Hymenopterous parasitoids associated with *Phyllonorycter coryli* (Nic.) and *Phyllonorycter nicellii* (Stt.) on hazel in Poland

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Abstract: The parasitoid complex associated with *Phyllonorycter coryli* and *P. nicellii* (Lepidoptera: Gracillariidae) leaf miners on hazel in Poland was examined and the contribution in the parasitoid community as well as the parasitization level of two species was defined. The parasitoid community of the nut leaf blister moth, *Phyllonorycter coryli* (Nic.), and the red hazel midget, *Phyllonorycter nicellii* (Stt.), was studied in three localities situated in Lublin. At each locality, leaves with visible mines were collected from the shrubs *Corylus avellana* L. and *C. maxima* Mill. cv. ‘Purpurea’. Of the 518 larvae and pupae of *Ph. coryli*, 47 individual of parasitoids were obtained. However, 152 individuals of parasitoids from 898 mines of *Ph. nicellii* successfully emerged. The highest contribution in the parasitoid complex of both leaf miner species was found for the chalcidoid family Eulophidae. The parasitoid community of *Ph. coryli* was made up of 11 species, 7 of which had not been recorded from this host before. Thirteen parasitoid species were reared from larvae and pupae of *Ph. nicellii*, 8 of which were recorded for the first time. The species from the genus *Chrysocharis* had the greatest contribution in the parasitoid fauna of *Ph. coryli*, while *Pediobius saulius* Walk. was dominant in the parasitoid fauna of *Ph. coryli*.

Key words: *Phyllonorycter coryli*, *Phyllonorycter nicellii*, Eulophidae, Gracillariidae, parasitoid complex, natural regulation

1. Introduction

Leaf miners are a large, highly specialized trophic group treated as parasites whose larvae feed inside the plant tissue. Often they are classified by the shape of the mine that they create. Many leaf-mining species are an important and conspicuous element of insect fauna, and plenty of them are known as pests of fruit trees and shrubs (Buszko, 1990). Of significant interest of this group are the *Phyllonorycter* species, the dominant genus of the family Gracillariidae (Lepidoptera). Mostly woody plants have been reported as their hosts, with the Fagaceae, Betulaceae, and Salicaceae especially favored (Beiger, 2004).

In Poland, 22 species of leaf miners are known to feed on hazel leaves. Two of them are from the genus *Phyllonorycter*, namely *Ph. coryli* (Nicelli 1851) and *Ph. nicellii* (Stainon 1851) (Beiger, 2004). They feed on the leaves of the common hazel (*Corylus avellana* L.) as well as on the purple-leaved hazel (*Corylus maxima* Mill. cv. ‘Purpurea’), which is frequently planted as an ornamental in gardens and urban environments (Dimić, 1971; Gantner et al., 2005; Górska-Drabik and Gantner, 2005, 2006). *Ph. coryli* is an upper-side miner (Askew and Shaw, 1974) and its mine begins as a small silvery spot; then the chamber increases and the skin folds. In turn, *Ph. nicellii* is an under-side miner and the mine is not too large, heavily bulging with a marbled pattern (Beiger, 2004).

*Phyllonorycter* leaf-miners are great subjects for examining the interaction between phytophagous and parasitoids (Askew and Shaw, 1974; Shaw and Askew, 1999; Stojanović and Marković, 2005; Yefremova and Mishchenko, 2008; Marković and Stojanović, 2012). In Poland so far, 55 parasitoid species of *Phyllonorycter* have been found. They are strongly represented by Chalcidoidea belonging to Hymenoptera (Vidal and Buszko, 1990; Marczak and Buszko, 1993; Sawoniewicz and Buszko, 1994). This paper identifies the complex of Hymenoptera parasitoids attacking *Ph. coryli* and *Ph. nicellii*, and defines their contribution in the parasitoid community as well as the level of their parasitization.

2. Materials and methods

2.1. Study area

Hazel leaves infested by *Ph. coryli* and *Ph. nicellii* were collected in 2002 and 2003 at the following three localities situated in Lublin (Poland) (22°34’E, 51°14’N):

- in a church garden, where the hazel shrubs grew in the vicinity of other ornamentals,
was defined.

coryli abundance of species in the parasitoid complex of
All obtained parasitoids were counted, and then the relative

2.3. Data analysis

The parasitoid nomenclature was verified after Noyes
had not been recorded from
Ph. coryli before.

