The European Zoological Journal

ISSN: 2475-0263 (Online) Journal homepage: www.tandfonline.com/journals/tizo21

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To cite this article: M. Sielezniew, I. Sielezniew, C. Bystrowski, A. Gwiazdowska, J. Hilszczański, R. Rutkowski, K. Sapieszko, M. R. Shaw & T. Jaworski (2026) Natural enemies in the last Central European population of the Danube Clouded Yellow butterfly (*Colias myrmidone*), The European Zoological Journal, 93:1, 32-45, DOI: 10.1080/24750263.2025.2601368

To link to this article: https://doi.org/10.1080/24750263.2025.2601368

THE EUROPEAN ZOOLOGICAL JOURNAL
2026, VOL. 93, NO. 1, 32–45
https://doi.org/10.1080/24750263.2025.2601368

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Natural enemies in the last Central European population of the Danube Clouded Yellow butterfly (*Colias myrmidone*)

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ABSTRACT

The Danube Clouded Yellow (*Colias myrmidone*) is one of the most endangered butterfly species in Europe. Despite growing knowledge about habitat requirements and major threats, little is known about its natural enemies, particularly parasitoids. To fill this gap, we undertook field studies on the last Central European *C. myrmidone* population which inhabits the Knyszyn Forest (north-eastern Poland). Reared parasitoids were identified based on morphological features and were further DNA-barcoded. At the adult stage, we documented only four cases of predation, all caused by spiders: *Araneus quadratus*, *Argiope bruennichi* and *Misumena vatia*. Egg mortality was primarily due to parasitism by *Trichogramma evanescens* (Trichogrammatidae), a common parasitoid of Lepidoptera, which is also used for pest control. Surprisingly, larval parasitism was rare, with only a few individuals affected by *Cotesia ancilla* (Braconidae: Microgastrinae), a regular parasitoid of *Colias* species. Cases of pseudohyperparasitism by *Lysibia nanus* (Ichneumonidae: Phygadeuontinae) were recorded. Pupal mortality was considerably higher, and the most common was the solitary parasitoid *Brachymeria femorata* (Chalcididae: Chalcidinae). Additionally, we reared the gregarious *Blapsidotes vicinus* (Ichneumonidae: Phygadeuontinae). Both species are widespread and regular parasitoids of exposed butterfly pupae. Furthermore, we obtained *Hoplocryptus murarius* (Ichneumonidae: Cryptinae) from two chrysalises, which was unexpected due to its usual association with *Auplopus carbonarius* (Hymenoptera: Pompilidae). Aside from pupal parasitism, we recorded a single case of pupal predation by a *Polistes* wasp (Vespidae: Polistinae). These findings provide crucial insights for the conservation of *C. myrmidone*, highlighting potential risks posed by natural enemies, including those manipulated against crop pests.

ARTICLE HISTORY
Received 25 September 2025
Accepted 4 December 2025

KEYWORDS
Barcoding; *Brachymeria femorata*; *Cotesia ancilla*; parasitoid pressure; *Trichogramma evanescens*

Introduction

The Danube Clouded Yellow *Colias myrmidone* (Esper, 1780) exemplifies one of the most dramatic declines among European butterflies. A severe contraction of its Western Palearctic range has been documented, with numerous local extinctions reported from Germany, Czechia, Hungary, Austria and Slovenia (Marhoul & Dolek 2012). Within the European Union, recent confirmed records are limited to Poland and Romania (Loos et al. 2022; Sielezniew et al. 2024), whereas the species' status in Slovakia remains uncertain. Populations persist in Belarus, Ukraine and Russia, although up-to-date and comprehensive data from these regions are lacking. According to the weighted Red List index, *C. myrmidone* ranks among the five most endangered butterfly species in Europe (Maes et al. 2019). Within the EU, it is classified as Critically Endangered (CR) (Van Swaay et al. 2010). Furthermore, it is designated a priority species for conservation under Annexes II and IV of the Habitats Directive.

The Danube Clouded Yellow is a xerothermophilous species associated with mosaic forest–steppe habitats, particularly dry grasslands on sun-exposed slopes, often adjacent to forests and/or interspersed with scattered trees and shrubs. Habitat suitability is primarily determined by the presence of abundant larval food plants, i.e. several species of *Chamaecytisus* Link (Marhoul & Dolek 2012; Sielezniew et al. 2019;

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Nippen et al. 2021). Recent studies have highlighted the requirement for relatively extensive and well-connected habitat patches to sustain viable populations (Sielezniew et al. 2024). The species is vulnerable to genetic impoverishment and heterogeneous *Wolbachia* infection, which may restrict gene flow due to cytoplasmatic incompatibility of infected and uninfected individuals (Gwiazdowska et al. 2025). Local population declines have also been linked to abiotic factors (Sielezniew et al. 2024), and *C. myrmidone* is considered highly vulnerable to the effects of climate change (Settele et al. 2008).

Grazing has been identified as a potential factor contributing to reduced survival of *C. myrmidone* eggs and larvae (Nippen et al. 2021). However, knowledge of the species' natural enemies remains extremely limited, particularly regarding parasitoids, which in many Lepidoptera can substantially reduce the survival of immature stages and drive pronounced fluctuations in population size (Razmi et al. 2011). Among butterflies, comprehensive data on parasitoid assemblages are scarce and typically limited to pests or a few well-studied taxa, including some of conservation concern (Shaw et al. 2009).

Identifying the complete spectrum of parasitoids associated with a given host is methodologically challenging due to highly variable and sometimes narrow windows of parasitism. Detectability also differs across different developmental stages: larvae are generally more accessible for sampling, and molecular tools have proven valuable for both identifying larval parasitoid taxa and quantifying parasitism rates (Konvičková et al. 2024). In contrast, eggs and pupae are considerably more difficult to locate and study (Mills 2005; Shaw et al. 2009). Nevertheless, intensive research has shown that some butterfly species experience high egg parasitism (Stefanescu et al. 2003), whereas in others this stage appears largely unaffected (Stefanescu et al. 2009).

Closely related butterfly species may share parasitoids, yet the composition of natural enemy assemblages often varies geographically and seasonally (Shaw et al. 2009; Stefanescu et al. 2009, 2012; Audusseau et al. 2021; Colom et al. 2022). These dynamics are further shaped by bottom-up effects, as host plant variation can significantly modulate parasitism dynamics (Lill et al. 2002). Furthermore, parasitism rates are frequently attenuated in recently colonized areas (Menéndez et al. 2008; Nakabayashi & Ohshima 2024), whereas the establishment of novel parasitoids in a region has been implicated in the decline of some butterfly species (Gripenberg et al. 2011). Importantly, highly specialized parasitoids of threatened butterflies may face an even greater extinction risk than their hosts, and yet they remain largely neglected in conservation strategies (Shaw & Hochberg 2001; Klapwijk & Lewis 2014; Dziekańska et al. 2020).

