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Changes in the Parasitism Rate and Parasitoid Community Structure of the Horse Chestnut Leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), in the Czech Republic

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Abstract: The horse chestnut leafminer, *Cameraria ohridella*, Deschka and Dimić, is a moth that has invaded most of Europe since it was first recorded in Macedonia near Lake Ohrid in 1985. It attacks horse chestnut trees and causes aesthetic and vitality problems. The parasitism rate, other mortality rates, and parasitoid structure were studied during a five-year survey at six sites in the Czech Republic. The results showed that the total parasitism rates varied from 1.9% to 20.5%, with an average of 7.2%, similar to other those published studies. The parasitism rate was significantly related to year, the developmental stage of *C. ohridella*, latitude, and greenery maintenance but not to *C. ohridella* population density, altitude, or area size. In contrast, the total other mortality rates varied from 13.7% to 59.5%, with an average of 31%, but overall temporal changes in the values indicated a declining trend. The parasitoid complex was predominantly polyphagous parasitoids of the family Eulophidae, similar to that found previously in south-eastern Europe. The results further revealed that the most abundant parasitoid species, *Minotetrastichus frontalis* (Nees), was gradually replaced by *Pediobius saulius* (Walker). The increasing abundance of *P. saulius* is thus an interesting adaptation of an autochthonous parasitoid to a new host.

Keywords: *Aesculus hippocastanum*; invasive pest; natural enemies; Eulophidae; parasitoids; parasitism rate; mortality rate

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1. Introduction

The horse chestnut leafminer, *Cameraria ohridella*, Deschka and Dimić (Lepidoptera: Gracillariidae), is a pest moth that attacks horse chestnut trees (*Aesculus hippocastanum* L. (Sapindales: Sapindaceae)). It was first recorded in 1985 in Macedonia in the area near Lake Ohrid [1], which probably represents the origin of this species [2,3]. Soon after it was first recorded, it started to spread and affect the aesthetics and vitality of the horse chestnut [4], which is a frequently planted ornamental tree species in many parks throughout Europe [5]. Since 2004, horse chestnut leaf miner has also spread in Asia Minor [6–8]. The moth reached the Czech Republic in 1993 [9], and, over the course of five years, it spread across the whole country [6,10].

Although the preferred host tree of *C. ohridella* is *A. hippocastanum*, it lays eggs and forms mines on other *Aesculus* species [11,12] and occasionally attacks sycamore maple (*Acer pseudoplatanus* L. (Sapindales: Sapindaceae)) [13]. Leaf damage inflicted by *C. ohridella* to *A. hippocastanum* in the Czech Republic can exceed 50% in some sites [14] and, together with the horse chestnut leaf blotch caused by *Guignardia aesculi* (Peck) V.B. Stewart (Botryosphaeriales: Botryosphaeriaceae), is the main cause of the decline in horse chestnut trees' ornamental aesthetics [15]. Heavy infestation can cause premature defoliation and negatively affects seed weight [4,16,17].

Despite the extensive efforts of scientists to develop various methods to control or support the control of the populations of *C. ohridella* [18–22], including the application of microbial biocontrol agents [23–31], no other effective and economical solution has yet been found without possible side effects on other organisms, including humans. We therefore need to rely on naturally occurring antagonists.

When invading new regions, leafmining moths often become quickly adopted and controlled by native parasitoids and other natural enemies [32,33]. *Cameraria ohridella*, as an invasive species, was adopted as a host by autochthonous enemies such as predators [34], pathogens [29,30,35], and parasitoids [36–39]. However, the combined impact of natural enemies has not been sufficient for the effective control of *C. ohridella* populations [36,38,40].

Many studies on *C. ohridella* parasitoids to identify natural control factors have been carried out since the first *C. ohridella* invasion in Europe [36–38,40–47]. While parasitism rates vary depending on the calculation method used [48], the reported rates are quite low, ranging between 0.5% and 45% [36,43,47,49–51].

The distribution of parasitoids is affected by geography, which often correlates with the climate and the host distribution [52]. Among geographical factors, altitude [53] and the direction of spread [7] are the most important. *Cameraria ohridella* expanded in the Czech Republic from south to north, so its direction of spread was correlated with latitude [7].

This study aimed to evaluate the effect of geographical variability and temporal changes on mortality rates caused by parasitism, together with other mortality factors during a period of five years in the Czech Republic. Our first objective was to determine whether parasitism and other mortality rates were related to altitude, the population density of *C. ohridella*, and other variables. The second objective was to describe the species within the parasitoid complex of the horse chestnut leafminer.

2. Materials and Methods

2.1. Sampling Sites

Samples of horse chestnut (only *A. hippocastanum* species) leaves infested with *C. ohridella* were collected in six sites representing the main regions of the Czech Republic (Figure 1). The trees sampled were either from city parks, formed alleys, or assemblages of trees near the road with lawn beneath with various intensities of care, e.g., mowing and raking. The altitude of sites ranged from 240 to 463 m above sea level (Table 1). The sampling was carried out over a five-year period from 2006 until 2010.