The parasitoid complex of Ph. coryli consisted of 11
species, including 10 species from the family Eulophidae
(Pediobius saulius (Walk.), Chrysocharis pentheus Walk.,
Chrysanthus nereus (Walk.), Pnigalio pectinicornis
(L.), P. soemius (Walk.), Synypsysericeicornis
(Nees), Synypsysericeicornis Walk., Cirrospilus lyncus Walk.,
Cirrospilus diallus Walk., and Pnigalio incompletus Bouček]
and 1 species from Braconidae, Apanteles circumscriptus
(Nees), as presented in Table 1. In this group four species
are endoparasitoids, while the others are ectoparasitoids
(Table 2). The ratio of ecto- to endoparasitoids was 24.23
(1:1). Ch. pentheus, Ch. nereus, P. pectinicornis,
P. soemius, C. diallus, P. incompletus, and A. circumscriptus
had not been recorded from Ph. coryli before.

As presented in the Figure, P. saulius had the highest
contribution (38.3%) in the parasitoid complex of
Ph. coryli, which parasitized 3.4% of the host (18 host
individuals were parasitized). The group of predominant
species also included S. sericeicornis (23.4%) and C.
lyncus (10.6%); their contribution in parasitisation was
respectively, 2.1% (11 parasitised individuals) and 0.9% (5
parasitised individuals), as shown in Table 1.

Table 2 shows the parasitoid complex of Ph. nicellii
was made up of 13 Hymenoptera species, including
12 chalcidoid species from the family Eulophidae
(P. saulius, Ch. pentheus, Ch. nereus, P. pectinicornis,
P. soemius, S. sericeicornis, S. gordinis, C. lycnis, C.
diallus, C. elegantissimus Westwood, M. frontalis, and
Neochrysocharis formosus (Westwood), and 1 species
from the family Braconidae (A. circumscriptus). Only five
parasitoids obtained from larvae and pupae of Ph. nicellii
are endoparasitoids, while the others are ectoparasitoids,
and their ratio was 97:55 (1.8:1) (Tables 1 and 2). P. saulius,
Ch. pentheus, N. formosus, P. pectinicornis, P. soemius,
C. lycnis, C. diallus, and M. frontalis were recorded for the
first time from Ph. nicellii as a host.

The species of the genus Chrysocharis had the greatest
effect on the abundance of Ph. nicellii. The contribution
of Ch. pentheus in the parasitoid complex was more than
twice that of Ch. nereus, as shown in the Figure. Their
participation in the total level of parasitism was 2.7 (24
host individuals were parasitized) and 5.9% (53 host
individuals were parasitized), respectively (Table 1). The
group of predominant species in the parasitoid complex of

2.2. Methods

At each locality, five C. avellana and C. maxima cv.
'Purpurea' shrubs were selected at random, from which
leaves, accessible at arm's length and with visible mines,
were collected. The infested leaves were collected every
10–14 days from June to October, resulting in a total of
579 mines of Ph. coryli and 1490 mines of Ph. nicellii.
Phyllonorycter species were identified on the basis of the
mine using the keys published by Hering (1957) and Beiger
(2004). All the colonized mines were placed in petri dishes
and then reared using Borkowski's (1969) directions.
When there were a few mines on a single leaf, they were
Cut from the larger piece of the leaf and individually placed
in a petri dish. The emerged adult moths and parasitoids
were collected, killed with ethyl acetate, prepared, and
identified. All the parasitoids reared were identified using
the keys published by Tobias (1986) and Trjapitzin (1978).
The parasitoid nomenclature was verified after Noyes
(2003). The obtained Hymenoptera specimens are kept at
the Department of Entomology, University of Life Sciences
in Lublin (Poland).

2.3. Data analysis

All obtained parasitoids were counted, and then the relative
abundance of species in the parasitoid complex of Ph.
coryli and Ph. nicellii was determined. The parasitization
level (C) of the leaf miners by particular parasitoid species
was defined.

