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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 community of *Ph. nicellii*, 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* (Stainton 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 (Dimič, 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 parasitoids 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):

1 - in a church garden, where the hazel shrubs grew in the vicinity of other ornamentals,

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2 - in the area of the Open Air Village Museum, where the shrubs of hazel grew along a path between the houses; around them different species of vegetables and blooming ornamental plants grew,

3 - near a very busy route to Warsaw, where the hazel grew.

No chemical treatments were used in any of the sites.

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} is frequency of species i in community 1,

p_{2i} is frequency of species i 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 *Minotetrastichus frontalis* (Nees) is a gregarious species, as shown in Table 2.

The parasitoid complex of *Ph. coryli* consisted of 11 species, including 10 species from the family Eulophidae [*Pediobius saulius* (Walk.), *Chrysocharis pentheus* Walk., *Chrysocharis nephereus* (Walk.), *Pnigalio pectinicornis* (L.), *Pnigalio soemius* (Walk.), *Sympiesis sericeicornis* (Nees), *Sympiesis gordius* 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. nephereus*, *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. nephereus*, *P. pectinicornis*, *P. soemius*, *S. sericeicornis*, *S. gordius*, *C. lyncus*, *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. lyncus*, *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. nephereus*, 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 parasitised), respectively (Table 1). The group of predominant species in the parasitoid complex of

Table 1. The contribution of Hymenoptera parasitoids associated with *Phyllonorycter coryli* (Nic.) and *Phyllonorycter nicellii* (Stt.).

Parasitoid species	*Phyllonorycter coryli* (Nic.)						*Phyllonorycter nicellii* (Stt.)					
	2002		2003		total	C (%)	2002		2003		total	C (%)
	♀	♂	♀	♂			♀	♂	♀	♂		
Chalciodoidea/Eulophidae												
Entedoninae												
Pediobius saulius (Walk.)	7	2	9	-	18	3.4	-	-	1	1	2 *	0.2
Chrysocharis pentheus Walk.	-	1	-	-	1 *	0.2	19	7	17	10	53 *	5.9
Chrysocharis nephereus (Walk.)	2	-	-	-	2 *	0.4	9	8	4	3	24	2.7
Neochrysocharis formosus Westwood	-	-	-	-	0	0.0	3	-	-	-	3 *	0.3
Eulophinae												
Pnigalio incompletus Bouček	1	-	-	-	1 *	0.2	-	-	-	-	0	0.0
Pnigalio pectinicornis (L.)	-	2	-	-	2 *	0.4	-	2	-	2	4 *	0.4
Pnigalio soemius (Walk.)	-	1	-	1	2 *	0.4	-	6	-	3	9 *	1.0
Sympiesis sericeicornis (Nees)	3	7	-	1	11	2.1	2	4	1	9	16	1.8
Sympiesis gordius Walk.	-	-	1	-	1	0.2	4	-	-	-	4	0.4
Cirrospilus lyncus Walk.	2	3	-	-	5	0.9	3	1	1	-	5 *	0.6
Cirrospilus diallus Walk.	1	-	-	-	1 *	0.2	1	-	-	-	1 *	0.1
Cirrospilus elegantissimus Westwood	-	-	-	-	0	0.0	1	-	-	-	1	0.1
Tetrastichinae												
Minotetrastichus frontalis (Nees)	-	-	-	-	0	0.0	1	3	10	1	15* (15)	1.7
Ichneumonoidea/Braconidae												
Microgastrinae												
Apanteles spp. (Nees)	-	-	1	-	1	0.2	-	-	4	-	4 (4)	0.4
Apanteles circumscriptus (Nees)	1	-	1	-	2 *	0.4	1	-	8	2	11	1.2
Total	17	16	12	2	47	9.0	44	31	46	31	152	16.9

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.