Table 1. Characteristics of the sampling sites.

Label	City	Local Name	Geographical Coordinates	Altitude(m)	Type	Greenery Area [1]	Care [2]
A	Praha	Obora Hvězda	50°4′57″ N, 14°19′54″ E	369	city park	88	2
B	Plzeň	Lochotínský park	49°45′37″ N, 13°21′49″ E	310	city park	36	1
C	Liberec	Jablonecká street	50°46′5″ N, 15°4′4″ E	382	group of trees	2	1
D	České Budějovice	Sady	48°58′38″ N, 14°28′34″ E	386	city park	3	2
E	Brno	Park Špilberk	49°11′40″ N, 16°36′12″ E	240	city park	21	1
F	Rožnov pod Radhoštěm	Hradisko	49°26′53″ N, 18°7′9 E	463	alley	166	0

[1] Size of the continuous area (in hectares) surrounding the sampled trees covered by lawn, shrubs, and trees.
[2] Intensity of greenery care: 0—no mowing or raking, 1—mowing 1–2 times per season, 2—mowing >2 times per season.

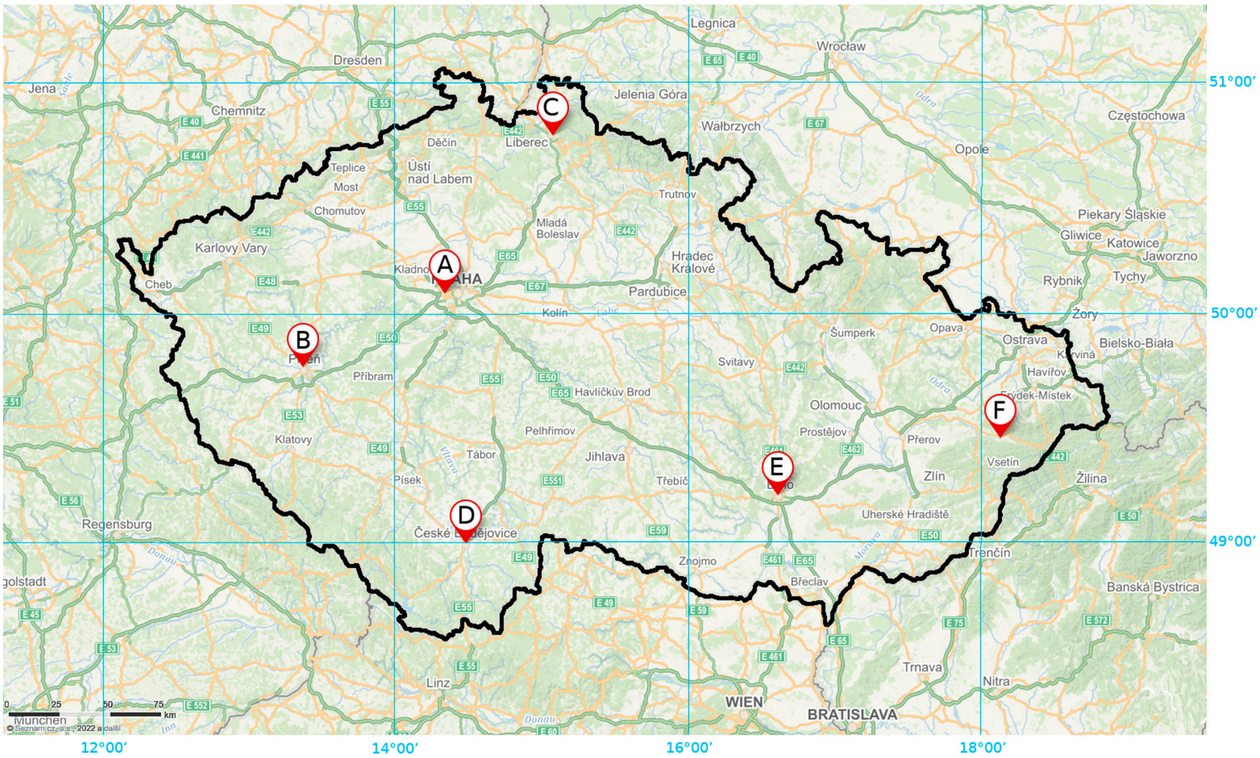


Figure 1. The map of the Czech Republic with marked sites where *Aesculus hippocastanum* leaf samples were collected. Source: Mapy.cz.

2.2. Leaf Sampling

Fifty horse chestnut leaves were randomly picked from the trees (maximum of 5 leaves per tree) up to 2 m above the ground at every site when the first adults of the first *C. ohridella* generation started to emerge. This occurred at approximately the phenological phase of the tree called 'chestnut appearing' [54]. The period of adult emergence was assessed for each site individually. The sampling dates thus differed (Table A1) because leaf miner phenology differed at every site and year. The leaves were put into plastic bags and transported to the laboratory in a cooler, where the leaves were processed immediately or stored in a refrigerator at 4–6 °C for a maximum of 2 days.

