The role of geography and host abundance in the distribution of parasitoids of an alien pest

Petra Nováková¹, Jaroslav Holuša² and Jakub Horák²

¹ Department of Game Management and Wildlife Biology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic
² Department of Forest Protection and Entomology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT

Chalcid wasps (Hymenoptera: Chalcidoidea) are probably the most effective and abundant parasitoids of the horse chestnut leaf miner (Cameraria ohridella), an alien pest in Europe that lacks specialized enemies. We studied how the species richness and abundance of chalcids are influenced by altitude, direction of an alien spread and host abundance of C. ohridella. We quantified the numbers and species richness of chalcid wasps and the numbers of C. ohridella that emerged from horse chestnut (Aesculus hippocastanum) leaf litter samples collected from 35 sites in the Czech Republic. Species richness of chalcids, which was considered an indicator of the possible adaptation of parasitoids to this alien host, was unrelated to C. ohridella abundance, direction of spread, or altitude. Chalcid abundance, which was considered an indicator of parasitism of the alien host, was strongly and positively related to C. ohridella abundance. Chalcid abundance was negatively related to direction of spread and positively related, although in a non-linear manner, to altitude. The relationship of chalcid abundance with direction of spread and altitude was weaker than that with C. ohridella abundance. The results provide evidence that biological control of the alien pest C. ohridella by natural enemies might develop in the future.

Subjects Biogeography, Ecology, Entomology, Parasitology

Keywords Aesculus hippocastanum, Horse chestnut leaf miner (Cameraria ohridella), Leaf blotch miner moths (Lepidoptera: Gracillariidae), Chalcid wasps (Hymenoptera: Chalcidoidea), Altitude, Spread direction

INTRODUCTION

The horse chestnut leaf miner, Cameraria ohridella Deschka and Dimic, 1986 (Lepidoptera: Gracillariidae), is causing ecological problems throughout Europe (Percival et al., 2011; Matosevic & Melika, 2012). This species, which may have originated in the Balkans (Valade et al., 2009), has increased its distribution (Sefrova & Lastuvka, 2001) within a relatively short time (Augustin, 2013). Although C. ohridella was not described until 1986, DNA analysis of herbarium specimens indicates that the species was present in Europe at least as early as 1879 (Lees et al., 2011).

In addition to causing aesthetic damage, mining by C. ohridella larvae may weaken or even kill horse chestnut trees (Aesculus hippocastanum L.)—the mining is nearly
constant throughout the growing season because the insect has multiple, overlapping generations (Matosevic & Melika, 2012). The weakened trees increase the dustiness in urban environments and reduce the food supply for game in non-urban environments (Percival et al., 2011). This alien pest also harms native fauna (Pere et al., 2010) and other tree species in Europe (Freise, Heitland & Sturm, 2004).

Because C. ohridella overwinters as pupa in leaves that have fallen to the ground, C. ohridella numbers can be reduced by leaf removal (Gilbert et al., 2003; Kehrli & Bacher, 2003). Leaf removal, however, is time consuming and thus expensive. In addition, the removed leaves must be properly composted to prevent leaf miner emergence in the following spring (Kehrli & Bacher, 2004). Burning is not always possible because of weather or local regulations. C. ohridella may also be controlled by the use of insecticides or pheromones but these methods have been inconsistent in reducing the abundance of this pest and may harm native fauna (Wagner et al., 1996; Sefrova, 2001). Although the application of synthetic inhibitors of chitin synthesis proved to be very effective (Blumel & Hausdorf, 1997; Percival, Banks & Keary, 2012), the residues of these inhibitors may be highly stable (i.e., persistent) on horse chestnut leaves (Nejmanova et al., 2006). From a long-term perspective, breeding of horse chestnut tree with resistance to C. ohridella is an option (Mertelik, Kloudova & Vanc, 2004).

