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First record of *Microctonus brassicae* in Czechia, a potential biological control agent against a primary oilseed rape pest

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Abstract

After the ban on treating oilseed rape seeds with neonicotinoids in European Union, cabbage stem flea beetles (*Psylliodes chrysocephala* Linnaeus, 1758) again became one of its main pests. In Czechia, the impact of this pest increases every year, given with the narrowing spectrum of suitable insecticides and growing damage to oilseed rape plants in autumn. Based on this scenario, it is appropriate to look for alternative options to control oilseed rape pests. One option could be supporting beneficial organisms. One of these organisms is the hymenopteran braconid parasitoid *Microctonus brassicae* (Haeselbarth, 2008), which parasitizes adult cabbage stem flea beetles. Its occurrence has now been confirmed outside Great Britain in Czechia and continental Europe respectively. Five male specimens of *M. brassicae* emerged from 50 collected adults of cabbage stem flea beetle by sweep netting from two localities in central Bohemia. This parasitoid of adult cabbage stem flea beetles and its larval parasitoids probably play an important role in the life cycle and population dynamics of this pest. Current knowledge about the biology, taxonomic classification and identification of this parasitoid is summarized in this paper.

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Introduction

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala*, Linnaeus 1758 (Coleoptera: Chrysomelidae), is currently a major pest of winter oilseed rape (*Brassica napus* var. *oleifera* M.) in Europe (Heimbach and Müller, 2013; Stará and Kocourek, 2019). Under central European conditions, this pest has one generation within a year (Williams, 2010). Adults feed on cotyledons and stems or perforate the first true leaves of emerging oilseed rape plants and other cruciferous crops (Kaufmann, 1941).

To keep the CSFB below the harmful threshold, only preparations with the active substance lambda-cyhalothrin or other pyrethroid insecticides (deltamethrin and cypermethrin) can currently be used in Czechia (Stará and Kocourek, 2019). However, the use of pyrethroids should not be continuous to avoid selection of resistant populations, as is now the case in Great Britain (Willis *et al*., 2020). Neonicotinoid insecticides such as thiacloprid, which have been traditionally used, have shown to be insufficiently effective under Czech conditions. During 2015-208, at least two populations of CSFBs (Prague and Potehy) showed tolerance to thiacloprid. Nevertheless, neonicotinoids are no longer directly approved for protection against CSFBs in European union (Stará and Kocourek, 2019). Thus, with the growing importance of this pest and the narrowing range of usable insecticides, farmers' interest in new CSFB control options is also growing. Given these circumstances, it is necessary to obtain more knowledge about the life cycle of this pest, its natural enemies (parasitoids) and their biology to create a supporting strategy for this important natural regulation system, which in the future could remain the only option for CSFB control. Before proceeding with chemical protection, it is appropriate to use agrotechnical preventive measures, such as sowing in well-prepared soil in the agrotechnical period. With this preventative measure, farmers can achieve evenly grown vegetation. Maintaining the dynamic growth of plants by growth regulators and balancing the nutritional state of soil also have a positive effect (Gendy and Marquard, 1989). Despite these direct precautions, ecosystem services provided by beneficial organisms at essentially no cost can be used (Polasky, 2008). These organisms include hymenopteran larval and adult pest parasitoids. These organisms can be attracted to fields by creating a suitable environment for them, such as flowering belts at the edge of a field or properly maintained landscape elements (e.g., draws and borders) that provide shelter and food. The reward for farmers can be maintaining pest populations below the harmful level and, consequently, decreasing the amount of chemicals applied to their fields (Hatt *et al*., 2018).

To date, only five parasitoids of CSFBs larvae have been identified in Europe (Ulber *et al*., 2010). These larval parasitoids belong to the Ichneumonidae and Braconidae families within the Hymenopteran order, and one species of larval hymenopteran parasitoid belongs to the Chalcidoidea superfamily. Of the parasitoids attacking adult CSFBs, two species, *Microctonus melanopus* (Ruthe, 1856) and *M. brassicae* (Haeselbarth, 2008), has been identified to date. The species of *M. melanopus* was raised from adult CSFBs in Great Britain and France. However, its biology and taxonomic classification is unclear (Ulber *et al*., 2010). In addition, this species is considered to be a parasitoid of some weevil species (Coleoptera: Curculionidae; *Ceutorhynchus assimilis* Paykull, 1792 (Harmon and McCaffrey, 1997); *C. obstrictus* Marsham, 1802 (Fox *et al*., 2004); *C. pallidactylus* Marsham, 1802 (Tobias, 1971); *C. leprieuri* Brisot, 1881 (Fulmek, 1968); and *Hypera meles* Fabricius, 1792 (Tobias, 1986)).