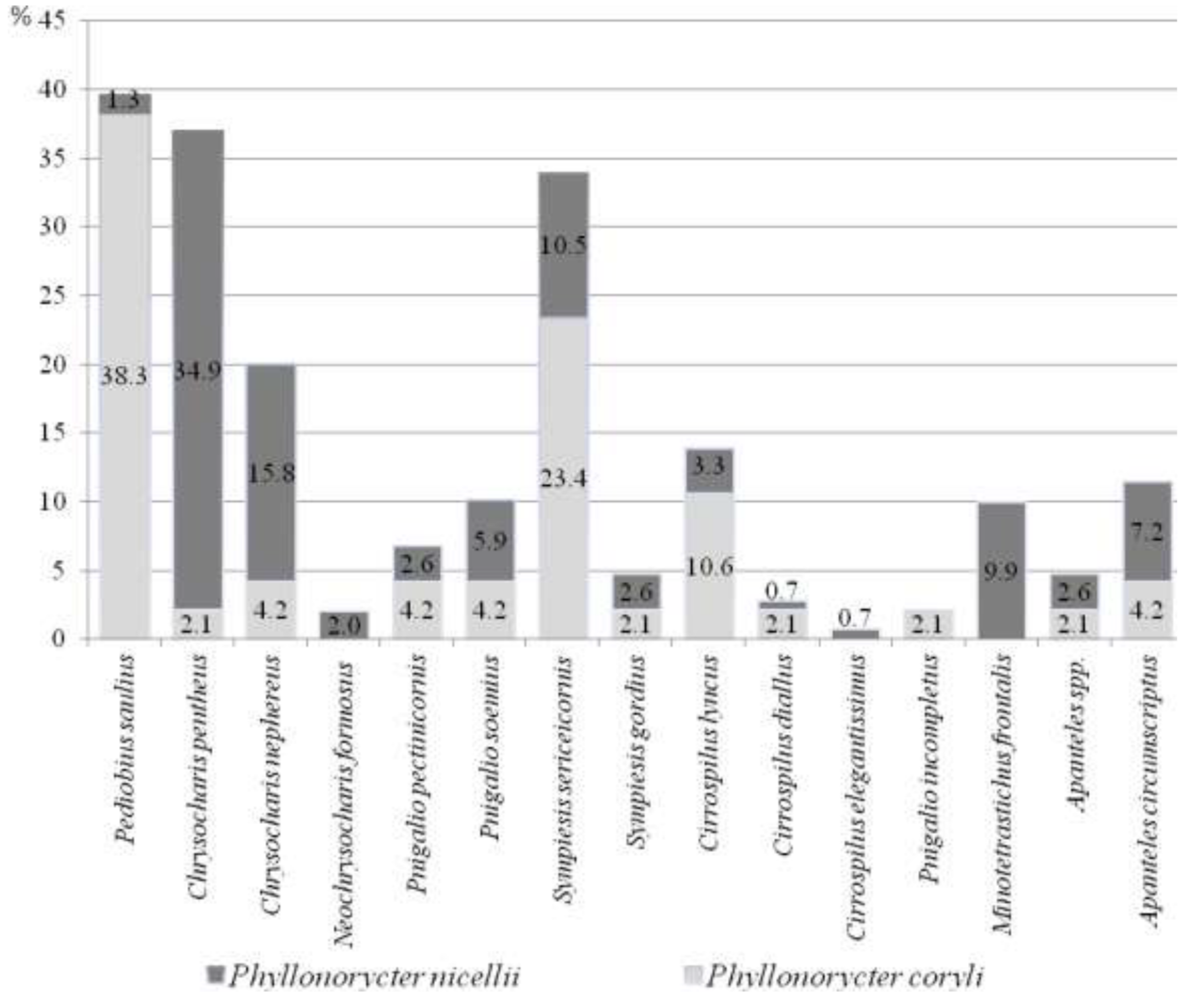

Figure. Relative abundance of species in the *Phyllonorycter* parasitoid complex.

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.), *C. 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. nicellii* 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. nicellii* 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 agrrimoniae* Frey (Vidal and Buszko, 1990), *Lyonetia clerkella* (L.) (Adachi, 1998), and *Phyllocnistis*

Table 2. Parasitoids recorded from *Phyllonorycter coryli* (Nic.) and *Phyllonorycter nicellii* (Stt.) and their status (according to the literature data).

Species	Status [a]					
	Primary	Secondary	Endoparasite	Ectoparasite	Solitary	Gregarious
Chalciodoidea/Eulophidae						
Entedoninae						
Pediobius saulius (Walk.)	+	+	+		+	
Chrysocharis pentheus Walk.	+	+	+		+	
Chrysocharis nephereus (Walk.)	+		+		+	
Neochrysocharis formosus Westwood	+		+		+	
Eulophinae						
Pnigalio incompletus Bouček	+			+	+	
Pnigalio pectinicornis (L.)	+	+		+	+	
Pnigalio soemius (Walk.)	+			+	+	
Sympiesis sericeicornis (Nees)	+	+		+	+	
Sympiesis gordius Walk.	+	+		+	+	
Cirrospilus lyncus Walk.	+	+[r]		+	+	
Cirrospilus diallus Walk.	+			+	+	
Cirrospilus elegantissimus Westwood	+			+	+	
Tetrastichinae						
Minotetrastichus frontalis (Nees)	+	+		+		+
Ichneumonoidea/Braconidae						
Microgastrinae						
Apanteles spp.(Ness)	+		+		+	+
Apanteles circumscriptus (Nees)	+		+		+	

[a] - The status of the parasitoid species is given according to Bouček and Askew (1968), Trjapitzin (1978), Zajančkauskas et al. (1979), Noyes (2003); Mafi and Ohbayashi (2010), Stojanović and Marković (2005), Górny (1979), Elekçioğlu and Uygun (2006), Doğanlar and Yiğit (2011), Van den Berg et al. (1988); [r] - rare

citrella Stt. (Mafi and Ohbayashi, 2010). 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 (Gracillaridae, 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 denticulella* (Thnbg.) (Goos, 1965; Górny, 1979; Piotrowski, 1980; Kadłubowski, 1981; Olszak, 1992; Górska-Drabik, 2007; 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.*

nicellii for the first time. So far, *Ph. nicellii* has not been also reported as a host of *P. saulius*, *Neochrysocharis formosus* (syn. *Achrysocharella formosa*), 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 *Ph. blancardella* (Kadłubowski, 1981; Balázs, 1997), *Ph. platani* (Staud.) (Vidal and Buszko, 1990; Marković and Stojanović, 2012), and *Ph. robiniella* (Clem.) (Stojanović and Marković, 2005). It was also obtained from *C. denticulella* (Górska-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* (Hübn.), *P. blancardella* (Tomov, 2002), *C. ohridella* (Grabenweger, 2003; Volter and Kenis, 2006; Bystrowski et al., 2008), *Ph. robiniella* (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 (Schauff et al., 1998). It was reported from *Ph. citrella* (Elekçioğlu 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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