The current research concerns the control of C. ohridella by natural enemies. Among the approximately 60 generalist parasitoids of C. ohridella (e.g., Grabenweger & Lethmayer, 1999; Toth & Lukas, 2005), Chalcid wasps (Hymenoptera: Chalcidoidea) are considered the most abundant and effective control agents (Grabenweger & Lethmayer, 1999). The overall parasitism rate of non-native C. ohridella by indigenous enemies is affected by temporal factors (i.e., miner residence time) and spatial factors (i.e., geography) (Grabenweger et al., 2010). In addition, the attack of alien pests by native natural enemies is often delayed—as a consequence, the alien pest often suffers little biological control early in its invasion (Godfray, 1994; Schonrogge & Crawley, 2000).

Many geographical factors influence the spread, expansion and distribution of organisms, and especially important predictors are altitude (Lomolino et al., 2010) or direction of spread (Sefrova & Lastuvka, 2001). These factors are often correlated with climate. Altitude, as an example, is known to well reflect geographical heterogeneity (Tognelli & Kelt, 2004). At the spatial scale of the Czech Republic, south-to-north expansion of C. ohridella was correlated with latitude of C. ohridella (Sefrova & Lastuvka, 2001). The relative importance of geography and host distribution on the distribution of parasitoids depends on the host-specificity of the parasitoids, i.e., the effect of host distribution becomes more important as host-specificity increases (Sivinski, Pinero & Aluja, 2000; Skillen, Pickering & Sharkey, 2000).

To our knowledge, C. ohridella lacks host-specific natural enemies (Grabenweger & Lethmayer, 1999; Toth & Lukas, 2005). Thus, we suspect that the number of species and individuals of non-specific parasitoids may be able to successfully respond to the high abundance of this alien pest only if the parasitoids are limited by geography only marginally.
The abundance of *C. ohridella* (based on data collected in the current study) is indicated by black circles, altitude is indicated by grey shading, and latitude is indicated by grey lines.

The main aim of this study was the answer on the question: How are the species richness and abundance of chalcid parasitoids influenced by geography and by the abundance of the alien pest, *C. ohridella*?

**MATERIAL AND METHODS**

**Sampling sites**

During 2002, we studied the parasitism of the horse chestnut leaf miner by chalcid wasps in 35 sites in the Czech Republic (Fig. 1). Each site contained a road lined with ≥ five horse chestnut trees that were infested with *C. ohridella*. These sites are typical of the patches with horse chestnut trees in the Czech Republic and are known to be highly suitable for *C. ohridella* development (*Sefrova & Lastuvka, 2001*).

**Study methods**

Horse chestnut leaf litter samples were collected from the soil surface under the crowns of horse chestnut trees that were distant from other tree species to minimize the possibility
that the litter was contaminated with leaves of other species. All samples were taken during the early spring before the emergence of parasitoids (Grabenweger, 2004). At each site, we collected 1 m$^2$ ($\approx$0.1 m height) of pure horse chestnut leaf litter.

All samples were covered with paper sheets and immediately transported to the laboratory, where the litter was placed in emergence traps (cardboard boxes 0.6 $\times$ 0.9 $\times$ 0.2 m) at 18–20$^\circ$. All arthropods that emerged from the litter were trapped in 70% ethanol. The adult C. ohridella and chalcids were counted daily. The ethanol was replaced daily and the preserved chalcids were identified to species.

**Dependent variables and environmental predictors**

Dependent variables included the number of chalcid species and the number of individuals of chalcid wasps that emerged from each litter sample. We used the list of Nováková & Nakladal (2008) for preliminary comparison of the parasitoid species and we found that all reared species are known to be associated with C. ohridella. It is indicated that species in their native areas are hosts of a higher number of species of parasitoids (Girardoz, Kenis & Quicke, 2006; Grabenweger et al., 2010). Thus, the number of chalcid species was considered an indicator of possible adaptation of parasitoids to the alien host, i.e., an increase in species would suggest an increase in adaptation. On the other hand, the number of chalcid individuals was considered a possible indicator of chalcid abundance and rate of parasitism of the alien host (e.g., Arneberg et al., 1998).

