

Parasitoid complex of leaf miner *Phyllonorycter comparella* (Lepidoptera, Gracillariidae) in Hungary

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Abstract – Between 2011 and 2014, 1,154 mines of *Phyllonorycter comparella* (Duponchel) were collected at 12 locations in Hungary and were put into single-mine rearing containers. A total of 574 parasitoid specimens belonging to 29 parasitoid species (26 Chalcididae, 2 Encyrtidae and 1 Braconidae) emerged. Of these species, 13 have not yet been mentioned in either international or in Hungarian literature as a parasitoid of the *P. comparella*. The species assemblages of the parasitoid complexes varied greatly among the sample sites. The primary dominant species of the total samples was found to be *Sympiesis sericeicornis* (Nees), an abundant idiobiont solitary ectoparasitoid. Among the species reared, we have found specialist parasitoids such as *Achrysocharoides scaposa* (Erdős) and even species never recorded from *Populus* (*Zagrammosoma variegatum* (Masi)) according to the Universal Chalcidoidea Database.

Phyllonorycter comparella / *Populus alba* / parasitoid assemblages

Kivonat – A *Populus alba*-n él *Phyllonorycter comparella* (Lepidoptera: Gracillariidae) levélaknázó magyarországi parazitoid együttese. 1154 *Phyllonorycter comparella* (Duponchel) levélaknát gyűjtöttünk és tettünk egyedi nevelésbe 2011–2014 között, 12 Magyarországi helyszínről. A nevelésből kikelt 574 parazitoid egyed alapján 29 fajt (26 Chalcididae, 2 Encyrtidae és 1 Braconidae) sikerült azonosítani. Ezek közül 13 fajt sem a nemzetközi sem a hazai szakirodalom korábban nem említ, mint a *P. comparella* parazitoidjait. Különböző mintagyűjtési helyszínek parazitoid komplexum fajegyüttesében eltérések mutatkoztak. A teljes minta elsődleges domináns faja a *Sympiesis sericeicornis* (Nees), egy gyakori idiobiont szoliter ektoparazitoid volt. A nevelésből kikelt fajok között olyan specialista fajokat is azonosítottunk, mint az *Achrysocharoides scaposa* (Erdős) valamint *Populus*ról eddig, a Nemzetközi Chalcidoidea Adatbázis (Universal Chalcidoidea Database) által még nem regisztrált *Zagrammosoma variegatum* (Masi) parazitoid fajt is.

1 INTRODUCTION

Phyllonorycter comparella (Duponchel) is a palaeartic species occurring all over the Eurasian continent (De Prins – De Prins 2014). Its main host plant is *Populus alba* (Gozmány 1956), but it can also be found on *Populus tremula* (Linné) and *Populus × canescens* (Ation) (De Prins – De Prins 2014). It is a bivoltine species; the adults fly from June till August and

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also in October (Sz cs 1977). The species overwinters in the adult stage. The blotch mines are located on the underside of the leaf between the leaf veins (Csóka 2003).

Based on our observations, *Ph. comparella* can locally occur at a very high density with several mines on a single leaf. Similar observations have been made by Yedfremova et al. in Russia (Ul'yanovsk Province) in 2007 (Yefremova et al. 2009). A number of papers predict a change in insect behaviour because of the effects of climate change (Cannon 1998, Csóka 1996, Csóka 1997, Dukes et al. 2009, Fleming – Volney 1995, Kirilenko – Sedjo 2007, Walther et al. 2002), and common and even rare species may become severe pests. Therefore, we believe that this species has the potential ability to cause severe outbreaks and possibly heavy damage on *Populus alba* and *Populus × canescens*.

It is always important to study the regulating potential and the species composition of the natural enemy complex of such a species.

The parasitoid complex of *Ph. comparella* has been inadequately studied thus far. Two of the main parasitoid databases publish a rather small number of parasitoids (De Prins – De Prins 2014, Noyes 2015). In Hungary only two researchers (Erd s 1956, Sz cs 1965, Sz cs 1979) reared parasitoids from *Ph. comparella*. The aim of this work is to summarize the knowledge of the parasitoid species developing in *Ph. comparella* in Hungary in comparison with the data available in European literature.

2 MATERIAL AND METHODS

We studied the parasitoid complexes of various leaf miners over a period of four years (2011–2014). A total of 1,154 leaf mines were collected from *P. alba* at 12 locations across Hungary (Table 1).

Single mine rearings were used. The mines were cut out from the leaf in order to avoid contamination from other parasitoids (i.e. egg or aphid parasitoids) which parasitize other insects on leaves. After a short drying period, the samples were placed individually into air ventilated plastic rearing tubes.