2.3. Estimation of Population Density of *C. ohridella*

The population density of *C. ohridella* was assessed as a percentage of the leaf area damaged by the larvae. The visual method proposed by Gilbert and Gregoire [55] was used to estimate leaf damage for each of the 50 compound leaves, and an average was calculated.

2.4. Mine Dissection, Rearing of Parasitoids, and Species Determination

The leaflets were carefully cut from the compound horse chestnut leaves, and 500 mines per sample were dissected under a stereomicroscope with a maximum of 10 mines selected from a single leaflet. The number of living, dead, and parasitized larvae and pupae were recorded. The developmental stage of larvae was determined according to Freise and Heitland [49] and recorded as follows: L1 and L2 = first and second larval instar, L3 = third larval instar, L4 = fourth larval instar (possible L5 instar was included), SP = first and second spinning instar, P = pupa and obviously emerged adults.

Parasitized individuals, including dead pupae, were put singly into plastic Petri dishes. The Petri dishes were stored in boxes at laboratory temperature in relatively high humidity provided by wet cotton wool on the bottom of plastic boxes. The content of Petri dishes was checked for emerged parasitoids daily, and parasitoid individuals were stored in a freezer for future determination.

Determination of parasitoid species was made by the first author according to Grabenweger et al. [56] and compared with specimens of the author's personal collection of parasitoids previously determined by specialists.

2.5. Calculation of Parasitism and Other Mortality Rates

Stage-specific parasitism rates and other mortality rates were calculated according to Volter and Kenis [47]:

$$PN_i = \frac{N_i}{\sum_{j=i}^{5}\left(N_j + D_j + A_j\right)} \quad (1)$$

$$PD_i = \frac{D_i}{\sum_{j=1}^{5}\left(N_j + D_j + A_j\right)} \quad (2)$$

where i (j) denotes the developmental stage (1: 1st and 2nd instar larva, 2: 3rd instar larva, 3: 4th instar larva, 4: spinning larva, 5: pupa; moths of the same generation were included in the number of living pupae), PN_i and PD_i denote parasitism and other mortality rates, respectively; N_i (N_j), D_i (D_j), and A_i (A_j) denote the number of parasitized, dead, or missing individuals and living individuals, respectively.

Total parasitism rates and total mortality rates were calculated using the following equations:

$$PtN = \sum_{i=1}^{5}\left[PN_i \prod_{j=0}^{i-1}\left(1 - PN_j - PD_j\right)\right] \quad (3)$$

$$PtD = \sum_{i=1}^{5}\left[PD_i \prod_{j=0}^{i-1}\left(1 - PN_j - PD_j\right)\right] \quad (4)$$

$$PN_0 = PD_0 = 0 \quad (5)$$

where PtN and PtD denote total parasitism and total other mortality rates, respectively. For other symbols, see Equations (1) and (2).

2.6. Statistical Analysis

A generalized linear model with a binomial distribution and logit link was used to analyze parasitism and other mortality rates data. The following predictors were used: year, altitude, latitude, size of continuous greenery area surrounding the sampled trees, developmental stage of *C. ohridella*, population density of *C. ohridella* expressed as leaf damage, and intensity of greenery care (mowing/raking, ranging from 0 to 2). The analysis was performed in SAS® Studio for Linux using the GLM procedure (PROC GENMOD) of SAS/STAT® module [57]. Since the data represented repeated measurements and thus could not be regarded as independent, the Generalized Estimating Equation (GEE) approach [58] was used to account for within-subject correlations, using option statement REPEATED in GENMOD procedure. We also conducted a Spearman correlation analysis [59] to reveal any significant shift in the ratio between two of the most abundant parasitoid species during the studied period. Data were analyzed using the PROC CORR function in SAS/STAT® module [57]. p values <0.05 were considered statistically significant in all tests.

3. Results

3.1. Dates of the Emergence of the First Generation and Population Density of *C. ohridella*

First adults of the first generation of *C. ohridella* were observed in June and July, depending on the site and year (Table A1). The population density of *C. ohridella* estimated as a percentage of the leaf area damaged by *C. ohridella* larvae varied substantially among sites and years, ranging from 2.0% in České Budějovice in 2007 to 48.7% in Plzeň in 2010 (Figure 2).