We studied three environmental predictors (Table 1) that might influence the species richness and abundance of parasitoids that emerge from litter samples. The number of C. ohridella adults that emerge (Host abundance) reflects C. ohridella abundance at each site. Altitude of the site reflects geographical heterogeneity and correlates with climate. The direction of spread (Spread direction) might well reflect the situation of species richness and abundance of parasitoids during the time of active spread of invasive species. Thus, the direction of spread was used as the third environmental predictor. As the spread of C. ohridella in the Czech Republic had south-to-north direction (Sefrova & Lastuvka, 2001; Sefrova, 2003), degrees of latitude were used.

**Statistical analyses**

All analyses were conducted in R 3.0.2 (R Development Core Team, 2013). The potential bias caused by spatial autocorrelation was controlled by Moran’s correlograms using the spdep package (Bivand, 2005). Because our data did not show spatial bias at any distance ($I < -0.1; P > 0.1$), we used traditional statistical methods.
Table 2  Relationships between the number of species and abundance of chalcid wasps (Hymenoptera: Chalcidoidea) that emerged from litter samples infested with *Cameraria ohridella* and predictors (Host abundance, Altitude and Spread direction) as indicated by hierarchical partitioning and generalized linear models.

| Dependent variable                  | AIC    | Predictor         | TEV    | z     | P    |
|-------------------------------------|--------|-------------------|--------|-------|------|
| Number of chalcid species per site  | 107.85 | Host abundance    | 13.3   | 1.0   | n.s. |
|                                     |        | Altitude          | 12.3   | −1.0  | n.s. |
|                                     |        | Spread direction  | 2.2    | −0.1  | n.s. |
|                                     | 541.33 | Host abundance    | 48.9   | 17.2  | <0.001|
| Number of chalcid individuals per site |        | Altitude          | 1.9    | 4.2   | <0.001|
|                                     |        | Spread direction  | 7.7    | −4.5  | <0.001|

**Notes.**
AIC, Akaike Information Criterion; TEV, % of total explained variance.

We then controlled for possible circular predicting and multicolinearity using the HH package (*Heiberger, 2009*) and the value of variance inflation factor (VIF). This showed that *C. ohridella* abundance was not correlated with the other studied predictors (Table 1), i.e., with altitude ($R = −0.3; P = n.s.$) or latitude ($R = −0.3; P = n.s.$). Data for the number of species and individuals of chalcids had Poisson distributions.

The variance explained by the predictors was computed using $R^2$ in hierarchical partitioning (package hier.part; *Walsh & Mac Nally, 2011*). The relationships between the dependent variables and the predictors were computed using generalized linear models and generalized additive models with the gam package (*Hastie, 2011*). Generalized additive models were fitted by spline function.

**RESULTS**
A total of 811 individuals (mean = 23.2 ± 4.3 SE; min = 1; max = 118) of eight chalcid wasp species (1.9 ± 0.2; 1–4) emerged from the 35 litter samples, namely: *Cirrospilus viticola* (0.1 ± 0.1), *Closterocerus trifasciatus* (0.8 ± 0.4), *Pediobius saulius* (2.1 ± 1.4), *Pniagalo agraules* (11.5 ± 2.9), *Pniagalo pectinicornis* (0.7 ± 0.3), *Pteromalus semotus* (1.4 ± 0.6) *Minotetrastichus frontalis* (7.1 ± 1.6) and *Sympiesis sericeicornis* (1 individual).