In this study, we investigated parasitoids and other natural enemies affecting the last remaining Central European population of *C. myrmidone* in the Knyszyn Forest, north-eastern Poland. Historically, the species was recorded across nearly half of the country's territory, but a gradual decline began in the mid-twentieth century, followed by a rapid population collapse around the turn of the millennium (Sielezniew et al. 2019, 2024). A thorough understanding the limiting factors, including the role of parasitoids, is therefore essential for developing effective conservation strategies.

Materials and methods

Life history of Colias myrmidone

The Danube Clouded Yellow (*Colias myrmidone*) is a moderately large pierid butterfly (Lepidoptera: Pieridae) with a wingspan of about 40–46 mm. Depending on locality and season, two or three generations may occur annually, with adults on the wing from May to September. While adults are relatively opportunistic in their use of nectar sources, larvae feed exclusively on a limited number of *Chamaecytisus* species. Females oviposit singly on the upper side of leaves of the sun-exposed plants. Eggs are initially pale yellow, turning orange during development. Newly emerged larvae feed by creating characteristic window-like traces, whereas later instars consume entire leaves, leaving stems partially defoliated – a damage pattern that may resemble that of other lepidopteran larvae. For pupation, *C. myrmidone* larvae remain near feeding sites, attaching themselves head-up to nearby shoots or occasionally to other adjacent plant species, usually at heights up to 20 cm. Both larvae and pupae are well-camouflaged. Development of summer and autumn generations takes approximately 6–7 weeks. In contrast, the spring generation overwinters as medium-sized larvae among fallen leaves of the host plant, undergoing seasonal colour change to brown (Marhoul & Dolek 2012; Nippen et al. 2021; Sielezniew et al. 2024 and unpublished data).

Study site

The study was conducted in the eastern part of the Knyszyn Forest, a large woodland complex in north-eastern Poland. Habitat patches occupied by *C. myrmidone* covers over 50 hectares clear-cuts and young tree plantations (Figure 1) composed predominantly of Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*), and less frequently of pedunculate oak (*Quercus robur*), lime (*Tilia* spp.) and European larch (*Larix decidua*). All sites are situated on former agricultural land, particularly on extensive pastures afforested 40–100 years ago. *Chamaecytisus ruthenicus*, along with various herbaceous species typical of dry grasslands, occurred in these areas prior to afforestation and has persisted in the resulting first-generation forests (Sielezniew et al. 2024).

Data collection

No targeted studies have been conducted on the natural enemies of *C. myrmidone* adults. Observations presented here were made in the course of an intensive mark–release–recapture (MRR) survey on adults carried out between 2022 and 2024. All generations were included: two generations in each of the first two years and three in the final year.

Observations of eggs were conducted in 2022 during the second (summer) generation. Ovipositing females were followed, and newly laid eggs were marked by tying a coloured ribbon around the plant shoot approximately 5 cm below the leaf bearing the egg. A total of 79 marked eggs were examined every 3–4 days until hatching or until they turned blackish in colour. Such eggs were collected along with the leaf and placed into individual 200-ml closed plastic containers, where they were kept under observation. Additional egg observations, primarily non-quantitative in nature, were carried out across all generations between 2022 and 2024.

To assess larval parasitism, a subset of 71 caterpillars were either monitored in the field or collected for captive rearing. Additionally, a total of 24 pupae were observed in situ from the onset of pupation until adult emergence or until signs of abnormal pigmentation appeared. Pupae exhibiting atypical colouration were collected and maintained under laboratory conditions until the emergence of parasitoids or confirmation of mortality.

In cases of premature mortality, most efforts were directed towards parasitoid identification, while predation observations were incidental. Given these limitations, it was not possible to assess the actual pressure exerted by the latter category of natural enemies. No statistical analyses were possible owing to the small sample size, which permitted only a qualitative assessment.

Figure 1. Example of *Colias myrmidone* habitat in the Knyszyn Forest.

Barcoding

Freshly reared parasitoid specimens were used as the source of biological material for genetic analyses. DNA was extracted with the GeneMATRIX Bio-Trace DNA Purification Kit (EURx). A single leg from each specimen was cut into small pieces, finely minced and incubated overnight at 56°C in lysis buffer containing Proteinase K. For very small taxa, such as *Trichogramma*, the entire insect was processed. Subsequent extraction followed the manufacturer's standard tissue protocol, and DNA extracts were stored at −20°C until further use.

Fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene were amplified using the primers LCO1490 (forward) and HCO2198 (reverse) (Folmer et al. 1994). Polymerase chain reactions (PCR) were performed in 25-µl volumes containing 12.5 µl GoTaq G2 Hot Start Green Master Mix (Promega), 1 µl of each primer, 8.5 µl nuclease-free water and 2 µl of DNA template. Thermal cycling consisted of an initial denaturation at 94°C for 7 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for 2 min, with a final extension step of 7 min at 72°C.

Amplification products were verified on 1.5% agarose gels (EURx, molecular-biology grade). Samples yielding a single band of the expected size were purified using ExoSAP-IT (Applied Biosystems, Waltham, MA, USA). Cycle sequencing was performed with the BigDye Terminator v3.1 kit (Applied Biosystems), and the products were purified using HighPrep DTR (MAGBIO) before capillary electrophoresis on an ABI 3500XL Genetic Analyser. Chromatograms were inspected and aligned in BioEdit v7.2.5 (Hall 1999). Resulting COI haplotypes were compared with reference sequences in GenBank and BOLD using BLAST and Barcode ID tools. Finally, the identified haplotypes were deposited in the GenBank database under specific accession numbers (see Results).

Results

Adults

During three years of intensive fieldwork, over 1800 adults were marked (Sielezniew et al. 2024 and unpublished data), yet only four cases of predation were documented. All involved spiders: *Araneus quadratus* (Clerck, 1757), *Argiope bruennichi* (Scopoli, 1772) and *Misumena vatia* (Clerck, 1757) (two cases) (Figure 2).

Eggs

In 2022, of the marked eggs ($n = 79$), 43.0% turned black and were subsequently confirmed as parasitized by *Trichogramma evanescens* Westwood, 1833 (Chalcidoidea: Trichogrammatidae) (Figure 3). Between one and six parasitoid individuals emerged from each egg. Only 10 eggs (12.7%) hatched successfully, while 35 eggs disappeared. Parasitism appeared to occur at various stages of egg development, as blackening was observed both in freshly laid (yellow–greenish) and more advanced (orange) eggs.

Figure 2. *Colias myrmidone* males captured by *Argiope bruennichi* (left) and *Misumena vatia*.

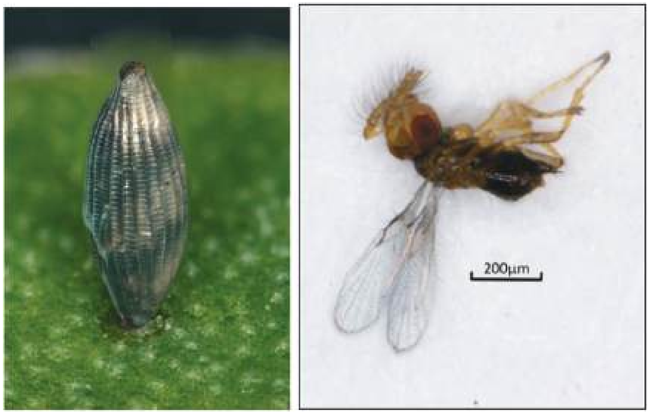

Figure 3. *Colias myrmidone* egg parasitized by *Trichogramma evanescens* (Trichogrammatidae) (left) and an adult wasp reared.