To date, the only confirmed parasitoid of adult CSFBs is *Microctonus brassicae* (Haeselbarth, 2008). This species was described in 2008 by E. Haeselbarth from Great Britain as a *Perilitus brassicae*, and in 2016, Gavin R. Broad transferred it to the genus *Microctonus* (Wesmael, 1835). The only occurrence of this parasitoid has been documented in Great Britain, where it was raised from collected adult CSFBs from three localities in Norfolk County. The accuracy of its morphological identification was also genetically confirmed by Jordan *et al*. (2020) by the barcoding method.

The aim of this work was to determine the presence of *Microctonus brassicae* in Czechia for future research on this potential biological control agent.

Materials and Methods

Adult cabbage stem flea beetles were collected by sweep netting from two Czech field sites within the central Bohemia region at the end of September 2020 (Šlapanice GPS:50.3216647N, 14.1097611E and Unhošť GPS: 50.0833436N, 14.1017786E localities; date of collection: 25.9.2020). Adult CSFBs were collected from winter oilseed rape and adjacent white mustard. Fifty individuals from each locality were maintained on potted oilseed rape (variety DK Exssence) or kohlrabi (*Brassica oleracea* var. *gongylodes*) in insect breeding cages (35×35×60 cm) with microperforated textile cover (Entosphinx Ltd, Czechia) in rooms with controlled environments at an L16 (22°C): D8 (20°C) photothermoperiod. Beetle colonies were checked three times a week for parasitoid emergence.

Upon emergence, wasps were collected with an exhaustor into a 100 ml polyethene bottle (Entosphinx Ltd, Czechia) that was used to kill the wasps with 1 ml of 99.7% ethyl acetate (Penta Ltd, Czechia). Some wasp specimens were also collected and preserved in 99% ethanol (Penta Ltd, Czechia) for future molecular analysis. Collected specimens in polyethene bottles were then glued on the entomological labels and morphologically identified under an Olympus SZX7 binocular microscope (Olympus Czech Group Ltd, Czechia) using a key by Haeselbarth (2008) and description by Jordan *et al.* (2020).

Results

The emerged parasitoids

The first adult wasp was detected within the beetle colonies maintained under controlled conditions approximately 2 months after collection (10.12.2020). Another four individuals hatched from the CSFBs during the following 20 days. All five individuals were identified as males.

Identification of *Microctonus brassicae*

All emerged wasp specimens were identified as *Microctonus brassicae* (Haeselbarth, 2008). This is the first report on this parasitic wasp species from Czechia and continental Europe. The species belongs to the hymenopteran parasitic family Braconidae. Together with Ichneumonidae, these families form one of the most species-rich families within Hymenoptera. In nature and in man-made ecosystems (agrocenoses), they are important natural regulators of pests. *M. brassicae* is further classified in the subfamily Euphorinae and the genus *Microctonus*. This subfamily is one of the many subfamilies (236 species known in Europe; de Jong Yde *et al*., 2021) within the Braconidae family and contains several small species parasitizing not only Coleoptera but also Hemiptera, Neuroptera, Psocoptera, Orthoptera and Hymenoptera (Quicke, 2015; Yu *et al*., 2016).

Male and female of *M. brassicae* are different in colour. According to Jordan *et al*. (2020), females are orange to brown, and males are predominantly black with yellow-brown legs (Figures 1 and 2). Females have 21 flagellomeres, while males have 25 to 26 flagellomeres. The mean size of the body from the head to the last metasomal segment is usually 2.5 mm in diameter, with the males being slightly larger (mean body length in males: 2.6 mm, females: 2.3 mm; Jordan *et al*., 2020). The genus *Microctonus* is characterized by two fused cells (submarginal and discal) in the front wing and an elongated first metasomal tergite (Figures 1 and 2) (Haeselbarth, 2008; Jordan *et al*., 2020).