The parasitoids that emerged were preserved in 95% ethanol, and later identified by Csaba Thuróczy and George Melika. The emerging leaf miner adults were also identified to make sure that the identification of the leaf mines was correct. After the identification process, the adult insects were kept in alcohol for further molecular analysis.

3 RESULTS AND DISCUSSION

From the samples collected during the four consequent years (2011–2014), a total of 574 parasitoid specimens emerged from 255 parasitized samples. These specimens belong to 29 parasitoid species (26, 2, and 1 species to the families Chalcididae, Encyrtidae and Braconidae, respectively). The total parasitization rate of our samples was 22.1% (n=1154 samples collected), and at different sample locations (with regard to the sample size) varied between 7.3% (n=55) and 75% (n=4) (Table 1).

Table 1. A summary of the sampling locations, the sample sizes and the parasitism rates of our rearings.

WGS84: the coordinates of the sample plots;

TMC: total leaf mines collected; TPM: total parasitized leaf mines;

TSE: total specimen reared; P%: percentage of parasitism.

Localities	WGS84 (GPS) coordinates		Sampling date	TMC	TPM	TSE	P%
	Lat. (N)	Long. (E)					
Ásotthalom	46°11'26.90"	19°46'25.00"	07.10.2013	147	21	25	14.3
			07.15.2014	238	73	99	30.7
Gödöll	47°36'06.70"	19°24'17.91"	07.27.2012	3	0	0	0
Gyöngyösoroszi	47°50'40.45"	19°54'03.54"	08.13.2012	5	0	0	0
Hort	47°40'52.51"	19°45'20.60"	09.08.2013	4	3	3	75
Jánd	48°06'56.28"	22°21'24.69"	06.19.2013	192	44	256	22.9
Kisk rös	46°35'12.72"	19°15'18.75"	07.08.2013	17	9	19	52.9
Kiskunhalas	46°22'13.47"	19°34'03.04"	07.13.2011	45	17	39	37.8
			07.15.2014	72	12	11	16.7
Mátrafüred	47°49'31.36"	19°58'28.60"	06.07.2011	39	18	22	46.2
			06.28.2011	47	13	28	27.7
			06.26.2012	108	1	5	0.9
			06.25.2013	56	8	8	14.3
Poroszló	47°39'02.11"	20°40'25.28"	07.11.2013	43	8	16	18.6
Püspökladány	47°20'04.55"	21°05'22.83"	07.11.2013	55	12	19	21.8
Tatabánya	47°31'48.96"	18°25'42.11"	10.19.2013	55	4	7	7.3
Törtel	47°05'15.56"	19°53'24.33"	07.08.2013	28	14	17	50
TOTAL				1,154	257	574	22.3

The identified parasitoid species of the complex is listed in Table 2.

We compared our species list with two of the most important on-line databases, the Universal Chalcidoidea Database, hosted by the British Natural History Museum (Noyes 2015) and the Global Taxonomic Database of Gracillariidae (Lepidoptera), hosted by the Belgian Biodiversity Platform (De Prins – De Prins 2014) and discovered that 15 species found during this research were not listed in these databases as parasitoids of the *Ph. comparella*.

From the samples collected at Ásotthalom, a total of 124 specimens belonging to 14 species have been identified. The parasitization rate at this place was 24.2% with 93 parasitized samples. The parasitoid complex was dominated by the synovigenic idiobiont solitary *Sympiesis sericeicornis* (Nees, 1834) (43%, n = 93 parasitised samples) and *Zagrammosoma talitzkii* (Boucek, 1961) (12.9%, n=93). Subdominant species were *Pediobius saulius* (Walker, 1839) (8.6%), *Minotetrastichus frontalis* (Nees, 1834) (8.6%), *Closterocerus trifasciatus* (Westwood, 1883) (7.5%) and a species belonging to the family Braconidae (7.5%). *S. sericeicornis* was also dominant at two other locations with small sample sizes (Kisk rös and Törtel) and present in nearly all samples from the 12 sampling locations. *Ageniaspis testaceipes* (Ratzeburg, 1848), was the dominant species of the samples collected at Jánd (n = 192 total samples collected) causing 43.2% (from n = 44 parasitized samples) of the leaf miner mortality.

Table 2. The checklist and the number of parasitoid species reared from *Phyllonorycter comparella* in Hungary. Species marked with "*" have been recorded for the first time from *P. comparella*.