Parasitized eggs were recorded in 18 of the 26 habitat patches of *C. myrmidone* where adult population ecology studies were conducted in 2022. In addition, *Trichogramma* parasitism was observed across all generations of *C. myrmidone* in each year of the study. For the third generation in 2024, field-monitored eggs ($n = 20$) showed an estimated parasitism-induced mortality of 75%.

Later in the season, in all three years of study, heavy damage to *Chamaecytisus* plants was observed, caused by the weevil *Sitona* (*Charagmus*) *gressorius* (Fabricius, 1792) (Coleoptera: Curculionidae) (Figure 4). This herbivory probably contributed to egg or early larval mortality of *C. myrmidone*, either directly through destruction at oviposition sites or indirectly by reducing available foliage.

Caterpillars and pupae

During the summer generation of 2022, 45 larvae were monitored. Of these, 15 (33.3%) disappeared from their host plants; however, it was not possible to determine whether their loss was due to predation or other causes. The remaining individuals successfully pupated, primarily on the larval host plants or on nearby vegetation within 20 cm of the last feeding site. In six cases, no butterfly emerged from the pupa. These atypical pupae were collected and maintained under laboratory conditions. From one of them, an individual of *Brachymeria femorata* (Panzer, 1801) (Chalcidoidea: Chalcididae: Chalcidinae) emerged through a characteristic gnawed hole (Figure 5). The same parasitoid species was simultaneously reared from a *Gonepteryx rhamni* (Linnaeus, 1758) pupa collected in the same habitat patch. The remaining abnormal pupae of *C. myrmidone* died for unknown reasons, conceivably including host-feeding by adult *B. femorata*.

Figure 4. *Colias myrmidone* eggs (left) on *Chamaecytisus ruthenicus* leaves heavily damaged by the lupin weevil *Sitona gressorius*, illustrating the potential negative impact on the butterfly.

Figure 5. An individual of *Brachymeria femorata* reared from a *Colias myrmidone* pupa.

In 2023, 15 caterpillars were monitored, of which eight disappeared – probably falling victim to insectivores – while the remaining individuals pupated low on the host plant or in its immediate vicinity. Additionally, one more pupa was discovered in the field. Of the eight pupae observed, only one successfully developed into an adult, as confirmed by the presence of exuvium. Six pupae exhibited discoloration (Figure 6) not typical for the species. In two cases, these changes were observed in more advanced pupae, with visible orange – but never black – pigmentation of the developing wings. Five of the discoloured pupae were collected for laboratory observation. From four of them, single individuals of *B. femorata* were reared; one pupa died from unknown causes. In one field case, a hole was observed in the cuticle of the pupa, probably resulting from an insectivore attack. A larva was visible inside that pupa, presumably also belonging to *B. femorata*, although no adult emerged to confirm this.

In 2024, prior to the emergence of the second generation, 16 pupae were located in the field on host plants and monitored for external changes. Ten pupae exhibiting abnormal discoloration were collected and maintained under laboratory conditions. Of these, six were parasitized by *B. femorata*, with adult wasps emerging between 22 July and 2 August. Additionally, single individuals of *Hoplocryptus murarius* (Börner, 1782) (Ichneumonidae: Cryptinae) (Figure 7) emerged from two pupae, while 10 individuals of *Blapsidotes vicinus* Gravenhorst, 1829 (Ichneumonidae: Phygadeuontinae) (Figure 8) emerged through a single hole from one pupa. Based on these results, pupal parasitoids were estimated to cause mortality of at least 56.25% of the pupae. One pupa died from unknown causes. Of the six pupae left in the field, two disappeared, while the remaining four successfully developed into adults, indicating a survival rate of 25% for the pupal stage of the summer generation in 2024.

The only clearly documented case of predation involved a direct attack on a pupa by a *Polistes* sp. (Vespidae: Polistinae) wasp. The wasp tore off part of the pupa and flew away with it (Figure 9).

Parasitism among larvae was rare and observed only in four of 10 individuals during the atypical third generation in 2024. From these cases, we reared exclusively the gregarious *Cotesia ancilla* (Nixon, 1974) (Braconidae: Microgastrinae) (Figures 10 and 11). Instances of pseudohyperparasitism by *Lysibia nanus* (Gravenhorst, 1829) (Ichneumonidae: Phygadeuontinae) (Figure 11) were also recorded from *C. ancilla* cocoon batches collected on larval food plants in the field. In spring 2024, when the adult population of the first generation was exceptionally low, only three post-overwintering caterpillars were located, and two of them were parasitized by *C. ancilla*.

Tachinid flies also appear to be potential parasitoids of *C. myrmidone*. In one case, a dipteran egg was observed attached to a larva (Figure 10); however, *Cotesia* individuals ultimately emerged, precluding

Figure 6. Examples of *Colias myrmidone* pupae exhibiting abnormal colouration due to parasitism by *Brachymeria femorata*.

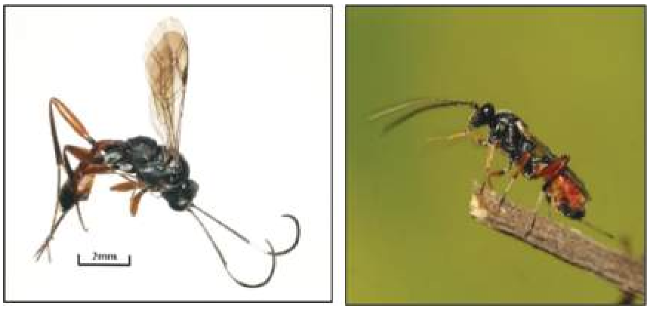

Figure 7. *Hoplocryptus murarius* (Ichneumonidae: Cryptinae) reared from a *Colias myrmidone* pupa.

Figure 8. Ten *Blapsidotes vicinus* (Ichneumonidae: Phygadeuontinae) individuals reared from a single *Colias myrmidone* pupa.

Figure 9. A single case of pupal predation by a *Polistes* wasp (left) and an example of pupal destruction close to adult emergence by an unknown predator.

identification of the fly species. In another larva found in 2022, multiple eggs – presumably of dipteran origin – were present (Figure 12). This larva exhibited arrested development, a response sometimes associated with excess parasitoid infection, and did not survive to later rearing stages.