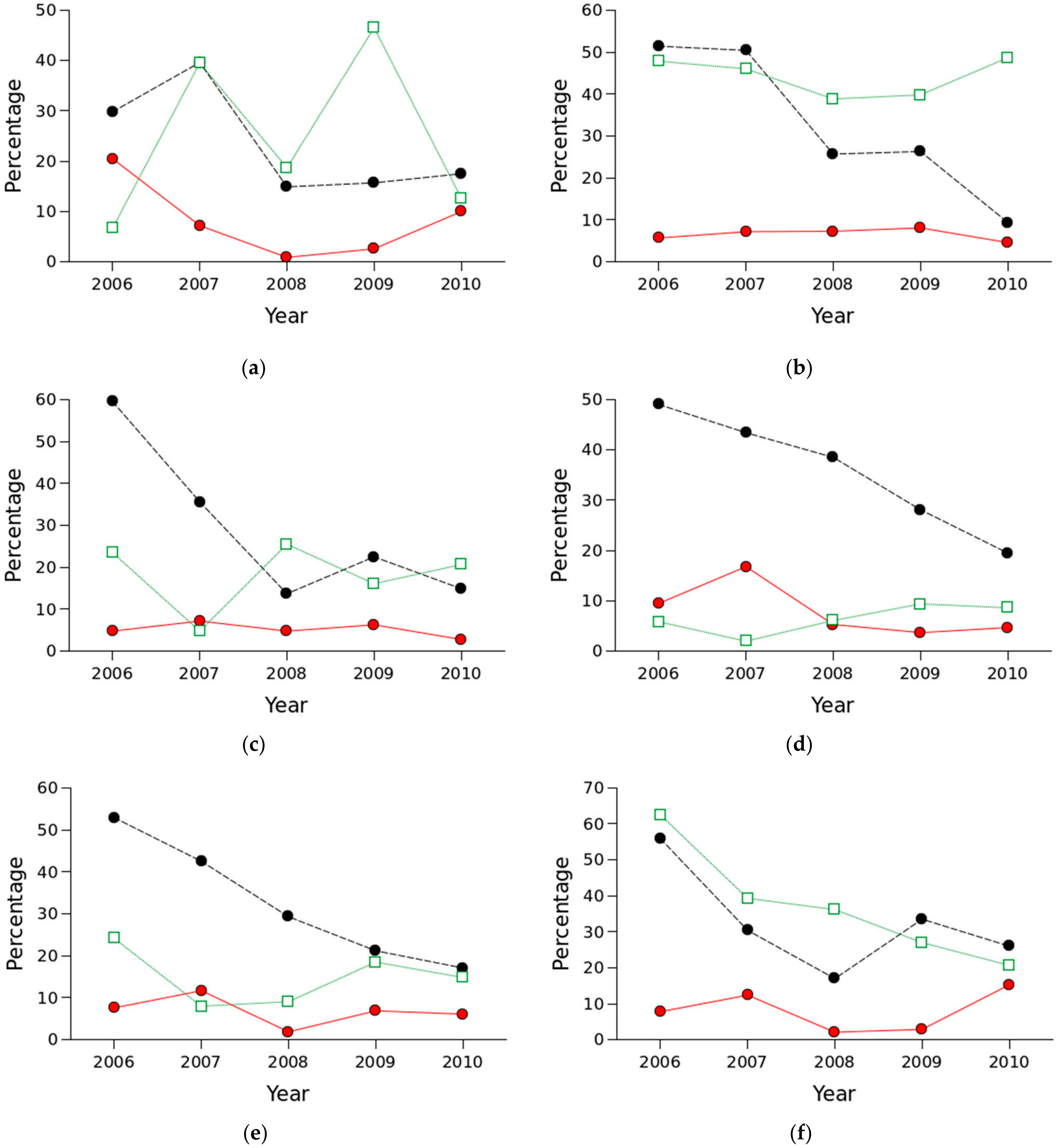


Figure 2. Total parasitism (full red line), total other mortality (dashed black line), and leaf damage inflicted by *C. ohridella* larvae (dotted green line, open squares) at six sites in the Czech Republic from 2006 to 2010. (**a**) Praha; (**b**) Plzeň; (**c**) Liberec; (**d**) České Budějovice; (**e**) Brno; (**f**) Rožnov pod Radhoštěm.

3.2. Parasitism and Other Mortality Rates

Parasitism and other mortality rates of the 15,000 mines dissected showed high variability among developmental stages, years, and sites (Figure 2, Table A1). Parasitism rates of feeding instars reached a maximum of only 1.2%, but were often lower. On the other hand, spinning stages' parasitism was much higher reaching a maximum of 6.4%. The highest parasitism rates were observed in pupae, in which they reached a maximum of 24.1%, with an average value of 7.3%. Besides the positive relationship between the developmental stage of *C. ohridella* and the parasitism rate, the statistical analysis revealed that the parasitism rate was negatively related to year, latitude, and greenery maintenance (Table 2).

Table 2. Results of the analysis of GEE parameter estimates.