Life-cycle

Microctonus brassicae is a solitary koinobiont endoparasitoid. Females lay their eggs on the pronotum or elytra, which is the most preferred site, but mouthparts and the tip of adult CSFB abdomens can also be chosen. The larva hatches from the egg and, after entering the body of the host, feeds on the haemolymph for most of its larval development (Jordan *et al*., 2020). Once the larva reaches its last (fifth) stage of development, it leaves the host's body through its anus and seeks shelter (mostly underground), where it pupates in a silk cocoon (Figure 3). The adult then hatches from the cocoon (Quicke, 2015). The time to complete the whole lifecycle from oviposition in the CSFB to enclosion of the adult took on average 43.5 days (Jordan *et al*., 2020). It is currently unknown whether adult CSFBs survive when parasitoid larvae leave their bodies. However, it is known that the larva of the parasitoid feeding on the body of the CSFB significantly affects its behaviour and, in the later stages of its development, the level of CSFB host plant (cruciferous plant) consumption. Cabbage stem flea beetle adults are sterile due to the presence of parasitoid larvae (Jordan *et al*., 2020), which also occurs in other species of the *Microctonus* genus (Loan and Holdaway, 1961). This fact can highly affect the future population density of pests if strongly parasitized. Thus, we will focus on this problem in the future in more detail.

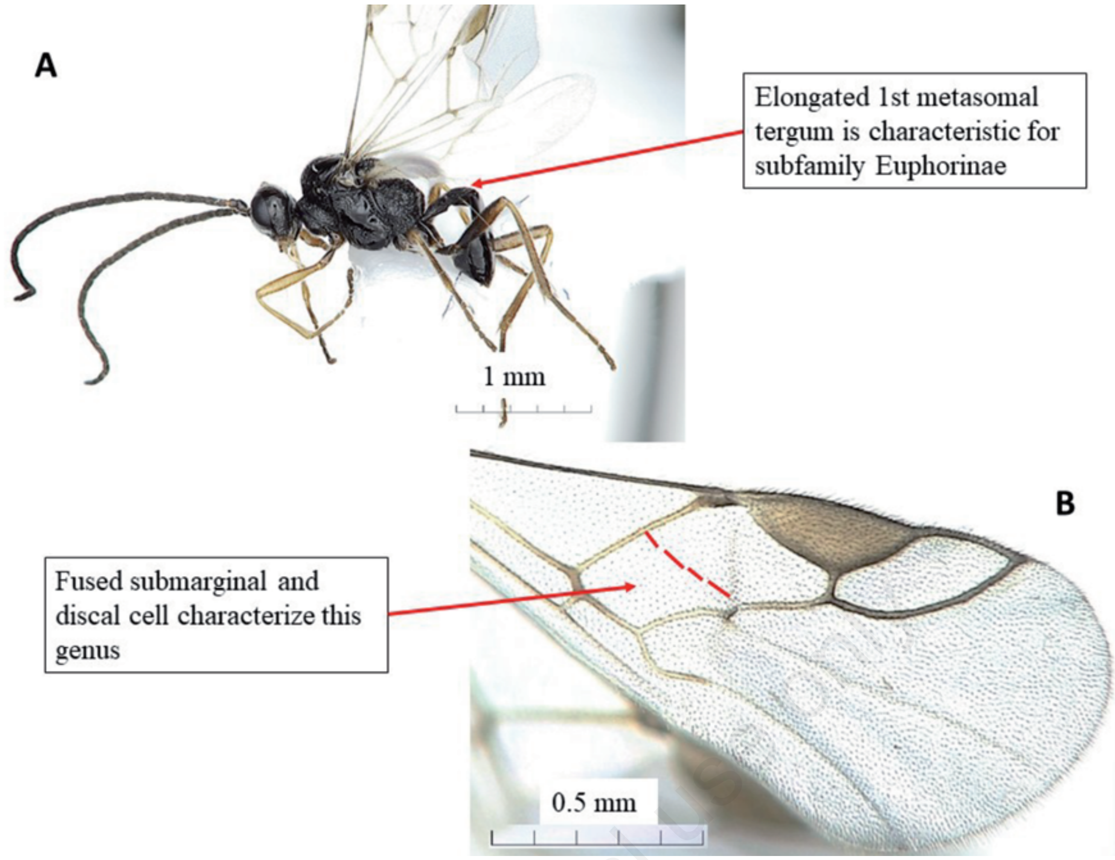

Figure 1. ***Microctonus brassicae*** **male emerged from the CSFB adult (Šlapanice locality 2020): A) Overall view B) detail of fore wing venation.**