Genetic analysis

Six parasitoid specimens were subjected to genetic analysis through amplification of the standard DNA 'barcode' gene. Successful amplification was achieved for all six individuals, and complete nucleotide sequences were obtained. Comparison of these sequences with genetic databases (GenBank and BOLD) indicated the presence of six species, with morphological identifications

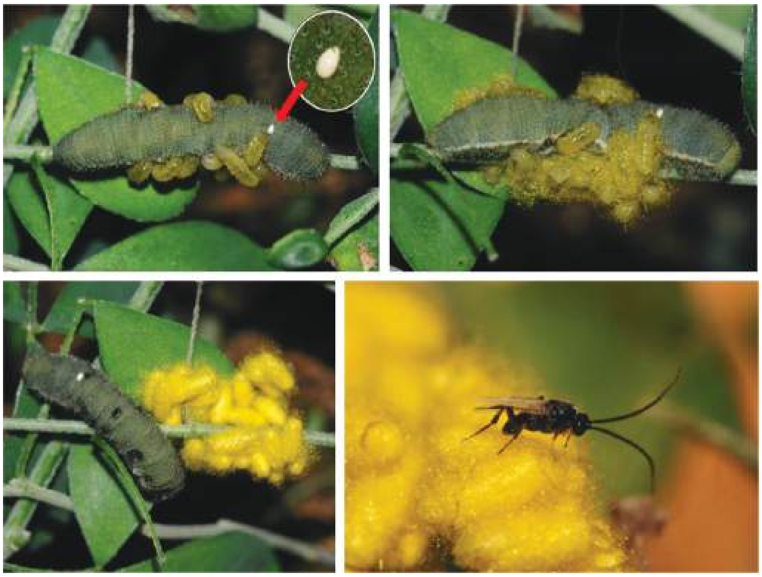

Figure 10. Larvae of *Cotesia ancilla* emerging from a *Colias myrmidone* caterpillar (upper left) and spinning cocoons (upper right); cocoons of *C. ancilla* (bottom left) and a newly emerged adult (bottom right). The red arrow shows an egg of an unidentified Tachinidae fly.

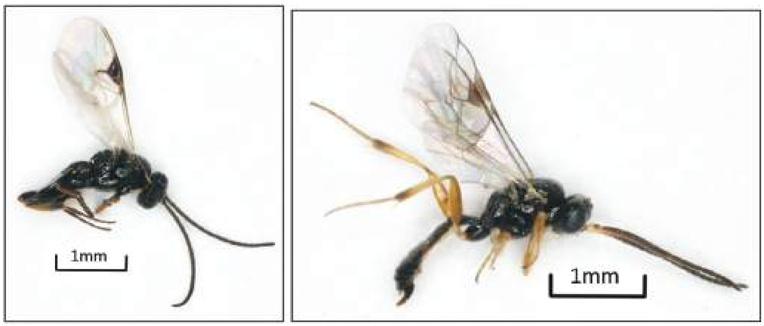

Figure 11. *Cotesia ancilla* and its solitary pseudohyperparasitoid *Lysibia nanus* (both images are of males).

Figure 12. *Colias myrmidone* caterpillar with visible eggs of an unidentified, probably dipteran, parasitoid.

consistent with the molecular results. Specifically, sequence comparisons revealed the presence of *Brachymeria femorata* (reference sequence from GenBank: Acc. no. KT175580.1; the identified haplotype Acc. no.: PX568623), *Hoplocryptus murarius* (reference sequence from BOLD no.: FBICA196-11; the identified haplotype Acc. no.: PX583445), *Trichogramma evanescens* (reference sequence from GenBank: Acc. no. MW722248.1 and BOLD no.: GBMNA19704-19; the identified haplotype Acc. no.:

PX568647), *Lysibia nanus* (reference sequence from GenBank: Acc. no. MG340558.1 and/or BOLD no.: DTNHM12280-24; the identified haplotype Acc. no.: PX568597), *Blapsidotes vicinus* (reference sequence from BOLD no.: FBICA206-11; the identified haplotype Acc. no.: PX583447), and *Cotesia ancilla* (reference sequence from BOLD no.: ASQSR274-11; the identified haplotype Acc. no.: PX583446), with all perfect matches in both BLAST and/or BOLD searches.

Discussion

Despite intensive field surveys performed in the present study, predation on adult *C. myrmidone* individuals was rarely observed and exclusively involved spiders. The recorded spider species are opportunistic generalist predators, lacking any notable specialization for butterfly predation (Pekár & Toft 2015; Michalko et al. 2019). *Misumena vatia*, an ambush predator associated with flowers, does not construct webs (Morse & Fritz 1982). *Araneus quadratus* and *Argiope bruennichi* are common orb-weavers, with the latter having expanded its range rapidly in recent decades and is now widespread across Europe (Wawer & Hajdamowicz 2018). Although spider abundance was not quantified, they appeared generally uncommon across seasons, which may account for the limited number of predation events recorded on the focal species.

The European mantis (*Mantis religiosa*) is another potential predator that has recently extended its distribution (Żurawlew et al. 2022) and is at the moment frequently encountered in the Knyszyn Forest; however, no predation on *C. myrmidone* has been observed to date. Using the methods applied, it was not possible to quantify actual predation pressure from *M. religiosa*, which is likely higher. Predation by invertebrates often leaves easily detected traces (vespids are an obvious exception), while vespids and vertebrate predators are likely to remove the prey. Consequently there are good reasons to believe that the rate of adult predation would have been underestimated, perhaps greatly, by our survey methods. Predators can significantly influence butterfly assemblages, as demonstrated in dragonfly studies conducted in wetter habitats (Tiitsaar et al. 2013).

Among natural enemies, parasitoids generally exhibit a higher degree of host specialization compared to predators. In this study, parasitoids were reared from all developmental (i.e. excluding adult) stages of *C. myrmidone*, with the egg parasitoid *T. evanescens* emerging as the most significant mortality factor. This species is considered relatively polyphagous, having been recorded from a range of unrelated Lepidoptera, including micromoths such as *Ostrinia nubilalis* (Hübner, 1796) (Crambidae) (Ivezić et al. 2021) and *Eupoecilia ambiguella* (Hübner, 1796) (Tortricidae) (Ricciardi et al. 2024). Intraspecific variation in host preference among *T. evanescens* strains has also been documented (Dijken et al. 1986). *Trichogramma* species are generally regarded as more habitat-specific than host-specific, with environmental and plant-related factors influencing various steps of the host selection process in females, thereby affecting parasitism rates (Romeis et al. 2005). Due to their parasitic efficiency, *Trichogramma* wasps are widely used as biological control agents against lepidopteran pests (e.g. Herz et al. 2007; Zang et al. 2021; El-Gepaly et al. 2024). Specifically, *T. evanescens* has been employed in the biological control of *Pieris rapae* (Linnaeus, 1758) (Pieridae) (Siam & El-Kholy 2023). Application of parasitoids like *Trichogramma* is usually regarded as desirable in comparison to chemical plant protection, but it may pose a risk to biodiversity (Ivezić & Trudić 2021).