Dependent Variable	Parameter	Estimate	Standard Error	Z	*p*
Parasitism rate	Intercept	440.340	136.875	3.22	0.001
	Year	−0.215	0.069	−3.13	0.002
	Altitude	0.001	0.001	1.41	0.157
	Latitude	−0.331	0.052	−6.32	<0.001
	Greenery area	−0.002	0.002	−1.00	0.319
	Developmental stage	1.165	0.091	12.81	<0.001
	C. ohridella population density	−0.009	0.007	−1.15	0.248
	Care (mowing, raking) intensity	−0.157	0.047	−3.31	0.001
Other mortality rate	Intercept	605.940	73.252	8.27	<0.001
	Year	−0.295	0.037	−8.06	<0.001
	Altitude	0.001	0.000	1.65	0.099
	Latitude	−0.315	0.019	−16.80	<0.001
	Greenery area	−0.001	0.001	−2.36	0.019
	Developmental stage	−0.515	0.053	−9.82	<0.001
	C. ohridella population density	0.001	0.002	0.44	0.662
	Care (mowing, raking) intensity	−0.059	0.015	−4.06	<0.001

Total parasitism rates were quite low and varied from 1.9% to 20.5%, with an average value of 7.2%. The highest fluctuation of parasitism rates during the study period was observed in Praha, České Budějovice, and Rožnov pod Radhoštěm (Figure 2).

Mortality caused by other factors was much higher than that caused by parasitism, especially in first/second larval instars and pupae, and varied from 1.4% to 52.9%. Third larval instars up to spinning stages suffered less from other mortality factors; which reached up to 5.8%. Data analysis revealed that in contrast to the parasitism rate, the other mortality rate was also significantly related to the greenery area.

Total other mortality rates varied from 13.7% to 59.5%, with an average value of 31%. The highest rates were found in 2006, followed by an obvious decreasing trend in all sites, except for the Praha site (Figure 2).

3.3. *Parasitoids of C. ohridella*

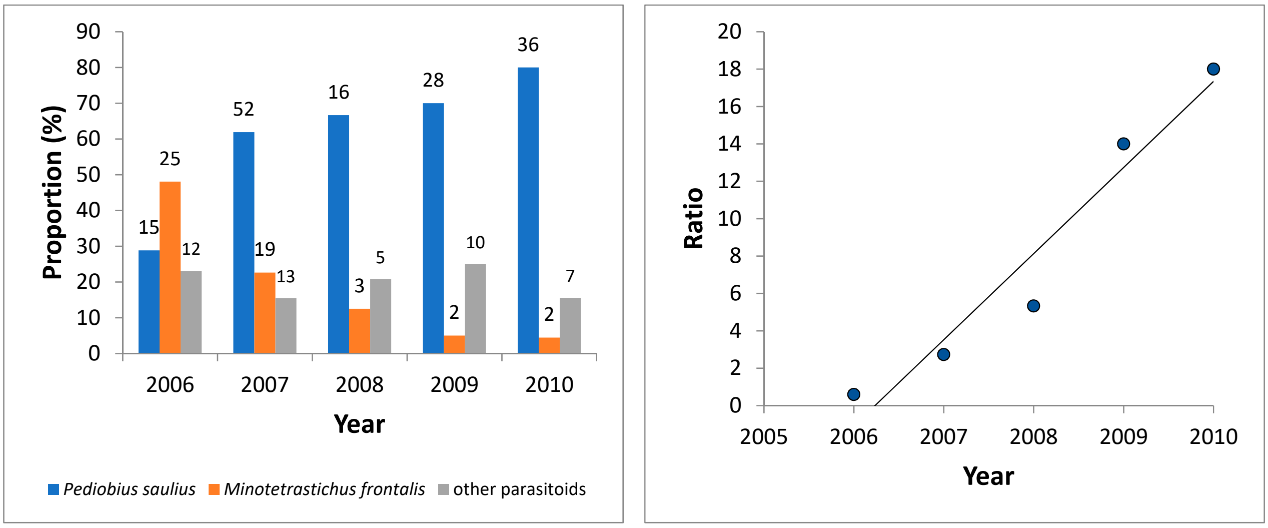
In total, eight hymenopteran parasitoid species were identified in samples from six sampling sites in the Czech Republic over five years (Table 3). Five Eulophidae species and an undetermined species of the genus *Chrysocharis*, one of the family Eupelmidae, an undetermined species of the genus *Pteromalus* (Pteromalidae), and two ichneumonids were reared in this study. The data show variability in species composition and abundance among sites and hosts (Table 3).

The most abundant parasitoid species was *Pediobius saulius* (Walker) at all sites (Figure A1). Because this species is considered a koinobiont, it is difficult to determine which host stage it attacks; nevertheless, it always emerged from pupae of *C. ohridella*, except for one instance in the spinning stage. The second most abundant parasitoid species was *Minotetrastichus frontalis* (Nees) (Figure A1). However, the comparison of individual years showed that the abundance of these two species changes over time (Figure 3a). The proportion of *M. frontalis* among the total parasitoid complex gradually decreased. On the other hand, it was the only species in this study with more than one larvae observed on one host individual. The multiparasitism was occasionally observed with *M. frontalis*/*P. saulius* and *M. frontalis*/*Closterocetus trifasciatus* (Westwood). *Closterocerus trifasciatus* was the only species reared from the first/second larval instar of *C. ohridella*.

