Pteromalid fauna (Hymenoptera, Pteromalidae) in oilseed rape (Brassica napus L.) fields in Bulgaria – species composition and perspectives for biological control

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Abstract
Parasitoid wasps belonging to the family Pteromalidae are widespread and abundant members of the insect communities in the temperate regions of the world. As many other chalcids do, pteromalids serve as natural enemies of the pests in various crops and play an important role in the biological control of these harmful insects. Here we present the results of a field study in Bulgaria which was focused on the diversity of family Pteromalidae in ten oilseed rape fields. All samples were collected by sweep netting on the border line or inside the crop field. A total of 93 pteromalid specimens belonging to 26 taxa were gathered. The most abundant genus was Mesopolobus – 67% of the sampled pteromalids. The most numerous species in the samples was Mesopolobus morjs – a well-known key parasitoid of the cabbage seed weevil, Ceutorhynchus obstrictus, in Europe. One species – Halticoptera patellana, is recorded for the first time in Bulgarian fauna. Clearfield oilseed rape fields had relatively higher parasitoid abundance and richness than the fields treated by conventional technology. In the present work we discuss the overall species composition of Pteromalidae obtained from the studied areas and present our point of view on the perspectives for biological control of oilseed rape pests.

Keywords
Distribution ecology, major pests, parasitoids
Introduction

Oilseed rape (Brassica napus L.) (Brassicaceae) is one of the most important sources of both vegetable oil and oil extraction meal worldwide. The oilseed rape oil amounts to 11.7% of the total world consumption of vegetable oils, being exceeded only by the soybean and palm oil production. Nowadays, wild B. napus forms are unknown and that leads to an assumption that the species diverged relatively recently through cultivation of its parental species in geographically close areas (Friedt and Snowdon 2010).

Due to its negative influence on crop production, the insect pests of the oilseed rape have been well studied in Europe. Three groups of pest are considered to invade oilseed crop fields in Europe – major pests, minor pests and incidental pests (Alford 2003). Among the first group, six species are found to be most abundant and with a key significance for the growing of the winter rape, namely the pollen beetle, Brassicogethes aeneus (Fabricius) (Nitidulidae), the cabbage seed weevil, Ceutorhynchus obstrictus (Marsham), the cabbage stem weevil, Ceutorhynchus pallidactylus (Marsham) and the rape stem weevil, Ceutorhynchus napi (Gyllenhal) (Curculionidae), the brassica pod midge, Dasineura brassicae Winnertz (Cecidomyiidae), and the cabbage stem flea beetle Psylliodes chrysocephala (L.) (Chrysomelidae). Recent studies on these harmful species have revealed at least 12 hymenopterans, which have been considered as key parasitoids of the pests’ larvae. They belong mainly to Ichneumonidae, Braconidae, Platygastridae, Pteromalidae and Eulophidae (Williams et al. 2005; Williams et al. 2005; Ulber et al. 2010). The total number of known egg and larval parasitoids is much higher, but most of them have wider host ranges and therefore various food sources, diminishing their impact on the oilseed rape pests. Besides the effects of hosts on the parasitoid diversity, the role of wildflower strips growing along field margins or within crops on the natural enemy populations in oilseed rape fields was recently studied (Hatt et al. 2018). This investigation revealed that the presence of flowering plants close to the crop fields positively affects the parasitoid abundance and increases the potential for biological control in these areas.

Nine pteromalid species are currently known to be associated with B. napus in Europe, mostly developing as parasitoids on Ceutorhynchus and Dasineura spp. (Herrström 1964; Kuhlmann and Mason 2002; Gibson et al. 2006a; Veromann et al. 2012; Noyes 2019). Among them five species have been reported from Bulgaria – Mesopolobus morys Walker, Pachyneuron muscarum L., Pteromalus cerealellae Ashmead, Trichomalus nanus Walker and Trichomalus perfectus Walker, but in papers not dealing with oilseed rape fields (Thompson 1958; Thuroczy 1990; Todorov 2013; Todorov et al. 2014).

According to Laufer et al. (2014) Clearfield is the combination of an imidazolinone-based herbicide and a corresponding plant, which is tolerant against the active ingredient of the herbicide. Cultivation of Clearfield oilseed rape aims towards a reliable control of broadleaf and grass weeds in post-emergence.