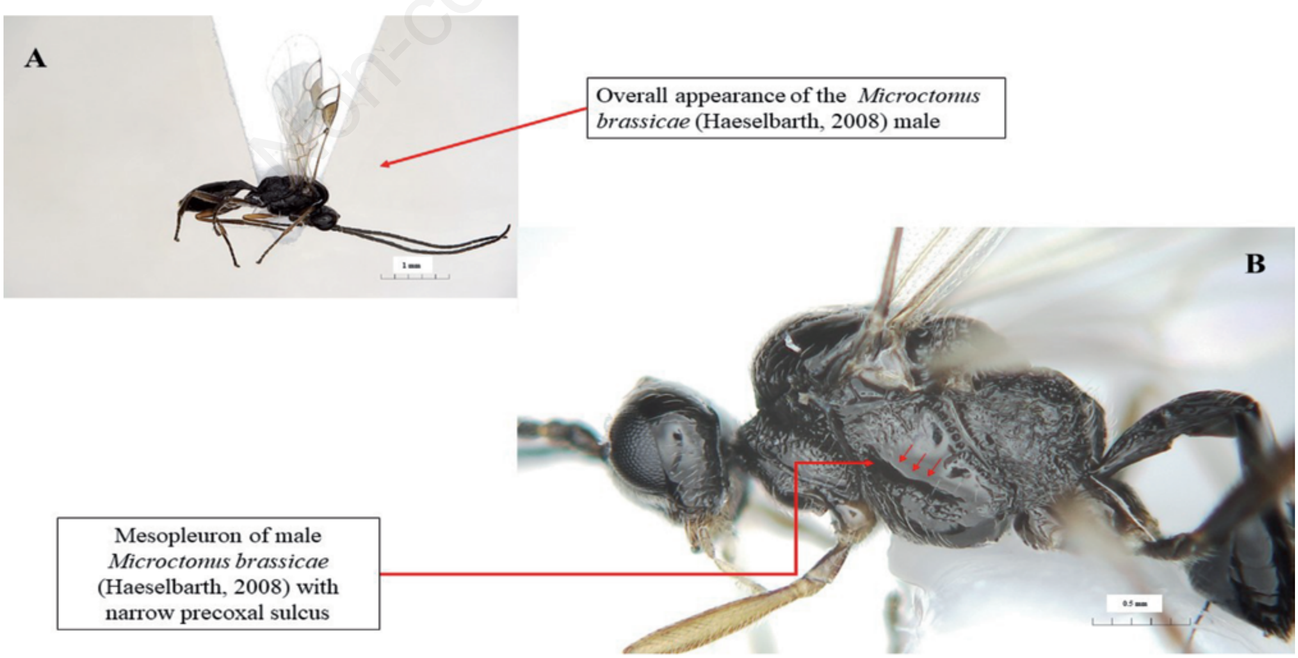

Figure 2. Male of *Microctonus brassicae*: A) Whole specimen B) Detail of Mesopleuron.