Table 3. Parasitoid species reared from *C. ohridella* and summary data regarding the sampling site and the developmental stage from 2006 to 2010.

Superfamily	Family	Species	Sampling Site [1]						Host Stage [2]				
			A	B	C	D	E	F	L1 and L2	L3	L4	SP	P
Chalcidoidea	Eulophidae	*Cirrospilus vittatus* (Walker)		1									1
		Closterocerus trifasciatus (Westwood)			1	4	2	2	1			2	6
		Chrysocharis sp.	1	2		4	1				1	4	3
		Minotetrastichus frontalis (Nees)	3	6	1	19	10	12		1		22	28
		Pediobius saulius (Walker)	11	28	23	26	19	40				1	146
		Pnigalio agraules (Walker)		7	2		2	1		1	3	5	3
		Pnigalio sp.		3			6			1	2	5	1
	Eupelmidae	*Eupelmus urozonus* (Dalman)					1					1	
	Pteromalidae	*Pteromalus* sp.		3								1	2
Ichneumonoidea	Ichneumonidae	*Itoplectis alternans* (Gravenhorst)		1								1	
		Scambus annulatus (Kiss)			2	1						3	

[1] See Table 1 for site descriptions. [2] Developmental stage of *C. ohridella* attacked: L1 and L2—first and second larval instar, L3—third larval instar, L4—fourth larval instar, SP—first and second spinning instar, P—pupa and obviously emerged adults.



(**a**) (**b**)

Figure 3. Temporal changes in the parasitoid species complex of *C. ohridella* at six sites in the Czech Republic: (**a**) proportion among all parasitoids; numbers above columns represent the number of parasitized host individuals; (**b**) changes in *P. saulis/M. frontalis* ratio fitted by linear trend.

There was an obvious increase in *P. saulius* proportion in the parasitoid complex during the five years of observation, while *M. frontalis* became less abundant (Figure 3b). A Spearman correlation analysis revealed that the *P. saulius/M. frontalis* ratio clearly increased over time ($r_s = 1$; $n = 5$; $p < 0.001$).

4. Discussion

4.1. Parasitism and Other Mortality Rates

The parasitism rates in our study were rather low and did not differ from the results published by other authors [36,43,47,49–51]. Our study showed that parasitoids do not often emerge from the first to fourth larval instars of *C. ohridella*, while higher parasitism is observed in older stages [47]. Pupal parasitism rates ranged from 0 to 12% and from 4 to 28% in Belgrade and Istanbul, respectively [60]. Similar values were observed in this study, where *P. saulius* was responsible for the majority of pupal parasitism. This species can, however, develop as a koinobiont, i.e., it can attack some younger stages of the host and finish its development in the host pupa, in contrast to other parasitoids reared during this study considered to be idiobionts. Because *P. saulius* was also a dominant species, the parasitism rates observed in individual *C. ohridella* stages might thus be biased.

Although different methods for calculating the parasitism rates were used in other studies, the ability of parasitoids to manage *C. ohridella* populations is still too low. Such low parasitism rates probably result from insufficient adaptation of the local parasitoids to the new host [52]. While the composition of the assemblage of parasitoids is latitude-dependent, the higher proportion of *P. saulius* has not yet improved the overall rate of parasitism. Nevertheless, in comparison with Volter and Kenis [47], a stronger pressure of parasitoids on pupae was seen in the present study.

Variability in parasitism rates between sampling sites could be due to different local climatic conditions and factors related to the city's characteristics, such as the structure of the streets and buildings near sampling sites and the prevailing wind direction. Our results confirmed that the parasitism rate was positively related to the developmental stage of the host and negatively related to year, latitude, and greenery care. A study on chalcid wasps that emerged from horse chestnut leaf litter samples collected from 35 sites in the Czech Republic [52] showed that wasp abundance, considered an indicator of parasitism, was also negatively related to latitude, but contrary to our findings, it was positively related to altitude as well as *C. ohridella* abundance. The positive correlation of the number of parasitoids with the number of moths that emerged from leaf litter in spring was confirmed in a study by Kopačka and Zemek [14].

High variability in the percentage of leaf area damaged by *C. ohridella* among sampling sites found in the present study is likely to be geographically related, but variability in leaf damage can be quite high, even within a city area [14]. A key factor seems to be the maintenance of public green areas, particularly raking and removing leaf litter in autumn or early spring, which is a recommended and effective way to regulate the damage caused by *C. ohridella* in the spring [20,61]. However, the removal of fallen horse chestnut leaves also reduces parasitoids that also overwinter in horse chestnut leaf litter [14,52,62]. Our results confirmed a significant negative effect of greenery maintenance intensity, i.e., mowing frequency, on the parasitism rate.