Parasitoids reared	Ásotthalom	Hort	Jánd	Kisk rös	Kiskunhalas	Mátrafüred	Poroszló	Püspökladány	Tatabánya	Törtel	TOTAL
Encyrtidae											
<i>Ageniaspis testaceipes</i> *	23		230	11				2	4		270
<i>Encyrtidae</i> sp.*					23	24					47
Eulophidae											
<i>Achrysocharoides attilis</i> *						1					1
<i>Achrysocharoides cilla</i> *					1	15					16
<i>Achrysocharoides scaposa</i>				1		3	12	14		4	34
<i>Aprostocetus</i> sp.					1						1
<i>Baryscapus nigroviolaceus</i>	2		2								4
<i>Baryscapus</i> sp.					2	4					6
<i>Chrysocharis</i> sp.	2										2
<i>Chrysocharis laomedon</i>			3								3
<i>Chrysocharis nephereus</i>	1	1			1						3
<i>Chrysocharis pentheus</i> *	1					1					2
<i>Chrysocharis</i> sp. "A"*									1		1
<i>Cirrospilus elegantissimus</i>			4			2					6
<i>Cirrospilus lyncus</i>			1		1		1				3
<i>Closterocerus trifasciatus</i> *	8		5	1	9	1			1		25
<i>Minotetrastichus frontalis</i>	8		2				2				12
<i>Neochrysocharis formosus</i>	5	1									6
<i>Pediobius pyrgo</i> *					2						2
<i>Pediobius saulius</i>	9		2		2	1				1	15
<i>Pnigalio agraulis</i>			2								2
<i>Pnigalio pectinicornis</i>			1								1
<i>Pnigalio soemius</i> *	1										1
<i>Sympiesis acalle</i> *	1										1
<i>Sympiesis dolichogaster</i> *						1					1
<i>Sympiesis sericeicornis</i>	42		3	6	2	9	1	3	1	8	75
<i>Zagrammosoma talitzkii</i> *	13				6					4	23
<i>Zagrammosoma variegatum</i> *						1					1
Braconidae											
<i>Braconidae</i> sp.	8	1	1								10
TOTAL	124	3	256	19	50	63	16	19	7	17	574

From the 12 species that were present in these samples, we identified only one parasitoid species belonging to the Braconidae family. At this location, the sub dominant species were *C. trifasciatus* (11.4%, n=44 parasitized samples) and *Cirrospilus elegantissimus* (9.1%, n = 44 parasitized samples). *Zagrammosoma talitzkii* was dominant in the samples collected at Kiskunhalas, representing 21.4% of the 28 parasitized samples. The species occurred at only 3 locations. The secondary dominant species of the complex was *C. trifasciatus* (17.9%, 28 parasitized samples) and an unidentified species from the Encyrtidae family. *Achrysocharoides cilla* (Walker, 1839) was only dominant in the samples collected at Mátrafüred, being present in 32.5% of the 40 parasitised samples.

The species also occurred in the samples from Kiskunhalas with an insignificant parasitization rate. The secondary dominant species was *S. sericeicornis* (22.5%, n = 40 parasitized samples). *Achrysocharoides scaposa* (Erdős, 1961) was the dominant species at Poroszló (12.5%, n = 8 parasitized samples and 43 total samples collected) and Püspökladány (17.5%, n = 12 parasitized samples and 55 samples collected) but reared from a relatively small sample size. The species has been observed in samples from five different collecting locations.

The samples from Hort and Tatabánya contained few parasitized samples with only 3 parasitoid species emerged with one specimen each. *S. sericeicornis* is a solitary idiobiont species and was the most frequent species with a high capacity for controlling the miners. As with *C. trifasciatus*, it has a wide range of hosts and both species can be also be secondary parasitoids (Askew 1979, Stojankovic and Markovic 2005, Noyes 2015).

A more specialized species with a narrow host range, the koinobiont gregarious *A. testaceipes*, was also abundant, but dominant only at one sample place. According to the Universal Chalcidoidea Database (Noyes 2015) *A. scaposa* has been reared only from *Ph. comparella*. We identified the species in 5 of our samples and it was dominant in 2 (Püspökladány and Poroszló).

The gregarious koinobiont *A. cilla* was identified from only 2 sample locations (Kiskunhalas and Mátrafüred). Despite its low abundance within the samples, at Mátrafüred it seems to be the major controlling agent of its host. *Zagrammosoma variegatum* (Masi, 1907), an idiobiont solitary ectoparasitoid, has never been described from any species living on *Populus* sp (De Prins and De Prins 2014, Noyes 2015). In our research, we only observed the species at Mátrafüred. At this location we identified another rare and semi-specialist species, *Achrysocharoides altilis* (Delucchi, 1954).