The number of parasitoid species that emerged was not significantly related to the studied predictors (Table 2). The number of chalcid individuals that emerged (i.e., chalcid abundance) was positively related to the number of *C. ohridella* that emerged from each sample, i.e., *C. ohridella* abundance explained nearly 50% of the variance in chalcid abundance. Chalcid abundance was negatively related with spread direction, and spread direction explained nearly 8% of the variance in chalcid abundance (Table 2). Unexpectedly, chalcid abundance was positively related with altitude (Table 2), although the response to altitude was not linear (Fig. 2 and Table 3). Altitude explained less than 2% of the variance in chalcid abundance (Table 2).

**DISCUSSION**
Data in the current study were collected when the invasive horse chestnut leaf miner (*C. ohridella*) had become fully established in the Czech Republic (*Sefrova & Lastuvka, 2016,* PeerJ, DOI 10.7717/peerj.1592).
Table 3  Relationships between the number of chalcid wasps (Hymenoptera: Chalcidoidea) that emerged from litter samples infested with *Cameraria ohridella* and predictors (Host abundance, Altitude and Spread direction) as indicated by the generalized additive model (DF = 1.5).

| Name                                      | AIC     | Deviance | Predictor          | Npar | χ²   | P     |
|-------------------------------------------|---------|----------|--------------------|------|------|-------|
| Number of chalcid individuals per site    | 495.71  | 56.02%   | Host abundance     | 5.5  | <0.01|       |
|                                           |         |          | Altitude           | 31.9 | <0.001|       |
|                                           |         |          | Spread direction    | 10.1 | <0.001|       |

Notes.  
AIC, Akaike Information Criterion; Npar χ², non-parametric value of χ².

Figure 2  Relationship between the abundance of chalcid wasps (Hymenoptera: Chalcidoidea) and three predictors. Host is abundance of *C. ohridella*; Altitude is m a.s.l.; and Direction is spread direction from north-to-south as indicated by the generalized additive model fitted by spline function with DF = 1.5.

Since then, this invasive pest has expanded throughout central Europe and has established its first populations on the British Isles (*Augustin, 2013*). Our results show that the abundance of parasitoids of *C. ohridella* was weakly related to predictors that are highly connected with geography, namely altitude and spread direction (linked to the latitude), but was relatively strongly related to *C. ohridella* abundance.

The number of parasitoid species was not significantly related to the studied predictors. This result indicates that the adaptation of indigenous parasitoid species to the alien pest was rather low, which is consistent with *Girardoz, Kenis & Quicke (2006)* and it seemed that most of the parasitoid species were generalists, which agrees with *Novakova & Nakladal (2008)*. On the other hand, parasitoid abundance was closely and positively related to *C. ohridella* abundance.

In addition to being closely related to *C. ohridella* abundance, the abundance of generalist parasitoids seemed relatively high, even though the emergence of *C. ohridella* and its parasitoids are indicated to be poorly synchronized (*Grabenweger, 2004*). Although parasitism rates as high as 50% have been reported for other leaf mining moths, the percentage of *C. ohridella* attacked by parasitoids is often low and does not usually reach...
20% (Grabenweger & Lethmayer, 1999; Novakova & Nakladal, 2008; Grabenweger et al., 2010). This low parasitism rate, which undoubtedly contributed to the heavy infestation of horse chestnut trees by C. ohridella in many places, probably results from former insufficient adaptation of the local parasitoids to this recently introduced leaf miner. If such adaptation is possible, it will most probably require more time (Zwölfer & Pschorn-Walcher, 1968).

On the other hand, we suspect that generalist parasitoids may adapt to C. ohridella given that their abundance increased with that of the pest although with delay. Grabenweger et al. (2010) hypothesized that the adjustment of specialist parasitoids requires more than a few decades. Recruitment and accumulation of native parasitoid species on introduced herbivores has been documented (Cornell & Hawkins, 1993), and exotic insects do not necessarily suffer lower enemy-induced mortality rates than natives (Hawkins, Cornell & Hochberg, 1997). A quick shift of native parasitoids to the new invasive host Tuta absoluta (Meyrick 1917) was observed in Italy (Zappala et al., 2012). Similarly, another recent study indicated that resident generalist parasitoids and predators can work in conjunction to hinder the invasion of a herbivore (Hogg et al., 2013). It follows that although natural enemies have not prevented invasion of Europe by C. ohridella, based on our results we could suppose that successful biological control of invasive C. ohridella by natural enemies may develop in the future—because the total amount of chalcid individuals can better reflect the rate of parasitism of the alien host than number of adapted parasitic species.