Trichogramma wasps are known often to parasitize freshly laid insect eggs (Herz et al. 2007); however, the host is arrested at the time of attack and, as idiobionts, they can be flexible (Shaw et al. 2009). In our study we observed that even advanced-stage (orange-coloured) *C. myrmidone* eggs produced this parasitoid. Due to their minute size, *Trichogramma* wasps possess limited flight capabilities and rely on a combination of short- and long-range cues to locate hosts (Romeis et al. 2005). One of the best-known examples involves *T. brassicae* Bezdenko, 1968, in which females are attracted by anti-aphrodisiac compounds transferred from male to female *Pieris brassicae* (Linnaeus, 1758) during mating. The wasps use this chemical cue to hitchhike on mated butterfly females and disembark during oviposition to parasitize the newly laid eggs deposited in clusters (Fatouros et al. 2005). However, such phoretic behaviour is unlikely in *C. myrmidone*, which lays eggs singly. Nevertheless, *Trichogramma* wasps are capable of associative learning and can use visual, host-related cues to enhance host location and parasitism efficiency (Keasar et al. 2001).

In maize crops, *O. nubilalis* is typically controlled using *T. brassicae*, but *T. evanescens* naturally occurs in Eastern European maize fields, with egg parasitism rates reaching up to 70% (Grushevaya 2020). This implies that maize expansion alone, even without supplemental *Trichogramma* releases, could increase threats to endangered species. Urgent research is needed, especially given evidence that *Trichogramma* readily disperses from crop fields to non-target habitats (Kuske et al. 2003).

The most specialized natural enemy recorded in our study was the larval parasitoid *C. ancilla*, which has so far been reared exclusively from species of the genus *Colias*, including *C. palaeno* (Linnaeus, 1761), *C. chrysotheme* (Esper, 1781), *C. hyale* (Linnaeus, 1758), *C. alfacariensis* Ribbe, 1905, *C. croceus* (Geoffroy, 1785) (Shaw et al. 2009) and *C. phicomone* (Esper, 1780) (sequenced specimens in NMS; recorded in BOLD). Many *Cotesia* species are known for their high host specificity, and such specialization may even drive cryptic speciation (Kankare et al. 2005). Females of *C. ancilla* oviposit tens of eggs into early larval instars of their hosts. The developing gregarious *Cotesia* larvae feed primarily on host haemolymph and fat bodies (Harvey et al. 2009), eventually emerging from the host, often during its final instar, and pupating in characteristically spun cocoons. Interestingly, *C. ancilla* was recorded only during the final year of our study and solely in the unusual third generation of *C. myrmidone*, which may indicate a recent colonization event. Alternatively, as shown in long-term studies on widespread butterfly species, parasitism rates may gradually increase over the season, potentially exerting selective pressure against the occurrence of an additional late-summer host generation (Stefanescu et al. 2022).

It is also worth noting that the only other potential host species co-occurring with *C. myrmidone* was *C. hyale*, which had increased its abundance in relation to *C. myrmidone*. Studies on another two pierids elsewhere, *Gonepteryx cleopatra* (Linnaeus, 1767) and *G. rhamni*, have demonstrated that parasitoids can be shared among closely related butterfly species (Colom et al. 2022). Notably, in 2025, when only three larvae of *C. myrmidone* were found – two of which were parasitized by *C. ancilla* – the adult population size was extremely low and *C. hyale* was completely absent from the study sites. A high rate of *Cotesia* parasitism, in addition to unfavourable weather conditions (in particular, an unusually cold May), may explain the collapse of both populations.

During our study, we also recorded the presence of *Lysibia nanus*, a solitary pseudohyperparasitoid that targets newly cocooned pre-pupae and pupae of (especially gregarious) braconid wasps belonging to the subfamily Microgastrinae (Schwarz & Shaw 2000). Although solitary with respect to each microgastrine cocoon in the brood, usually most or all of the cocoons of gregarious species will be attacked. Adult females use herbivore-induced plant volatiles to locate their parasitoid host; these volatiles are emitted by plants in response to herbivory by caterpillars that have themselves been parasitized by primary parasitoids (Poelman et al. 2012). *Lysibia nanus* injects a permanently paralysing venom into the host before laying a single egg on the immobilized body. The emerging larva initially consumes host haemolymph but, as it grows, it transitions to feeding on other tissues, ultimately consuming the host entirely. *L. nanus* demonstrates exceptional efficiency in resource utilization; adults emerging from *Cotesia glomerata* (Linnaeus, 1758) cocoons exhibit body masses comparable to those of their unparasitized hosts (Harvey et al. 2009).

We were unable to confirm the identity of the only potential dipteran parasitoid of *C. myrmidone* recorded during this study. Although parasitism by *C. ancilla* was observed concurrently, only this braconid species was successfully reared. According to Tschorsnig (2017), eight tachinid species have been documented as parasitoids of *Colias* butterflies: *Exorista japonica* (Townsend, 1909) (*C. erate*), *E. larvarum* (Linnaeus, 1758) (*C. croceus*), *Phryxe nemea* (Meigen, 1824) (*C. palaeno*), *P. vulgaris* (Fallén, 1810) (*C. erate*), *Bactomyia aurulenta* (Meigen, 1824) (*C. erate*), *Pales pavida* (Meigen, 1824) (*C. croceus*), *Aplomyia confinis* (Fallén, 1820) (*C. erate* and *C. hyale/C. crocea*) and *Pseudoperichaeta nigrolineata* (Walker, 1853) (*C. erate*). Considering ecological factors and egg placement, one or more of the three latter species appear to be the most plausible candidates in our case.

In contrast to low levels of larval parasitism, pupal mortality was particularly high, with three species of idiobiont parasitoids recorded. We estimated that pupal parasitoids could account for up to 70% mortality of *C. myrmidone* pupae. The most frequent was *B. femorata*, a solitary parasitoid of exposed Lepidoptera pupae, previously reported to parasitize several butterfly species, including *Pieris brassicae*, *Melitaea didyma* (Esper, 1778), *M. deione* (Geyer, 1832) and *Maniola jurtina* (Linnaeus, 1758) (Shaw et al. 2009). While *Brachymeria* females are suspected to preferentially attack freshly formed pupae, our observations suggest that more advanced stages of development can also be parasitized, as *B. femorata* emerged also from pupae in which butterfly pigmentation was already visible. The gregarious *Blapsidotes vicinus* was also identified as a pupal parasitoid: this species is a widespread and regular parasitoid of exposed butterfly pupae, with known hosts including *Pieris rapae*, *Vanessa atalanta* (Linnaeus, 1758), *Polygonia c-album* (Linnaeus, 1758) and *Euphydryas desfontainii* (Godart, 1819) (Shaw et al. 2009).