To compare parasitoid community of Ph. coryli and Ph.
nicellii the Renkonen similarity index was calculated as

\[ S_R = \sum_i \min (p_{1i}, p_{2i}), \]

where

\[ p_{1i} \text{ is frequency of species } i \text{ in community 1}, \]

\[ p_{2i} \text{ is frequency of species } i \text{ in community 2} \]

(Balmer, 2002).

This index ranges from 0 to 1 (±SE). If species occur in
both communities in the same proportions the value of the
index is supposed to be close to 1.

3. Results

Of the 518 larvae and pupae of Ph. coryli, 47 individuals
of parasitoids were obtained and the contribution of
parasitization was 9%. However, 152 individuals of
parasitoids from 898 mines of Ph. nicellii successfully
emerged, and so this number was 16.9% (Table 1). A total
of 14 parasitic hymenopteran species of two families were
obtained from both leaf-mining species. Most parasitoids
belonged to the chalcidoid family Eulophidae, while the
family Braconidae included only one species and some
specimens classified only to the genus Apanteles. Among
the obtained parasitoids, 7 species can be both primary
and secondary parasitoids, while only Minotetrastrichus
frontalis (Nees) is a gregarious species, as shown in Table 2.

As presented in the Figure, the primary parasitoids
affected the abundance of Ph. nicellii. The contribution
of Ch. pentheus in the parasitoid complex was more than
twice that of Ch. nereus, as shown in the Figure. Their
participation in the total level of parasitism was 2.7 (24
host individuals were parasitized) and 5.9% (53 host
individuals were parasitized), respectively (Table 1). The
group of predominant species in the parasitoid complex of

The index ranges from 0 to 1 (±SE). If species occur in
both communities in the same proportions the value of the
index is supposed to be close to 1.
Table 1. The contribution of Hymenoptera parasitoids associated with *Phyllonorycter coryli* (Nic.) and *Phyllonorycter nicellii* (Stt.).

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th><em>Phyllonorycter coryli</em> (Nic.)</th>
<th><em>Phyllonorycter nicellii</em> (Stt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
</tr>
<tr>
<td>Chalcidoidea/Eulophidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pedioius saulius</em> (Walk.)</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><em>Chrysocharis pentheus</em> Walk.</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Chrysocharis nephereus</em> (Walk.)</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>Neochrysocharis formosus</em> Westwood</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eulophinae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pnigalio incompletus</em> Bouček</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Pnigalio pectinicornis</em> (L.)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Pnigalio soemius</em> (Walk.)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Symposies seriecornis</em> (Nees)</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td><em>Symposies gordinis</em> Walk.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Cirrospilus lyncus</em> Walk.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Cirrospilus diallus</em> Walk.</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Cirrospilus elegantissimus</em> Westwood</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tetrastichinae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Minotetrasichius frontalis</em> (Nees)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ichneumonoidea/Braconidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microgastrinae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Apanteles spp.</em> (Nees)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Apanteles circumscriptus</em> (Nees)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>16</td>
</tr>
</tbody>
</table>

C – contribution to parasitization, *new parasitoid species for the host*, ( ) – number of hosts parasitized

*Ph. nicellii* also included *S. sericeicornis* and *M. frontalis* as shown in the Figure.

Both in the parasitoid complex of *Ph. coryli* and *Ph. nicellii*, the proportion of females was higher than of males and the sex ratio was 29:18 (1.6:1) and 90:62 (1.5:1), respectively (Table 1).

Renkonen’s similarity between the parasitoid community of *Ph. corylus* on the upper surface and *Ph. nicellii* on the under surface was 0.376 (±0.007). This low value indicates that relative species abundance was not similar among communities. The species belonging to the genus *Chrysocharis* made up a greater part of the parasitoid complex of under-side mines, while *P. saulius* was dominant in the parasitoid fauna of upper-surface mines.