In respect to the insecticide treatment of B. napus pests, a number of chemical agents belonging mostly to carbamates, pyrethroids and organophosphates have been tested in the past and nowadays are usually used in crop fields (Alford et al. 1991;
Murchie et al. 1999; Cook et al. 2004; Hansen 2004; Hansen 2008; Petraitienë et al. 2012). Besides the pests’ mortality, chemical control may also have a negative effect on numerous beneficial insect species (Ruberson et al. 1998; Romeis et al. 2006; Karise et al. 2007; Wen et al. 2021). Conversely, the rate of parasitism on the pest larvae can reach a high percentage in rape not treated with insecticides (Murchie et al. 1997).

A suitable ecological structure within the agroecosystems obtained by suitable alternatives to the conventional agricultural systems provides resources such as food for adult natural enemies and influence their abundance and diversity (Landis et al. 2000; Möller et al. 2021).

The purpose of this study was to 1) obtain data about the biodiversity of Pteromalidae in oilseed rape fields in Bulgaria, and 2) assess the effect of the two production systems used, namely conventional and Clearfield technology, on the pteromalid assemblages.

**Materials and methods**

The field study was carried out in ten oilseed rape fields situated on the southern foots of Sarnena Gora Mountains and in the western and south-eastern part of the Thracian Lowland, Bulgaria (Fig. 1, Table 1), during late April and the second half of May, 2018. Details about the oilseed crops and management practices at the localities selected are shown in Table 2.

![Figure 1](image-url). Approximate location of the studied areas (white rectangles) in Bulgaria.
To assess the diversity of Pteromalidae, which could be potential parasitoids of oilseed rape pests in the crops, we used two classic sweep netting techniques: 1. sweeping with following catch of the target insects with an aspirator; 2. sweeping with immediate storage of all insects in vials of 70% ethanol. The first method was conducted by collecting three samples at one transect per site in the crop field. Each transect was 200 m long and samples were taken in the starting, middle and ending points, making 20 movements and walking 10 meters for every sample. The second method was conducted by walking a 100 m transect along the field margin. At every 20 m the insects were removed from the net. All samples were collected on sunny days, preferably in the morning between 09.30 and 11.30 a.m. or in the late afternoon (16.00 p.m. onwards). Collected material was stored in 70% ethanol, dehydrated with 99% ethanol and dried with HMDS following Heraty and Hawks (1998). Identification of the taxa was performed using the keys in Bouček (1963), Graham (1969), Bouček and Rasplus (1991), Mitroiu (2010), Klimmek and Baur (2018) and Gibson (2009). Nomenclature verification was performed following de Jong et al. (2014), Noyes (2019) and GBIF.org. (2021).

**Results**

A total of 93 pteromalid specimens were collected, from which 86 were identified. They belong to 15 valid species in 14 genera (Table 3). Nine species and two taxa identified at most to generic level were found in the samples conducted by the first method inside the crop fields. Nine species and nine taxa identified at most to generic level were caught by the second method along the field margin. Only three species were gathered by both of the collecting methods – *Macroglenes penetrans* (Kirby), *M. morys* (Walker) and *Pteromalus sequester* Walker (Table 3). Most species and also the taxa that were not identified to species level were represented by only one specimen in our material. The most abundant and widespread pteromalid was *M. morys*, averaging 60.2% of all collected specimens. It was followed by *Pteromalus semotus* (Walker) with 6.5% and *Pachyneuron aphidis* (Bouche) with 4.3%. Crop fields with a relatively high presence of pteromalids, in terms of both the number of specimens and the number of species, were Momino selo (site 6 – 27 individuals, nine species) and Kostievo (site 9 – 19 ind., nine species).