The most surprising observation in our study was the emergence of *Hoplocryptus murarius* from two *C. myrmidone* pupae. Although host data for this species are scarce, it is generally considered a parasitoid of *Auplopus carbonarius* (Scopoli, 1763) (Hymenoptera: Pompilidae). Larvae of *A. carbonarius* feed on paralysed spiders, and *H. murarius* parasitizes the barrel-like brood cells created from small mud pellets by the host females. Hosts of other European *Hoplocryptus* are thought to be solitary, aboveground-nesting aculeate Hymenoptera (Apidae, Crabronidae, Vespidae), including their own parasitoids (Chrysididae and Gasteruptiidae), often nesting in stems of plants such as *Rubus* and *Rosa*. Behavioural cues probably involve oviposition through plant tissue to reach the host. Nearctic representatives of the genus have been reported to parasitize other insects developing in stems (Hymenoptera: Cephidae; Coleoptera: Cerambycidae; Lepidoptera: Crambidae) (Schwarz 2007). Given the general ecology of *Hoplocryptus* wasps, it cannot be excluded that in our study *H. murarius* acted as a hyperparasitoid, ovipositing on *B. femorata* larvae developing inside *C. myrmidone* pupae, which provided the necessary enclosed structure. The typical host, i.e. *A. carbonarius*, is widespread and not habitat-specific in Poland, and it is recorded in the same region (Wiśniowski 2009). Therefore, the observed association could just represent an accidental interaction.

We recorded a single case of predation by a *Polistes* wasp on *C. myrmidone* pupa. *Polistes* social wasps are major generalist predators, with lepidopteran caterpillars being among their preferred prey, which they capture to feed their carnivorous larvae. These wasps are considered valuable biological control agents against pests such as *Pieris* spp. on cabbage (Rayor et al. 2007), but they are also significant natural enemies impacting monarch butterfly (*Danaus plexippus* (Linnaeus, 1758)) populations (Rayor 2004; Baker & Potter 2020). The disappearance of *C. myrmidone* larvae – noted by Nippen et al. (2021) – and pupae could be also attributed to predation by vespids, although other factors, such as bird predation, cannot be ruled out.

Eggs and larvae can also be accidentally destroyed by folivorous insects. In the case of *C. myrmidone* in the Knyszyn Forest, a potential threat is the large lupin beetle *Sitona* (*Charagmus*) *gressorius* (Curculionidae), which is currently expanding its range in Poland (Wiater 2004). *Chamaecytisus ruthenicus* appears to be one of its preferred food plants. We observed heavy foliage damage on some plants; however, further studies are needed to assess the impact of this beetle on the premature stages of *C. myrmidone*.

Conclusions

Our study demonstrates that parasitoids can constitute a substantial mortality factor in *Colias myrmidone* populations. Given the absence of previous data, all parasitoid records reported here constitute new natural enemy records for this species. An open question remains regarding the stability of the parasitoid assemblage, particularly whether any taxa have recently colonized the study area. Notably, no egg or larval parasitoids have been reported from Romania, where field studies on immature survival of *C. myrmidone* have been conducted (Nippen et al. 2021). Nevertheless, it is evident that natural enemies may pose a significant risk, especially when populations are simultaneously subjected to habitat degradation, fragmentation and climate change. Parasitoids are recognized as key drivers of Lepidoptera population dynamics (Berryman 1996); however, it remains unclear whether all parasitoid species observed in the Knyszyn Forest are of natural origin. The widespread use of *Trichogramma* wasps in biological control, particularly against *O. nubilalis* in maize (Bzowska-Bakalarz et al. 2020), and the historical expansion of maize cultivation in Poland – including the study region – may have strongly influenced the abundance of the most important parasitoid observed in our study. Potential conflicts between preserving biodiversity and successful pest control are often recognized in theory, but in the case of *C. myrmidone* and *T. evanescens*, known to have a potentially wide host repertoire, the need for specific research seems urgent.

Acknowledgments

The Regional Director for Environmental Protection in Białystok issued relevant permissions for sampling *Colias myrmidone prematures*. We thank three anonymous reviewers for valuable comments on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This article has received financial support from the General Directorate of the Polish State Forests under a project entitled ″Forest management for endangered species: conservation of the Danube Clouded Yellow (Colias myrmidone) in the Knyszyn Forest" (contract no. EZ.271.3.7.2021).

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References

Audusseau H, Ryrholm N, Stefanescu C, Tharel S, Jansson C, Champeaux L, Shaw MR, Raper C, Lewis OT, Niklas Janz N, Schmucki R. 2021. Rewiring of interactions in a changing environment: Nettle-feeding butterflies and their parasitoids. Oikos 130(4):624–636. DOI: 10.1111/oik.07953.

Baker AM, Potter DA. 2020. Invasive paper wasp turns urban pollinator gardens into ecological traps for monarch butterfly larvae. Scientific Reports 10(1):9553. DOI: 10.1038/s41598-020-66621-6.

Berryman AA. 1996. What causes population cycles of forest Lepidoptera? Trends in Ecology & Evolution 11(1):28–32. DOI: 10.1016/0169-5347(96)81066-4.

Bzowska-Bakalarz M, Bulak P, Bereś PK, Czarnigowska A, Czarnigowski J, Karamon B, Pniak M, Bieganowski A. 2020. Using gyroplane for application of *Trichogramma* spp. against the European corn borer in maize. Pest Management Science 76(6):2243–2250. DOI: 10.1002/ps.5762.

Colom P, Traveset A, Shaw MR, Stefanescu C. 2022. Spatial variation in relative abundances of two butterfly species sharing both host plant and natural enemies. BioRxiv 11.23.517710. DOI:10.1101/2022.11.23.517710.

Dijken MJV, Kole M, Lenteren JCV, Brand AM. 1986. Host-preference studies with *Trichogramma evanescens* Westwood (Hym., Trichogrammatidae) for *Mamestra brassicae*, *Pieris brassicae* and *Pieris rapae*. Journal of Applied Entomology 101(15):64–85. DOI: 10.1111/j.1439-0418.1986.tb00834.x.

Dziekańska I, Nowicki P, Pirożnikow E, Sielezniew M. 2020. A unique population in a unique area: The Alcon Blue butterfly and its specific parasitoid in the Białowieża Forest. Insects 11(10):687. DOI: 10.3390/insects11100687.

El-Gepaly HM, Abdelhameed KM, Shakl SY, Saleh AA, Hafez EE. 2024. Performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) eggs at laboratory and field conditions. Scientific Reports 14(1):26831. DOI: 10.1038/s41598-024-77347-0.

Fatouros NE, Huigens ME, Van Loon JJA, Dicke M, Hilker M. 2005. Chemical communication: Butterfly anti-aphrodisiac lures parasitic wasps. Nature 433(7027):704. DOI: 10.1038/433704a.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5):294–299. DOI: 10.1371/journal.pone.0013102.

Gripenberg S, Hamer N, Brereton T, Roy DB, Lewis OT. 2011. A novel parasitoid and a declining butterfly: Cause or coincidence? Ecological Entomology 36(3):271–281. DOI: 10.1111/j.1365-2311.2011.01269.x.

Grushevaya I 2020. Prevalence rates of *Trichogramma evanescens* (Westwood) in *Ostrinia nubilalis* (Hübner) population in the south of Russia in 2013-2018. BIO Web Conferences 18:00012. DOI: 10.1051/bioconf/20201800012.