In 3.5% of the 257 parasitized mines we found more than one species of coexisting parasitoids. On these rare occasions, both parasitoid species/specimens are able to complete the larval development. Such events/phenomena are termed the coexistence of multiple parasitoids, a phenomenon among parasitoids of various insects that is well known and has been documented for a long time (Askew – Shaw 1979, Amarasekare 2000a, Amarasekare 2000b, Klapwijk – Owent 2011, Borer et al. 2004, Hackett-Jones et al. 2009, László – Tóthmérész 2013). The coexistence of two parasitoids foraging on a single host occurs when the distribution of the parasitoids is sufficiently aggregated (May and Hüssel 1981, Klopfer and Ives 1997), which triggers intra and interspecific competition (MacArthur and Levins 1967). The successful development of the parasitoids on a single host requires the partitioning or sharing of the food resource (Harvey et al. 2013) by partial host consumption (Miller 1982) and/or the separation of the temporal niche (Hackett-Jones et al. 2009, Amarasekare 2000a) In this case, the competition between the parasitoid species is delimited by the earlier mentioned requirements.

In our samples we registered 9 cases of multiple parasitism. In most cases (6 of 9) the parasitoid couple was composed from a koinobiont and an idiobiont parasitoid, where the koinobiont is a primary parasitoid and the idiobiont is a secondary generalist parasitoid. Therefore, we might say that it is likely that the first (primary) encounter could be the

koinobiont endoparasitoid. On one occasion, we registered two idiobiont ectoparasitoids that had completed their stages.

The most surprising finding was the presence of three koinobiont species from a single mine, which is a rather rare event (Askew 1979, Miller 1982).

Next follows a brief overview on the biology of the parasitoid pairs emerged from single mines (abbreviations: En = endoparasitoid; Ec = ectoparasitoid; G = gregarious; S = solitary; K = koinobiont; I = idiobiont. Read as follows: En,G,K = Endoparasitoid, Gregarious, Koinobiont):

- 1 female specimen of *Achrysocharoides attilis* (En,G,K) +
1 male specimen of *Baryscapus* sp. (Ec,G,I)
- 1 female specimen of *Braconidae* (En,S,K) +
1 male specimen of *Pnigalio agraulis* (Ec,S,I)
- 2 female specimens of *Achrysocharoides scaposa* (En,G,K) +
1 female and 1 male specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 male specimen of *Baryscapus nigroviolaceus* (En,S,K) +
1 female specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 female specimen of *Chrysocharis nephereus* (En,S,K) +
1 female specimen of *Pnigalio soemius* (Ec,S,I)
- 1 male specimen of *Pediobius saulius* (En,S,K) +
1 female specimen of *Zagrammosoma talitzkii* (Ec,S,I)
- 1 female specimen of *Braconidae* (En,S,K) +
1 female specimen of *Minotetrastichus frontalis* (Ec,G,I)
- 1 female specimen of *Zagrammosoma talitzkii* (Ec,S,I) +
1 male specimen of *Sympiesis sericeicornis* (Ec,S,I)
- 1 female specimen of *Chrysocharis nephereus* (En,S,K) +
1 female specimen of *Neochrysocharis formosus* (En,G,K) +
1 male specimen of *Braconidae* (En,S,K).

The two relevant online databases (Noyes 2015, De prins and De Prins 2014) list only a small number (19) of parasitoid species associated with the *P. comparella*, reflecting a small amount of studies regarding species load of the leaf miners parasitoid complexes. In this work we contribute 13 species of parasitoids as first records for the host *Phyllnorycter comparella*. Overall, we summarize that 36 parasitoid species have been recorded from *Ph. comparella* so far, including 29 species listed in the present publication. The species composition of the parasitoid complexes of sample sites varied greatly. It is acceptable to say that the parasitoid species of the leaf miner presented a habitat-specific occurrence as found by Hawkins (1994) and Gibbson et al. (1997). Common species, with a generally wide host range, being abundant in nearly all places represented the highest number of species of the entire complex (18 species). The endoparasitoid species, like the *A. testasceipes*, had the biggest impact on the leaf miner *P. comparella*.

As found at the sample sites, the *Ph. comparella* leaf miner can sometimes be very abundant (Yefremova 2009, personal observation). Climate change might trigger outbreaks of this species (as seen with other insects), causing heavy damage to its host plant (Kyle et al. 2014).

Nowadays many forests are composed from a low diversity of trees due to economic forest use; this low diversity of trees may help to intensify the outbreaks of many species (Jactel and Brockerhoff 2007). A number of papers show that diverse habitats contain more complex food-webs with a number of natural enemy species (Paine 1966, Polis and Strong 1996). It is always important to study the species composition of both native (Sz cs et al. 2013) and invasive (Sz cs et al. 2014) species with outbreak ability.

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