The relationship to the spread direction fairly well illustrated that the number of parasitoids is decreasing with increasing distance from the area of origin. On the other hand, the increasing number of individuals of parasitoids was higher in higher altitudes, which is not common (Lomolino et al., 2010). This might correlate with relatively high altitude of the Lake Ohrid and surrounding areas in Macedonia and Albania, which is the area of origin of C. ohridella (Valade et al., 2009). The result appears to indicate that aliens are more vulnerable to enemies in conditions that are close to their former area of distribution (e.g., Roy et al., 2011).

**CONCLUSIONS**

The number of parasitoid species that emerged from leaf litter infested with the horse chestnut leaf miner, C. ohridella, was not significantly related to C. ohridella abundance, altitude or spread direction, a finding which possibly indicates a delayed response of indigenous enemies to the expansion of their hosts. Although the abundance of generalist parasitoids was weakly related to altitude and spread direction, it was strongly related to C. ohridella abundance. Our results indicate a potential for biological control of C. ohridella by generalist parasitoids.

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Author Contributions
• Petra Nováková conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools.
• Jaroslav Holuša contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
• Jakub Horák analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability
The following information was supplied regarding data availability:
The raw data has been supplied as Data S1.

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REFERENCES

Arneberg P, Skorping A, Grenfell B, Read AF. 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society of London B: Biological Sciences 265:1283–1289 DOI 10.1098/rspb.1998.0431.

Augustin S. 2013. Distribution of Cameraria ohridella. The Natural History Museum. Available at http://www.nhm.ac.uk/nature-online/species-of-the-day/biodiversity/alien-species/cameraria-ohridella/index.html.

Bivand R. 2005. The Spdep Package. Comprehensive R archive network. Version 0.3-13. Available at http://www.cran.r-project.org.

Blumel S, Hausdorff H. 1997. Versuche zur Kontrolle von Cameraria ohridella Deschka & Dimić mit insektiziden Wachstumsregulatoren. Forstschutz Aktuell 21:16–18.
Cornell HV, Hawkins BA. 1993. Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders. *The American Naturalist* **141**:847–865 DOI 10.1086/285512.

Freise JF, Heitland W, Sturm A. 2004. Host-plant range of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic (Lepidoptera, Gracillariidae), a pest of the white flowering horse-chestnut, *Aesculus hippocastanum* in Germany. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* **14**:351–354.

Gilbert M, Svaton A, Lehmann M, Bacher S. 2003. Spatial patterns and infestation processes in the horse chestnut leafminer *Cameraria ohridella*: a tale of two cities. *Entomologia Experimentalis et Applicata* **107**:25–37 DOI 10.1046/j.1570-7458.2003.00038.x.

Girardoz S, Kenis M, Quicke D. 2006. Recruitment of native parasitoids by an exotic leafminer, *Cameraria ohridella*: host-parasitoid synchronization and influence of the environment. *Agricultural and Forest Entomology* **8**:49–56 DOI 10.1111/j.1461-9555.2006.00281.x.

Godfray HCJ. 1994. *Parasitoids: behavioral and evolutionary ecology*. Princeton: Princeton University Press.

Grabenweger G. 2004. Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), by native European parasitoids: a synchronisation problem. *European Journal of Entomology* **101**:189–192 DOI 10.14411/eje.2004.023.