Gwiazdowska A, Rutkowski R, Sielezniew M. 2025. Conservation genetics of the endangered Danube Clouded Yellow butterfly *Colias myrmidone* (Esper, 1780) in the last Central European stronghold: Diversity, *Wolbachia* infection and Balkan connections. Insects 16(2):220. DOI: 10.3390/insects16020220.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Harvey JA, Wagenaar R, Bezemer TM. 2009. Interactions to the fifth trophic level: Secondary and tertiary parasitoid wasps show extraordinary efficiency in utilizing host resources. Journal of Animal Ecology 78(3):686–692. DOI: 10.1111/j.1365-2656.2008.01516.x.

Herz A, Hassan SA, Hegazi E, Khafagi WE, Nasr FN, Youssef AI, Agamy E, Blibech I, Ksentini I, Ksantini M, Jardak T, Bento A, Pereira JA, Torres L, Souliotis C, Moschos T, Milonas P. 2007. Egg parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) in olive groves of the Mediterranean region. Biological Control 40(1):48–56. DOI: 10.1016/j.biocontrol.2006.08.002.

Ivezić A, Rugman-Jones P, Malausa T, Ris N, Ignjatović-Ćupina A. 2021. Molecular identification of *Trichogramma* species parasitizing *Ostrinia nubilalis* in corn and pepper in south–east border of Europe. International Journal of Pest Management 67(4):346–357. DOI: 10.1080/09670874.2020.1779383.

Ivezić A, Trudić B. 2021. Parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), natural enemies of European corn borer *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae). Journal of Central European Agriculture 22(4):787–797. DOI: 10.5513/JCEA01/22.4.3247.

Kankare M, Stefanescu C, Van Nouhuys S, Shaw MR. 2005. Host specialization by *Cotesia* wasps (Hymenoptera: Braconidae) parasitizing species-rich Melitaeini (Lepidoptera: Nymphalidae) communities in north-eastern Spain. Biological Journal of the Linnean Society 86(1):45–65. DOI: 10.1111/j.1095-8312.2005.00523.x.

Keasar T, Ney-Nifle M, Mangel M, Swezey S. 2001. Early oviposition experience affects patch residence time in a foraging parasitoid. Entomologia Experimentalis et Applicata 98(2):123–132. DOI: 10.1046/j.1570-7458.2001.00766.x.

Klapwijk MJ, Lewis OT. 2014. Spatial ecology of host–parasitoid interactions: A threatened butterfly and its specialised parasitoid. Journal of Insect Conservation 18(3):437–445. DOI: 10.1007/s10841-014-9653-5.

Konvičková H, John V, Konvička M, Rindoš M, Hrček J. 2024. High hymenopteran parasitoid infestation rates in Czech populations of the *Euphydryas aurinia* butterfly inferred using a new molecular marker. Journal of Hymenoptera Research 97:29–42. DOI: 10.3897/jhr.97.113231.

Kuske S, Widmer F, Edwards PJ, Turlings TCJ, Babendreier D, Bigler F. 2003. Dispersal and persistence of mass released *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in non-target habitats. Biological Control 27(2):181–193. DOI: 10.1016/S1049-9644(02)00191-3.

Lill JT, Marquis RJ, Ricklefs RE. 2002. Host plants influence parasitism of forest caterpillars. Nature 417(6885):170–173. DOI: 10.1038/417170a.

Loos J, Schröer C, Becker T, Kastal A, Kortmann E, Dolek M. 2022. Mosaic landscapes provide conservation pockets for an endangered species: *Colias myrmidone* in Romania. Insect Conservation and Diversity 15(3):359–369. DOI: 10.1111/icad.12561.

Maes D, Verovnik R, Wiemers M, Brosens D, Beshkov S, Bonelli S, Buszko J, Cantú-Salazar L, Cassar L-F, Collins S, Dincă V, Djuric M, Dušej G, Elven H, Franeta F, Garcia-Pereira P, Geryak Y, Goffart P, Gór Á, Hiermann U, Höttinger H, Huemer P, Jakšić P, John E, Kalivoda H, Kati V, Kirkland P, Komac B, Kőrösi Á, Kulak A, Kuussaari M, L'Hoste L, Lelo S, Mestdagh X, Micevski N, Mihoci I, Mihut S, Monasterio-León Y, Morgun DV, Munguira ML, Murray T, Nielsen PS, Ólafsson E, Óunap E, Pamperis LN, Pavlíćko A, Pettersson LB, Popov S, Popović M, Pöyry J, Prentice M, Reyserhove L, Ryrholm N, Šašić M, Savenkov N, Settele J, Sielezniew M, Sinev S, Stefanescu C, Švitra G, Tammaru T, Tiitsaar A, Tzirkalli E, Tzortzakaki O, van Swaay CAM, Viborg AL, Wynhoff I, Zografou K, Warren MS. 2019. Integrating national red lists for prioritising conservation actions for European butterflies. Journal of Insect Conservation 23(2):301–330. DOI: 10.1007/s10841-019-00127-z.

Marhoul P, Dolek M. 2012. Action plan for the conservation of the Danube Clouded Yellow *Colias myrmidone* in the European Union. Brussels, Belgium: European Commission.

Menéndez R, González Megías A, Lewis OT, Shaw MR, Thomas CD. 2008. Escape from natural enemies during climate-driven range expansion: A case study. Ecological Entomology 33(3):413–421. DOI: 10.1111/j.1365-2311.2008.00985.x.

Michalko R, Pekár S, Entling MH. 2019. An updated perspective on spiders as generalist predators in biological control. Oecologia 189(1):21–36. DOI: 10.1007/s00442-018-4313-1.

Mills NJ. 2005. Parasitoids and predators. Insect sampling in Forest ecosystems (S.R. Leather, ed.). Malden, MA: Blackwell Science. pp. 254–278.

Morse DH, Fritz RS. 1982. Experimental and observational studies of patch choice at different scales by the crab spider *Misumena vatia*. Ecology 63(1):172–182. DOI: 10.2307/1937042.

Nakabayashi Y, Ohshima I. 2024. Geographical variation in parasitoid communities and the cause of enemy-free space in a range-expanding myrmecophilous lycaenid butterfly. Biological Journal of the Linnean Society 141(1):17–32. DOI: 10.1093/biolinnean/blad060.

Nippen P, Dolek M, Loos J. 2021. Preserving *Colias myrmidone* in European cultural landscapes: Requirements for the successful development from egg to higher larval stages at a natura 2000 site in Romania. Journal of Insect Conservation 25(4):643–655. DOI: 10.1007/s10841-021-00332-9.

Pekár S, Toft S. 2015. Trophic specialisation in a predatory group: The case of prey-specialised spiders (Araneae). Biological Reviews 90(3):744–761. DOI: 10.1111/brv.12133.

Poelman EH, Bruinsma M, Zhu F, Weldegergis BT, Boursault AE, Jongema Y, van Loon JJA, Vet LEM, Harvey JA, Dicke M. 2012. Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. PLOS Biology 10(11): e1001435. DOI: 10.1371/journal.pbio.1001435.

Rayor LS 2004. Effects of monarch larval host plant chemistry and body size on *Polistes* wasp predation. The Monarch Butterfly: Biology and Conservation. Oberhauser K, Solensky M, editors. Ithaca, NY: Cornell University Press. pp. 39–46.