<table>
<thead>
<tr>
<th>Sampling field</th>
<th>Location</th>
<th>Nearest settlement</th>
<th>Coordinates</th>
<th>Altitude, m a.s.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>TL</td>
<td>Malak Chardak</td>
<td>42°16.73'N, 24°38.80'E</td>
<td>198</td>
</tr>
<tr>
<td>Site 2</td>
<td>SG</td>
<td>Zelenikovo</td>
<td>42°23.20'N, 25°02.85'E</td>
<td>281</td>
</tr>
<tr>
<td>Site 3</td>
<td>SG</td>
<td>Zelenikovo</td>
<td>42°22.75'N, 25°04.86'E</td>
<td>288</td>
</tr>
<tr>
<td>Site 4</td>
<td>TL</td>
<td>Stalevo</td>
<td>42°03.43'N, 25°23.85'E</td>
<td>171</td>
</tr>
<tr>
<td>Site 5</td>
<td>TL</td>
<td>Dobrich</td>
<td>42°01.41'N, 25°32.13'E</td>
<td>129</td>
</tr>
<tr>
<td>Site 6</td>
<td>TL</td>
<td>Momino selo</td>
<td>42°17.51'N, 24°52.83'E</td>
<td>175</td>
</tr>
<tr>
<td>Site 7</td>
<td>TL</td>
<td>Stryama</td>
<td>42°15.31'N, 24°50.86'E</td>
<td>174</td>
</tr>
<tr>
<td>Site 8</td>
<td>TL</td>
<td>Malak Chardak</td>
<td>42°16.66'N, 24°37.73'E</td>
<td>201</td>
</tr>
<tr>
<td>Site 9</td>
<td>TL</td>
<td>Kostievo</td>
<td>42°10.28'N, 24°36.78'E</td>
<td>175</td>
</tr>
<tr>
<td>Site 10</td>
<td>TL</td>
<td>Kostievo</td>
<td>42°09.66'N, 24°37.61'E</td>
<td>178</td>
</tr>
</tbody>
</table>
Sampling fields with the lowest presence of pteromalids were Malak Chardak (site 1 – 1 ind.) and Stryama (site 7 – 2 ind., one species). The number of specimens (total number: 76 ind.; mean number ± SE: 12.67 ± 4.16 ind.) captured in the crops managed by the Clearfield technology were higher than those in crops with conventional technology of oilseed rape production (total number: 22 ind.; mean number ± SE: 5.50 ± 3.23 ind.) (Fig. 2A). Similarly, the abundance of pteromalid taxa was higher in crops with Clearfield technology compared to the conventionally treated ones (Clearfield – total number: 30 taxa; mean number ± SE: 5.00 ± 1.39 taxa; conventional system: total number: 10 taxa; mean number ± SE: 2.50 ± 1.19 taxa) (Fig. 2B).

**Discussion**

The use of an insect net is a well-known active method for collecting hymenopteran insects in vegetation. Weather, vegetation type and age, weight of net, type of mesh,
and handler skill are some of the factors affecting net collections (Marshall et al. 1994). Although a higher number of taxa was obtained by sweeping with immediate storage of the insects than collection of the target species captured in the net by aspirator, assessing relative sampling effort makes comparisons between collecting techniques problematic. As the purpose of this study was to investigate the diversity of Pteromalidae wasps in oilseed rape fields in Bulgaria, using a combination of sweep netting techniques we found a relatively high number of taxa. Ulber et al. (2010) reported 80 species of hymenopteran parasitoids of the pests of oilseed rape in Europe, including nine pteromalid species belonging to 

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Number of specimens</th>
<th>Collecting date</th>
<th>Sampling site (according to Table 1)</th>
<th>Presence in Clearfield sites</th>
<th>Presence in conventional sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorocytus cf. longispinus</td>
<td>1 (♀)</td>
<td>27.IV.</td>
<td>6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chlorocytus cf. phalaridis</td>
<td>1 (♀)</td>
<td>22.IV.</td>
<td>10</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chlorocytus cf. spicatus</td>
<td>1 (♀)</td>
<td>24.IV.</td>
<td>6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chlorocytus sp.</td>
<td>1 (♀)</td>
<td>30.V.</td>
<td>9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chlorocytus spicatus (Walker)</td>
<td>1 (♀)</td>
<td>30.V.</td>
<td>6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Cyrtogaster vulgaris Walker</td>
<td>1 (♂)</td>
<td>16.V.</td>
<td>9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Halictoptera patellana (Dalman)</td>
<td>1 (♀)</td>
<td>19.IV.</td>
<td>2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Macroglenes penetrans (Kirby)</td>
<td>3 (♂)</td>
<td>27.IV.; 16.V.</td>
<td>5, 9, 10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesopolobus incultus (Walker)</td>
<td>1 (♀)</td>
<td>21.IV.</td>
<td>6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Mesopolobus morys (Walker)</td>
<td>56 (55 ♀, 1 ♂)</td>
<td>19–27.IV.; 27–28.V.</td>
<td>2, 3, 4, 5, 6, 8, 9, 10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesopolobus sp.</td>
<td>1 ♀</td>
<td>28.V.</td>
<td>5</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Pachyneuron aphidis (Bouche)</td>
<td>4 (3 ♀, 1 ♂)</td>
<td>28–30.V.</td>
<td>2, 9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pachyneuron muscarum (Linnaeus)</td>
<td>1 (♀)</td>
<td>28.V.</td>
<td>2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pteromalus cf. chlorospilus</td>
<td>1 (♀)</td>
<td>14.V.</td>
<td>5</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Pteromalus intermedius (Walker)</td>
<td>1 (♀)</td>
<td>19.IV.</td>
<td>3</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pteromalus paparum (Walker)</td>
<td>1 (♀)</td>
<td>22.IV.</td>
<td>9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pteromalus semenius (Walker)</td>
<td>6 (♂)</td>
<td>14.V.; 27–30.V.</td>
<td>5, 6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pteromalus sequester Walker</td>
<td>2 (♀)</td>
<td>22, 27.IV.</td>
<td>6, 10</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pteromalus sp. undes. J (Graham, 1969 – p. 496, 556)</td>
<td>1 (♀)</td>
<td>14.V.</td>
<td>4</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Spathanga subpunctata Förster</td>
<td>1 (♀)</td>
<td>30.V.</td>
<td>9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sphingidae cf. interita</td>
<td>1 (♀)</td>
<td>27.V.</td>
<td>5</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Sphingidae sp. 27.V. Bouček</td>
<td>2 (♀)</td>
<td>27.V.</td>
<td>7</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Spintherus dubius Ashmead</td>
<td>1 (♀)</td>
<td>22.IV.</td>
<td>10</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Stenomalina cf. epistena</td>
<td>1 (♀)</td>
<td>30.V.</td>
<td>1</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Syntaxis sp.</td>
<td>1 (♂)</td>
<td>30.V.</td>
<td>6</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Tritoneptis cf. klugi</td>
<td>1 (♀)</td>
<td>29.V.</td>
<td>5</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