The other mortality rates combined all other mortality factors except parasitism, including the influence of predators such as birds, spiders, or some insects [34]; insect pathogens; and environmental stress. Fungi were observed on some *C. ohridella* cadavers, but it was not possible to determine whether the fungal growth was caused by entomopathogenic or saprophytic fungi on already dead individuals. Although this study did not focus on this, natural infections of *C. ohridella* by entomopathogenic fungi have been previously reported [29,30,35]. The other mortality rates of the first and second larval instars were higher in the present study compared to previous studies [47], which could indicate that the controlling factors have a higher impact on the young larval stages. In comparison to parasitism rates, other mortality rates were much higher and negatively related to the greenery area size as well as greenery care.

There was no obvious difference between the total other mortality rates recorded in the present study and those of Volter and Kenis [47]. The cause of the decline in other mortality rates during the study period observed in all sites is unclear, and further studies should be conducted to elucidate the validity of this trend.

4.2. Parasitoids of C. ohridella

The parasitoid species found at all sites in the Czech Republic do not differ from the records in the literature [36–38,40–47,63–66]. The abundance of individual species recorded by us was similar to the data reported from Bulgaria, Greece, Croatia, Macedonia, and Yugoslavia or generally from south-eastern Europe [36,40]. The most abundant parasitoid species in 2006 was *M. frontalis*, but the abundance of *P. saulius* increased in all consecutive years, while the abundance of *M. frontalis* declined. According to published reports, *P. saulius* was not recorded in Bayern (Germany) in 1998, while *M. frontalis* was the most abundant species [50]. The most abundant parasitoid species were *M. frontalis* together with *Pnigalio agraules* (Walker) in Wien and Gerasdorf (Austria) from 1996 to 1999, with no presence or very rare occurrence of *P. saulius* [43,51]. *Minotetrastichus frontalis* and *P. agraules* were also the most abundant species in Bayern in 2000 [49] and leaf litter collected in the Czech Republic in early spring 2002 [52]. The latter study also reported findings of *P. saulius*, contrary to Volter and Kenis [47], who did not detect this species between 2001 and 2003 in the western Czech Republic. It was also found in Ilava (Slovakia), but rarely [47]. In contrast, *P. saulius* dominated in Lipica (Slovenia) [47]. *Pediobius saulius* was not present on *C. ohridella* in 2003–2004 in Olten (Switzerland), Jübeck (Germany), and Brixen (Italy) at all, but it was present and even the most abundant species in Bulgaria in 2003–2004 [42].

These data suggest that the original parasitoid complexes in different countries started to change. There is a clear tendency that *P. saulius*, a parasitoid already known from other hosts in Yugoslavia, Hungary, Czech Republic, Slovakia, Germany, France, Switzerland, and Italy [42,67], is having a gradually increasing impact on *C. ohridella* from the south to north. The mechanism of its sudden appearance or increasing influence on *C. ohridella* is not clear, but it could be attributed to its adaptation to a new host species. It is assumed that when the parasitoid species adapt to *C. ohridella*, then they can follow its geographic dispersal. This was, for example, confirmed for *Pnigalio mediterraneus* Ferriére and Delucchi using molecular markers [68]. While generalist parasitoids may adapt to *C. ohridella* with a shorter time lag, the adjustment of specialist parasitoids probably requires more than a few decades [36]. Despite currently low parasitism rates reported in previous studies (e.g., [39,52]) as well as our findings, the successful biological control of this invasive pest by natural enemies may develop in the future.

5. Conclusions

Our study showed that the parasitism rate remained relatively low and that it was significantly related to year, *C. ohridella* population density and developmental stage, and latitude, but not to altitude or greenery area size and its maintenance. The parasitoid complex is predominantly formed by generalist parasitoids of the family Eulophidae, and it seems that the role of increasing *P. saulius* abundance does not positively influence the parasitism rate, but higher parasitism of *C. ohridella* pupae by *P. saulius* in combination with other mortality factors could improve the control of *C. ohridella* in the future and could be much more effective than *M. frontalis*, which does not exclusively attack pupae. Mortality in the earlier juvenile stages of hosts is caused by many factors, and the pupa, as the last juvenile stage, can be vulnerable to attack by *P. saulius*. If the abundance of *P. saulius* continues to increase, this species might become an important agent for more efficient biological control of *C. ohridella* populations in the future.

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Appendix A

Table A1. Parasitism and other mortality rates (%) on the different developmental stages of *C. ohridella* at six sites in the Czech Republic from 2006 to 2010.