4. Discussion

In Poland so far about 140 parasitoid species of mining moths have been found. They are represented in particular by Chalcidoidea. Species from the families Braconidae and Ichneumonidae are less numerous (Vidal and Buszko, 1990; Marczak and Buszko, 1993; Sawoniewicz and Buszko, 1994). The parasitoids obtained are typical for leaf miners, but many of them were not reported from the studied *Phyllonorycter* species before.
In the present study, the Hymenoptera community was most numerously represented by the family Eulophidae (13 species), while from Braconidae only one species was stated. Many previous reports confirmed the great contribution of parasitic Hymenoptera from the family Eulophidae in reducing the number of leaf-mining moths. According to Gates et al. (2002), as much as 80% of leaf miners parasitoids are representatives of Eulophidae. They appear in especially great numbers in apple orchards, where mining pests occur, mainly *Stigmella malella* (Stainton 1854) and *Phyllonorycter blancardella* (Fabr.) (Olszak, 1992). So far, 8 species of parasitic Hymenoptera have been recorded from *Ph. coryli*, namely *Chrysocharis laomedon* (Walk.), *Ch. lyncus*, *Elachertus inunctus* Nees, *Pediobius alcaeus* (Walk.), *P. saulius*, *S. gordius*, *S. sericericornis* (Noyes, 2003), and *Chrysocharis phryne* (Walk.) in Poland (Vidal and Buszko, 1990). On the other hand, a complex of 12 parasitoid species of *Ph. nicelli* was reported: *Achrysocharoides splendens* (Delucchi), *Ch. laomedon*, *Ch. nephereus*, *E. inunctus*, *S. gordius* (Noyes, 2003), *Achrysocharoides atys* (Walk.), *S. sericericornis*, *Minotetrastichus frontalis* (syn. *Tetrastichus ecus*), *Cirrospilus elegantissimus*, *Pnigalio longulus* (Zetterstedt), *S. gordius*, and *P. alcaeus* in Poland (Vidal and Buszko, 1990). The present study identified seven new parasitoid species associated with *Ph. coryli* and eight new species associated with *Ph. nicelli* as a host.

Among the parasitoids obtained, *Ch. pentheus*, *P. pectinicornis*, *P. soemius*, and *C. diallus* were not earlier reported from either *Phyllonorycter* species. *Ch. pentheus* is a solitary endoparasitoid of different leaf miners from Diptera and Coleoptera, mainly however, of Lepidoptera from the Nepticulidae and Gracillaridae (Trjapitzin, 1978; Hansson, 1985). It was reported from *Stigmella floslactella* (Haw.) (Szczepański, 1983), *Fomoria* (*Ectoedemia*) *septembrella* (Stt.), *Leucoptera laburnella* (Stt.), *L. listratella* (H-S), *Ectodemia agrimoniae* Frey (Vidal and Buszko, 1990), *Lyonetia clarkella* (L.) (Adachi, 1998), and *Phyllocnistis*
The second is *P. pectinicornis*, a solitary ectoparasitoid, which may act as a either primary or secondary parasitoid. It was reared from numerous species of leaf-mining moths (Gracillariidae, Nepticulidae, Elachistidae, Gelechidae, Lyonetidae, Tischeriidae) (Noyes, 2003). In Poland, it has been reported from *Ph. blancardella*, *S. malella*, *L. malifoliella* (Costa), *Lyonetia clerkella*, and *Callisto denticulata* (Thnbg.) (Goos, 1965; Górny, 1979; Piotrowski, 1980; Kadiłubowski, 1981; Olszak, 1992; Górska-Drabik and Napiórkowska-Kowalik, 2009). Likewise, *P. soemius* has not been reported from leaf miners colonizing hazel, although Bouček and Askew (1968) reared it from larvae of many *Phyllonorycter* species. *P. soemius* is a polyphagous ectoparasitoid of about 90 leaf miner species and gall maker larvae of Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Noyes, 2003). On the other hand, *C. diallus* develops on the larvae of numerous leaf-mining moths, mostly of Gracillariidae and Nepticulidae (Noyes, 2003). Recently it was reported from *Ph. citrella* (Lo Pinto et al., 2005), *Cameraria ohridella* Desch. & Dem. (Volter and Kenis, 2006), and *Phyllonorycter issikii* (Kumata) (Yefremova and Mishchenko, 2008). The same information corresponded to *C. lyncus*, which was reported from *Ph. citrella* Stt. (Mafi and Ohbayashi, 2010).
nicellii for the first time. So far, Ph. nicellii has not been also reported as a host of P. saulius, Neochrysocharis formosus (syn. Acrhysochara formaosa), or Minotetrastichus frontalis. The first one is a larval endoparasitoid of small moths from Gracillariidae, Bucculatricidae, Tortricidae, Yponomeutidae, and Gelechiidae, and sometimes may act as a hyperparasitoid (Trjapitzin, 1978). It can develop on larvae of many Phyllonorycter species, like P. blanardella (Kadłubowski, 1981; Balázs, 1997). Ph. platani (Staud.) (Vidal and Buszko, 1990; Marković and Stojanović, 2012), and Ph. robinax (Clem.) (Stojanović and Marković, 2005). It was also obtained from C. denticulata (Górsk-drabik and Napiórkowska-Kowalik, 2009), Tischeria sp. (Nikitenko et al., 2005), and C. ohridella (Horváth, 2006). In turn, N. formosus is distributed worldwide and colonized more than 100 leaf-mining species from Coleoptera, Hemiptera, Diptera, Lepidoptera, and Hymenoptera (Noyes, 2003). M. frontalis is also the most common polyphagous parasitoid of various groups of leaf miners (Noyes, 2003). It was reared from Phyllonorycter corylifoliella (Hüb.), P. blanardella (Tomov, 2002), C. ohridella (Grabenweger, 2003; Volter and Kenis, 2006; Bystrowski et al., 2008), Ph. robinax (Stojanović and Marković, 2005), and Ph. platani (Marković and Stojanović, 2012).