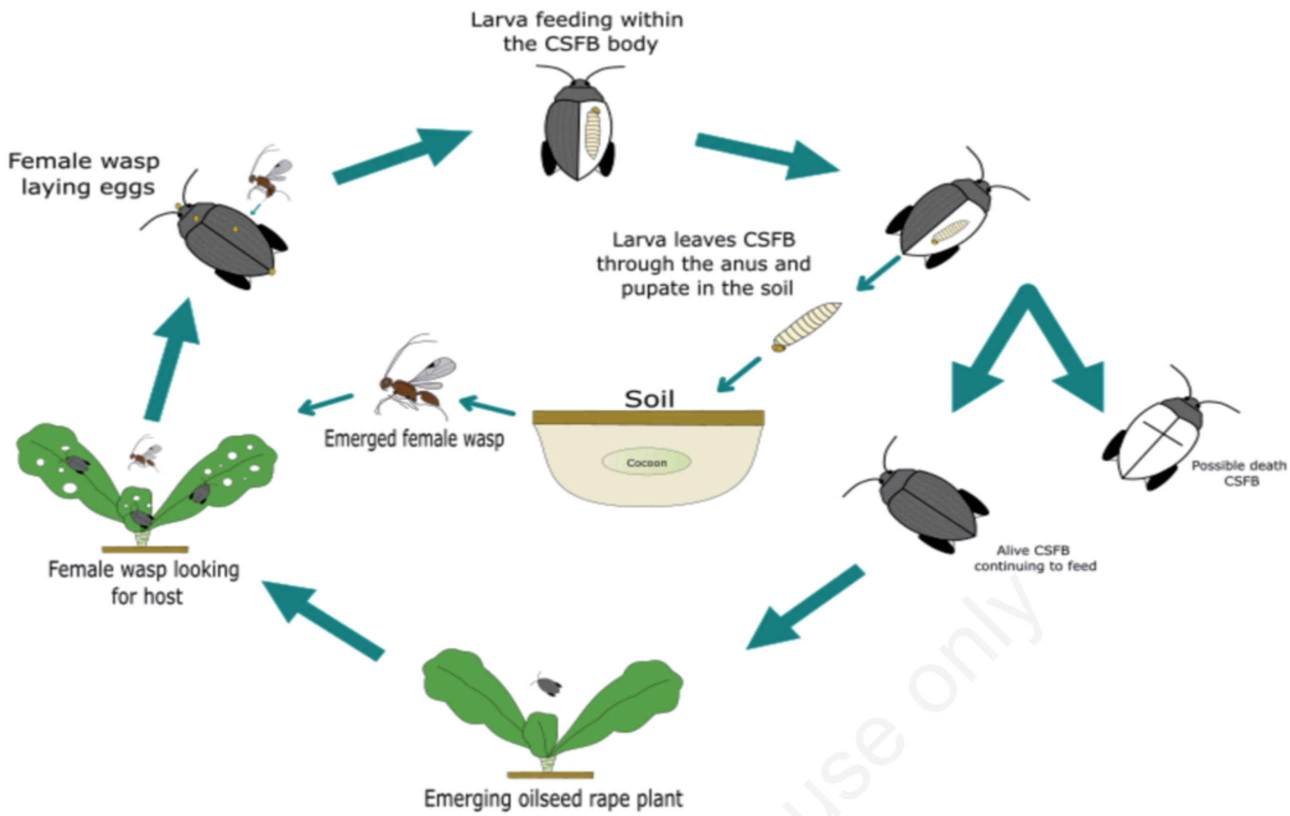

Figure 3. Life cycle of *Microctonus brassicae* and its host cabbage stem flea beetle.

Discussion and Conclusions

Braconid wasps of the genus *Microctonus* have been successfully used for the biological control of pests (Aeschlimann, 1983; Barratt *et al*., 2007). Two species can serve as an example: *M. aethiopoides* (Loan, 1975) and *M. hyperodae* (Loan, 1974). These two species were successfully introduced into New Zealand as a biological control agent of two weevil pests (*Sitona discoideus* Gyllenhal, 1834, and *Listronotus bonariensis* Kuschel, 1955) in alfalfa pastures and graminaceous crops. For both species of parasitoids, the average parasitisation rate was over 50% (Prestidge *et al*., 1991; Kean and Barlow, 2000). Similar results were obtained by Jordan *et al*. (2020) from Great Britain in the case of *M. brassicae* parasitoids of CSFBs in oilseed rape. This study by Jordan *et al*. (2020) served as a template for this and future research. The laboratory experiments with CSFBs exceeded the average parasitisation rate by over 44%. The study also described in more detail the life cycle of this parasitoid and the possibilities of breeding and reproducing in captivity. The protocol for breeding this parasitoid in captivity, which has been successfully described, indicates the possibility of its use for biological control of cruciferous crops against CSFBs in the future. However, it is necessary to optimize laboratory breeding for the conditions in Czechia and then to test the functionality of this bioagent in practice. Another possibility of using this parasitoid in the protection of cruciferous plants outside the laboratory is the release of parasitized adult CSFBs back into nature. However, it is necessary to accurately identify parasitized adults and determine the degree of parasitism of CSFB populations (Jordan *et al*., 2020) across Czechia.

In this article, the first report of the occurrence of the parasitoid *M. brassicae* from Czechia and continental Europe is presented. The report on its occurrence shows that due to the great distance between Great Britain and Czechia and the likely widespread distribution of this species, there is potential for its use in the biological control of oilseed rape against CSFBs.

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