Grabenweger G, Kehrli P, Zweimüller I, Augustin S, Avtzis N, Bacher S, Freise J, Girardoz S, Guichard S, Heitland W, Lethmayer C, Stolz M, Tomov R, Volter L, Kenis M. 2010. Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. *Biological Invasions* **12**:2797–2813 DOI 10.1007/s10530-009-9685-z.

Grabenweger G, Lethmayer C. 1999. Occurrence and phenology of parasitic Chalcidoidea on the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae). *Journal of Applied Entomology* **123**:257–260 DOI 10.1046/j.1439-0418.1999.00347.x.

Hawkins BA, Cornell HV, Hochberg ME. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology* **78**:2145–2152 DOI 10.1890/0012-9658(1997)078[2145:PPAPAM]2.0.CO;2.

Hastie T. 2011. *GAM: generalized additive models*. R Package Version 1.04.1. Available at [http://www.cran.r-project.org](http://www.cran.r-project.org).

Heiberger RM. 2009. *HH: statistical analysis and data display: Heiberger and Holland*. R package version 2.1-32. Available at [http://www.cran.r-project.org](http://www.cran.r-project.org).

Hogg BN, Wang XG, Levy K, Mills NJ, Daane KM. 2013. Complementary effects of resident natural enemies on the suppression of the introduced moth *Epiphyas postvittana*. *Biological Control* **64**:125–131 DOI 10.1016/j.biocontrol.2012.10.008.

Kehrli P, Bacher S. 2003. Date of leaf litter removal to prevent emergence of *Cameraria ohridella* in the following spring. *Entomologia Experimentalis et Applicata* **107**:159–162 DOI 10.1046/j.1570-7458.2003.00043.x.
Kehrli P, Bacher S. 2004. How to safely compost Cameraria ohridella-infested horse chestnut leaf litter on small compost heaps. Journal of Applied Entomology 128:707–709 DOI 10.1111/j.1439-0418.2004.00915.x.

Lees DC, Lack HW, Rougerie R, Hernandez-Lopez A, Raus T, Avtzis N, Augustin S, Lopez-Vaamonde C. 2011. Tracking origins of invasive herbivores using herbaria and archival DNA: the case of the horse-chestnut leafminer. Frontiers in Ecology and the Environment 9:322–328 DOI 10.1890/100098.

Lomolino MV, Riddle BR, Whittaker RJ, Brown JH. 2010. Biogeography. 4th edition. Sinauer.

Matosevic D, Melika G. 2012. Diversity of parasitoid assemblages of native and alien leaf miners in Croatia. Sumarski List 136:367–376.

Mertelik J, Kloudova K, Vanc P. 2004. Occurrence of Aesculus hippocastanum with high degree of resistance to Cameraria ohridella in the Czech Republic. Acta fytotechnica et zootechnica 7:204–205.

Nejmanova J, Cvacka J, Hrdy I, Kuldova J, Mertelik J, Muck Jr A, Nesnerova P, Svatos A. 2006. Residues of diflubenzuron on horse chestnut (Aesculus hippocastanum) leaves and their efficacy against the horse chestnut leafminer, Cameraria ohridella. Pest Management Science 62:274–278 DOI 10.1002/ps.1165.

Novakova P, Nakladal O. 2008. Pripádova studie parazitoidu klinenky jirovcove (Cameraria ohridella Deschka et Dimic, 1986) v Ceske republice v letech 2001–2005. Zpravy Lesnického Výzkumu 53:12–21.

Percival GC, Banks J, Keary I. 2012. Evaluation of organic, synthetic and physical insecticides for the control of horse chestnut leaf miner (Cameraria ohridella). Urban Forestry & Urban Greening 11:426–431 DOI 10.1016/j.ufug.2012.07.001.

Percival GC, Barrow I, Noviss K, Keary I, Pennington P. 2011. The impact of horse chestnut leaf miner (Cameraria ohridella Deschka and Dimic; HCLM) on vitality, growth and reproduction of Aesculus hippocastanum L. Urban Forestry & Urban Greening 10:11–17 DOI 10.1016/j.ufug.2010.11.003.