Rayor LS, Mooney LJ, Renwick JA. 2007. Predatory behavior of *Polistes dominulus* wasps in response to cardenolides and glucosinolates in *Pieris napi* caterpillars. Journal of Chemical Ecology 33(6):1177–1185. DOI: 10.1007/s10886-007-9283-4.

Razmi M, Karimpour Y, Safaralizadeh M, Safavi S. 2011. Parasitoid complex of cabbage large White butterfly *Pieris brassicae* (L.) (Lepidoptera, Pieridae) in Urmia with new records from Iran. Journal of Plant Protection Research 51 (3):248–251. DOI: 10.2478/v10045-011-0041-9.

Ricciardi R, Benelli G, Di Giovanni F, Lucchi A. 2024. The European grape berry moth, *Eupoecilia ambiguella* (Lepidoptera: Tortricidae): Current knowledge and management challenges. Crop Protection 180:106641. DOI: 10.1016/j.cropro.2024.106641.

Romeis J, Babendreier D, Wäckers FL, Shanower TG. 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids – underlying mechanisms and implications. Basic and Applied Ecology 6(3):215–236. DOI: 10.1016/j.baae.2004.10.004.

Schwarz M. 2007. Revision der westpaläarktischen Arten der Gattung *Hoplocryptus* Thomson (Hymenoptera, Ichneumonidae). Linzer biologische Beiträge 39(2):1161–1219.

Schwarz M, Shaw MR. 2000. Western Palaearctic Cryptinae (Hymenoptera: Ichneumonidae) in the National museums of Scotland, with nomenclatural changes, taxonomic notes, rearing records and special reference to the British check list. Part 3. Tribe Phygadeuontini, subtribes Chiroticina, Acrolytina, Hemitelina and Gelina (excluding *Gelis*), with descriptions of new species. Entomologist's Gazette 51:147–186.

Settele J, Kudrna O, Harpke A, Kühn I, van Swaay C, Verovnik R, Warren M, Wiemers M, Hanspach J, Hickler T, Kühn E, van Halder I, Veling K, Vliegenthart A, Wynhoff I, Schweiger O. 2008. Climatic risk atlas of European butterflies. BioRisk 1:1–712. DOI: 10.3897/biorisk.1.

Shaw MR, Hochberg ME. 2001. The neglect of parasitic Hymenoptera in insect conservation strategies: The British fauna as a prime example. Journal of Insect Conservation 5(4):253–263. DOI: 10.1023/A:1013393229923.

Shaw MR, Stefanescu C, Van Nouhuys S. 2009. Parasitoids of European butterflies. In: Settele J, Shreeve M, Konvicka H, van Dyck H, editors. Ecology of butterflies in Europe. Cambridge: Cambridge University Press. pp. 130–156.

Siam AN, El-Kholy MY. 2023. Compatibility of bio and natural compounds with *Trichogramma evanescens* (Westwood) for *Pieris rapae* management in cauliflower. Asian Journal of Research in Agriculture and Forestry 9(3):139–148. DOI: 10.9734/ajraf/2023/v9i3216.

Sielezniew M, Deoniziak K, Dziekańska I, Nowicki P. 2019. Dispersal in a metapopulation of the critically endangered Danube Clouded Yellow butterfly *Colias myrmidone*: Implications for conservation. Journal of Insect Conservation 23 (2):291–300. DOI: 10.1007/s10841-019-00126-0.

Sielezniew M, Jaworski T, Sielezniew I, Deoniziak K, Bystrowski C, Hilszczański J, Nowicki P. 2024. Clear-cuts support the metapopulation of a critically endangered butterfly. Forest Ecology and Management 562:121939. DOI: 10.1016/j.foreco.2024.121939.

Stefanescu C, Askew RR, Corbera J, Shaw MR. 2012. Parasitism and migration in southern Palaearctic populations of the painted lady butterfly, *Vanessa cardui* (Lepidoptera: Nymphalidae). European Journal of Entomology 109(1):85–94. DOI: 10.14411/eje.2012.011.

Stefanescu C, Colom P, Jm B, Horsfield D, Komac B, Miralles A, Shaw MR, Ubach A, Gutiérrez D. 2022. Larval parasitism in a specialist herbivore is explained by phenological synchrony and host plant availability. Journal of Animal Ecology 91 (5):1010–1023. DOI: 10.1111/1365-2656.13689.

Stefanescu C, Pintureau B, Tschorsnig HP, Pujade-Villar J. 2003. The parasitoid complex of the butterfly *Iphiclides podalirius feisthamelii* (Lepidoptera: Papilionidae) in north-east Spain. Journal of Natural History 37(4):379–396. DOI: 10.1080/713834709.

Stefanescu C, Planas J, Shaw MR. 2009. The parasitoid complex attacking coexisting Spanish populations of *Euphydryas aurinia* and *Euphydryas desfontainii* (Lepidoptera: Nymphalidae, Melitaeini). Journal of Natural History 43(9–10):553–-568. DOI: 10.1080/00222930802610444.

Tiitsaar A, Kaasik A, Teder T. 2013. The effects of seasonally variable dragonfly predation on butterfly assemblages. Ecology 94(1):200–207. DOI: 10.1890/12-0541.1.

Tschorsnig HP 2017. Preliminary host catalogue of Palaearctic Tachinidae (Diptera). Available: http://www.nadsdiptera.org/Tach/WorldTachs/CatPalHosts/Home.html.

Van Swaay C, Cuttelod A, Collins S, Maes D, Munguira ML, ŠaŠić, M,Settele J, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhoff I. 2010. European red list of butterflies. Luxembourg: Publication Office of the European Union.

Wawer W, Hajdamowicz I. 2018. Spatial niche separation on the periphery range of *Argiope bruennichi* and its effect with *Araneus quadratus* (Araneae: Araneidae). Environmental Entomology 47(1):26–31. DOI: 10.1093/ee/nvx173.

Wiater J. 2004. Występowanie *Sitona (Charagmus) gressorius* (FABRICIUS, 1792) (Coleoptera: Curculionidae: Entiminae) w Polsce. Wiadomości entomologiczne 23(4):233–238.

Wiśniowski B. 2009. Spider-hunting wasps (Hymenoptera: Pompilidae) of Poland. Diversity, identification, distribution. Ojców, Poland: Ojców National Park.

Zang LS, Wang S, Zhang F, Desneux N. 2021. Biological control with *Trichogramma* in China: History, present status and perspectives. Annual Reviews of Entomology 66(24):1–22. DOI: 10.1146/annurev-ento-060120-091620.

Żurawlew P, Kutera M, Orzechowski R, Czyżewski S, Radzikowski P, Grobelny S, Cymbała R, Kolago G, Malkiewicz A, Bury J, Gwardjan M, Sępioł B, Brodacki M. 2022. The European mantis *Mantis religiosa* (Linnaeus, 1758) (Mantodea: Mantidae) in Poland – the present status and changes in range. Fragmenta Faunistica 65(1):35–53. DOI: 10.3161/00159301FF2022.65.1.035.