identified as parasitoids of *Ph. populifoliella* [Ermolaev *et al*., 2016]. A comparison of the parasitoid complexes of the Izhevsk and Glazov localities indicated their low similarity ($K_S = 0.49$). Izhevsk is a larger city located more than 140 km south of Glazov. While eulophids *Sympiesis sericeicornis*, *S. gordius* and *Chrysocharis laomedon* were predominant parasitoid species in Izhevsk, *S. gordius*, *Chrysocharis laomedon* and *Ch. nephereus* prevailed in Glazov (Table 3). The total number of *Ph. populifoliella* parasitoids in Udmurtia, identified in the two studies, amounted to 40 species. For comparison, in 13 similar investigations in the Moscow Province, only 20 parasitoids of this miner were reared [Ermolaev, 2019].

The parasitoid complexes associated ecologically with poplars *P. balsamifera* and *P. × jackii* turned out to be similar both qualitatively and quantitatively (Table 3). This can be explained by the genetic proximity of the poplar species and, apparently, by the affinity of the induced infochemical signals used by the parasitoids to search for the miner. The only significant difference ($P < 0.05$) was found for *Cirrospilus elegantissimus*: this ectoparasitoid was more abundant on *P. × jackii*.

Our study of the outbreak of the native aspen leaf miner *Phyllonorycter apparella* (Herrich-Schäffer, 1855) (Gracillariidae), carried out in 2014–2017 near Izhevsk, demonstrated the development of the miner parasitoid assemblage over time [Ermolaev *et al*., 2022]. At the same time, there is an annual change in the dominant species and an increase in the number of species comprising this complex. Changes in the structure of the parasitoid assemblage in the *Ph. apparella* outbreak were based on multitrophic interactions between the species. The number of species in the assemblage can increase in two ways: through increased competition for the host among the primary parasitoids and through emergence of new cases of hyperparasitism. According to the Universal Chalcidoidea Database [2024], among parasitoids of *Ph. apparella* the greatest number of cases of hyperparasitism is associated with a primary braconid parasitoid, *Pholetesor circumscriptus* (Fig. 4, a). In our previous study [Ermolaev *et al*., 2022], it was revealed that the share of *Ph. circumscriptus* in the parasitoid complex of *Ph. apparella* decreased annually. Share of the species 92.4 ± 3.7, 14.9 ± 3.6, 9.7 ± 1.4 and 4.1 ± 1.7% has been declining over consecutive years, 2014–2017. It is possible that *Ph. circumscriptus* acts as a kind of edificator species. In the complex of *Ph. populifoliella* the greatest number of cases of hyperparasitism is also associated with *Ph. circumscriptus* (Fig. 4, b). It can be assumed that, parasitoid complexes ecologically associated with the same genera of miners and their host plants may have similar edificator species.

Conclusion

The assemblage of hymenopteran parasitoids associated with the poplar leaf miner *Ph. populifoliella* in Glazov Town includes 18 species of family Eulophidae and one species each from families Pteromalidae and Braconidae. Four species of them, namely *Cirrospilus elongatus*, *Sympiesis laevifrons*, *Baryscapus pospelovi* (Eulophidae) and *Pteromalus semotus* (Pteromalidae) were reported as the miner parasitoids for the first time. The parasitoid complexes ecologically associated the with poplars species, *Populus balsamifera* and *P. × jackii* turned out to be similar both qualitatively and quantitatively. The rate of parasitism of *Ph. populifoliella* caterpillars and pupae was relatively low.

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