Sampling Site [1]	Year	Sampling Date	Parasitism [2]					Other Mortality [2]				
			L1 and L2	L3	L4	SP	P	L1 and L2	L3	L4	SP	P
A	2006	27 July	0	0	0	2.9	24.1	19.6	2.9	0	0	10.3
	2007	20 June	1.0	0	0	0.6	8.7	30.8	2.2	0.4	0	10.7
	2008	2 July	0	0	0.3	0	0.8	12.2	1.2	0.8	0	1.2
	2009	2 July	0	0.2	0	0	2.6	9.0	0.5	0	0	6.9
	2010	2 July	0.2	0	0	0.9	10.8	13.4	0	0	1.8	3.1
B	2006	15 July	0	0.8	0.4	2.2	4.8	26.0	1.6	0.8	1.7	32.7
	2007	17 June	0	0.3	0.3	3.0	7.4	28.2	4.3	0.9	1.5	27.1
	2008	29 June	0.2	0.5	1.0	6.4	1.8	19.8	3.2	2.7	1.7	0
	2009	27 June	0.2	0	0.7	3.8	6.1	21.0	1.7	0.3	0	5.1
	2010	26 June	0	0	0	0	4.9	6.0	0	0	0	3.7
C	2006	26 July	0	0	0	2.9	2.9	13.2	4.1	0	0	52.9
	2007	29 June	0.2	0	0	0.3	9.2	22.0	4.3	0.6	0	13.4
	2008	10 July	0	0	0	0.3	5.1	8.6	0.4	0.7	0	4.6
	2009	9 July	0.2	0	0.3	0.4	6.6	12.6	1.9	0.3	0	9.4
	2010	13 July	0.2	0	0	0.9	2.1	13.6	0.5	0	0	1.1
D	2006	17 July	0	0.4	0.4	3.8	11.1	34.2	3.7	1.2	0	19.4
	2007	20 June	1.2	0.6	0.6	4.5	17.9	25.6	4.2	1.0	0.7	20.8
	2008	1 July	0.2	0.2	0.6	0.7	5.4	18.2	5.2	3.9	0	17.8
	2009	2 July	0.2	0	0	0.4	4.2	22.2	4.1	0	0	3.6
	2010	2 July	0.4	0	0.7	1.0	3.6	15.6	2.6	0	0	2.2
E	2006	10 July	0	0.7	1.1	5.3	3.9	26.6	1.1	0.8	1.6	36.2
	2007	12 June	0.4	0.4	0	1.8	15.6	29.4	5.8	1..9	0	12.2
	2008	25 June	0	0	0.3	0.4	1.7	17.6	1.5	0.3	1.2	11.7
	2009	25 June	0.2	0.8	0.3	0	6.9	1.4	4.3	0.3	0	6.9
	2010	25 June	0	0.2	0.4	1.5	5.1	12.4	1.7	0	0	3.8
F	2006	25 July	0	0	0	0.7	10.3	26.6	1.9	0	0	39
	2007	27 June	0.2	0	0	1.7	13.4	14	2.2	2.2	0	15.6
	2008	7 July	0	0	0.3	0	2.2	12.6	1.2	0	0	3.9
	2009	8 July	0.2	0	0.4	1.2	2.4	28	3.1	0	0	4.8
	2010	7 July	0.6	0.3	0	0.5	18.1	21	0.6	0	0	5.8

[1] See Table 1 for description of sites. [2] Developmental stages: L1 and L2—first and second larval instar, L3—third larval instar, L4—fourth larval instar, SP—first and second spinning instar, P—pupa and obviously emerged adults.

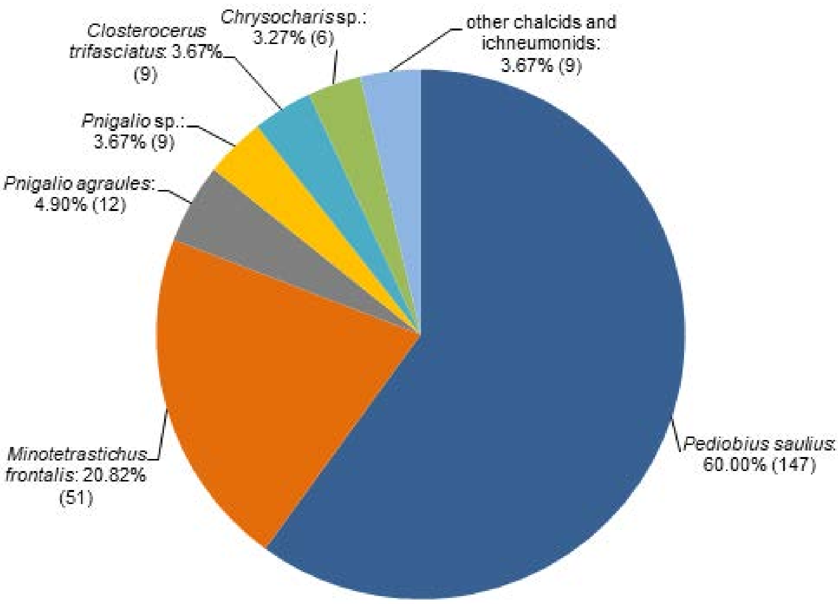

Figure A1. Parasitoid complex of *C. ohridella* at the six sites in the Czech Republic from 2006 to 2010. Numbers in brackets indicate absolute numbers.

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