Mesopolobus morys was the only pteromalid species occurring in our samples, which was included in the list of species associated with oilseed rape pests in Europe by Ulber et al. (2010). It is very common and widely distributed and has been reported from various natural and agricultural habitats. Mesopolobus morys develops as a larval ectoparasitoid mostly on the curculionids belonging to Ceutorhynchus spp. (Rosen 1964; Kuhlmann and Mason 2002; Noyes 2019) and rarely on Protapion apricans (Herbst).
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and *Protapion trifolii* (Linnaeus) (OILB 1971). Being recognized as one of the three key parasitoids of the cabbage seed weevil, *C. obstrictus*, (Ulber et al. 2010), *M. morys* plays an important role in the biocontrol of this pest.

The rest of the identified taxa, which have been found during the investigation, can be divided into the following groups:

Parasitoid species potentially associated with hosts feeding on *B. napus* or relative Brassicaceae plants, growing around the crop fields

**Pachyneuron aphidis** (Bouche)

Widespread pteromalid species distributed all over the world. Similar to most species in this genus, *P. aphidis* is a polyphagous hyperparasitoid of many Aphididae or other plant sucking Hemiptera mostly through their Braconidae, Aphelinidae and Encyrtidae primary parasitoids (Gibson 2001; Noyes 2019). This life strategy is not beneficial for crop field production due to the negative effect of the hyperparasitism on the natural enemies of the plant pests. However, in the case with oilseed rape, *P. aphidis* is not of great importance. It is not commonly associated with these plants and has been reported from crucifers in only four papers (OILB 1971; Kamijo and Takada 1973; Haeselbarth 1985; Gibson 2001).

**Pachyneuron muscarum** (L.)

Widely distributed Palearctic species with similar life-history and hosts as *P. aphidis*. It is known from *B. napus* fields (Rosen 1964; Graham 1969; OILB 1971) and probably could affect more or less negatively its natural enemies, but does not attack any of the oilseed rape key parasitoids.
**Pteromalus semotus** (Walker)

Common and widely distributed Palearctic species, introduced in New Zealand for the purpose of the biological control of some Lepidoptera. It is known to attack *C. obstrictus* (as *C. assimilis*) on *Brassica oleracea* but this association seems to be incidental because only two individuals (1% of the total parasitoids) emerged from the host larvae (Dmoch and Sulgostowska 1986).

Parasitoid species biologically similar to other oilseed rape associated pteromalids

**Chlorocytus spicatus** (Walker)

This species has eight known host associations (Noyes 2019), but only one may be discussed in the light of our study. It is recorded by Vidal (1993) as primary parasitoid of one unidentified *Ceutorhynchus* sp. but not from *B. napus* or other Brassicaceae host plant. Among the European *Chlorocytus*, only one – *Chlorocytus diversus* (Walker), has been found to attack some of the oilseed rape pests in Europe (cabbage seed weevil, *C. obstrictus*) (Ulber et al. 2010), but this pteromalid is not considered as a key species. Thus, *C. spicatus* appear to be of negligible or no importance for biocontrol in the *B. napus* crop fields.