Among the reared eulophid species, Ch. nephereus and P. incompletus have not been reared from Ph. coryli before. However, Ch. nephereus was reported from many leaf-mining moths, including Ph. nicellii (Noyes, 2003). P. incompletus infests leaf-mining Lepidoptera (Schaufl et al., 1998). It was reported from Ph. citrella (Elekcióglu and Uygun, 2006), Ph. corylifoliella (Mineo and Sinacori, 1998), and Tuta absoluta (Meyrick 1917) (Doğanlar and Yiğit, 2011).

Based on our data, A. circumscriptus from Braconidae is mentioned for the first time as a parasitoid of Ph. coryli. A. circumscriptus is a common solitary parasitoid of Phyllonorycter species and some related families of mining Lepidoptera (Nixon, 1973). It was reported as a parasitoid of numerous moth species from the families Gracillariidae and Elachistidae (Marczak and Buszko, 1993).

In the present study, the most abundant species in the parasitoid complex of Ph. coryli were P. saulius and S. sericeicornis, which totally parasitized 5.5% of the host. The other 9 species obtained comprised 3.5% of the total parasitization. Likewise, the greatest share in parasitization of Ph. nicellii was also by two species – Ch. pentheus and Ch. nephereus, which totally parasitized 8.6%, while the other 11 species parasitized 8.2% of the host. The obtained results seem to confirm the view and the results of previous reports. They submit that the entomophagous complex usually includes one or a few species of special importance for the number regulation of the phytophagous insects. The other, often quite numerous species destroy only a small percentage of the pest population (Szmidt, 1971; Szujecki, 1980; Adachi, 1998; Del Bene and Gargani, 2003; Bystrowski et al., 2008; Yefremova and Mishchenko, 2008; Marković and Stojanović, 2012).

According to Askew and Shaw (1974), the parasitoid complexes of the upper-surface and under-surface Phyllonorycter mines generally are qualitatively and quantitatively similar. In the present study, the relative abundance of parasitoid species was not similar among communities. The reason for this is probably the great number of parasitoid species associated with the genus Phyllonorycter.

In modern crop protection, where the number of used insecticides is still reduced, special attention to the role of Hymenoptera parasitoids should be paid. Their participation in natural regulation of phytophagous is indisputable. In this manuscript, we report the results of research that provides an important contribution to the knowledge extension of the Hymenoptera parasitoid complex associated with Phyllonorycter leaf miners on hazel. The results from our study revealed that the parasitoid community of Ph. coryli was made up of 11 species, 7 of which had not been recorded from this host before. Thirteen parasitoid species were reared from larvae and pupae of Ph. nicellii, 8 of which were recorded for the first time.

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References