Pere C, Augustin S, Tomov R, Peng LH, Turlings TC, Kenis M. 2010. Species richness and abundance of native leaf miners are affected by the presence of the invasive horse-chestnut leaf miner. Biological Invasions 12:1011–1021 DOI 10.1007/s10530-009-9518-0.

R Development Core Team. 2013. R: a language and environment for statistical computing. Version 3.0.2. Vienna: The R Foundation for Statistical Computing. Available at http://www.R-project.org/.

Roy HE, Handley LJL, Schonrogge K, Poland RL, Purse BV. 2011. Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? BioControl 56:451–468 DOI 10.1007/s10526-011-9349-7.

Schonrogge K, Crawley MJ. 2000. Quantitative webs as a means of assessing the impact of alien insects. Journal of Animal Ecology 69:841–868 DOI 10.1046/j.1365-2656.2000.00443.x.
Sefrova H. 2001. Control possibility and additional information on the horse-chestnut leafminer *Cameraria ohridella* Deshka & Dimic (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 49:121–127.

Sefrova H. 2003. Invasions of Lithocolletinae species in Europe-causes, kinds, limits and ecological impact (Lepidoptera, Gracillariidae). *Ekologia* 22:132–142.

Sefrova H, Lastuvka Z. 2001. Dispersal of the horsechestnut leafminer, *Cameraria ohridella* Deschka & Dimic, 1986, in Europe: its course, ways and causes (Lepidoptera: Gracillariidae). *Entomologische Zeitschrift* 111:194–198.

Sivinski J, Pinero J, Aluja M. 2000. The distributions of parasitoids (Hymenoptera) of *Anastrepha* fruit flies (Diptera: Tephritidae) along an altitudinal gradient in Veracruz, Mexico. *Biological Control* 18:258–269.

Skillen EL, Pickering J, Sharkey MJ. 2000. Species richness of the Campopleginae and Ichneumoninae (Hymenoptera: Ichneumonidae) along a latitudinal gradient in eastern North American old-growth forests. *Environmental Entomology* 29:460–466 DOI 10.1603/0046-225X-29.3.460.

Tognelli MF, Kelt DA. 2004. Analysis of determinants of mammalian species richness in South America using spatial autoregressive models. *Ecography* 27:427–436 DOI 10.1111/j.0906-7590.2004.03732.x.

Toth P, Lukas J. 2005. Parasitic Ichneumonoidea on the horse chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Slovakia. *Journal of Pest Science* 78:151–154 DOI 10.1007/s10340-005-0086-5.

Valade R, Kenis M, Hernandez-Lopez A, Augustin S, Mari Men A, Magnoux E, Rougerie R, Lakatos F, Roques A, Lopez-Vaamonde C. 2009. Mitochondrial and microsatellite DNA markers reveal a Balkanic origin for the highly invasive Horse-Chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). *Molecular Ecology* 18:3458–3470 DOI 10.1111/j.1365-294X.2009.04290.x.

Wagner DL, Peacock J, Carter JL, Talley SE. 1996. Field assessment of *Bacillus thuringiensis* on nontarget Lepidoptera. *Environmental Entomology* 25:1444–1454 DOI 10.1093/ee/25.6.1444.

Walsh C, Mac Nally R. 2011. Package ‘hier.part’. Available at http://www.cran.r-project.org.

Zappala L, Bernardo U, Biondi A, Cocco A, Deliperi S, Delrio G, Giorgini M, Pedata P, Rapisarda C, Garzia GT, Siscaro G. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bulletin of Insectology* 65:51–61.

Zwölfer H, Pschorn-Walcher H. 1968. Wie verhalten sich Insektenparasiten gegenüber eingeschleppten, faunenfremden Wirten? *Anzeiger für Schädlingskunde* 41:51–55 DOI 10.1007/BF02150940.