Parasitoid species that have not been reported from any Brassicaceae-associated hosts and probably only use *B. napus* as a source of flower nectar or honeydew

**Cyrtogaster vulgaris** Walker

Well-known solitary pupal parasitoid of various dipteran hosts, mainly Agromyzidae, Chloropidae and Lonchopteridae, which has been reported to attack only one coleopteran species – *Bruchidius marginalis* (Fabricius) (Chrysomelidae), but probably as secondary parasitoid (Andriescu and Mitroiu 2001). According to its hosts that mostly develop on Asteraceae, Fabaceae, and Poaceae, *C. vulgaris* is not a common pteromalid species inside the oilseed rape fields, but certainly can be found in surrounding areas.

**Halictoptera patellana** (Dalman)


**Macroglenes penetrans** (Kirby)

This pteromalid is a well-known natural biological agent of two cecidomyiid (Cecidomyiidae) wheat pests – the wheat fly, *Contarinia tritici* (Kirby), and the orange wheat
blossom midge, *Sitodiplosis mosellana* (Gehin). Common species in the grasslands and meadows but usually not numerous.

**Mesopolobus incultus** (Walker)

Primary and secondary parasitoid on some weevils belonging to *Apion* Herbst, *Protapion* Schilsky (Apionidae) or *Gymnetron* Schoenherr (Curculionidae), mostly associated with legumes (*Trifolium* sp.) (Fabaceae) and sometimes with plantains (*Plantago* sp.) (Plantaginaceae) (Graham 1969; Garrido Torres and Nieves-Aldrey 1992).

**Pteromalus intermedius** (Walker)

Not very common species known as primary parasitoid of some fruit flies (Tephritidae), mostly associated with Asteraceae and rarely with Chenopodiaceae, Lamiaceae and Tamaricaceae (Graham 1969; Garrido Torres and Nieves-Aldrey 1999; Askew et al. 2001).

**Pteromalus puparum** (Linnaeus)

Cosmopolitan species, which is known to attack a great number of hosts, mostly belonging to the butterfly families Nymphalidae, Papilionidae and Pieridae. It has not been recorded from *B. napus* in Europe. A study of Gibson et al. (2006b) based on voucher specimens of three species that McLeod (1953) listed as imported from Europe and released in British Columbia (USA), reported *P. puparum* as associated with *C. obstrictus*. However, the authors consider that the material likely represents an incorrect host association because of potential contamination of mass-reared seedpods by the diamondback moth, *Plutella xylostella* (L.) (Plutellidae).

**Pteromalus sequester** Walker

Cosmopolitan species, known as parasitoid mostly on coleopterans belonging to Apionidae, Bruchidae and Curculionidae associated with legumes (Noyes 2019).

**Spalangia subpunctata** Förster

This species belongs to the small subfamily Spalangiinae – specialized pupal parasitoids of dipteran hosts in manure piles or animal feces. Its presence in a sample from site 9R (Kostievo) can be explained with the presence of livestock herds feeding on the surrounding grasslands.

**Sphegigaster stepicola** Bouček

Rarely collected species in Bulgaria, with Palearctic range, known to attack larvae of a few Agromyzidae (Diptera) in grasses (Noyes 2019).
*Spintherus dubius* Ashmead

One of the most commonly collected pteromalid species in the natural or semi-natural grassland habitats in Bulgaria. *S. dubius* can be found almost everywhere from the sea level to the highly elevated mountainous meadows. It is associated mostly with *Apion* species on clovers (*Trifolium*) (Noyes 2019).

In order to interpret the biological potential of a certain parasitoid species controlling a certain pest one depends mostly on one’s research experience but this should be confirmed by field or laboratory experiments. Such experiments, in most cases, are planned after a lot of theoretical assumptions in line with our current knowledge. Thus, the results presented here should be considered as a base for future studies, at least regarding some of the established parasitoids. According to the insecticides used in studied crops, a clear difference between Clearfield and conventional fields is presented (Table 2). Different chemical agents could be a possible reason for the difference in the number of specimens and the abundance of species in studied areas. However, comparative investigations between the effects of insecticides on beneficial insects in Clearfield vs conventional crops have not been conducted until now. More detailed laboratory and field studies about the parasitoid communities and their resistance to pesticide treatment in the discussed types of crops are necessary.

**Conclusion**

The most abundant species, *M. morys*, was also the only pteromalid species in our samples previously reported as oilseed rape associated. Its presence indicates a high biocontrol potential, at least against the cabbage seed weevil, *C. obstrictus*. For the present, no other species found in this study can be considered as useful in the biological control against the *B. napus* pests.

The pteromalid fauna established in the crops with Clearfield technology was more abundant according both to the number of specimens and number of taxa compared to the crops treated with conventional technology of oilseed rape production.

The high portion of unidentified taxa (12%), probably undescribed species, represents a typical picture for the natural fauna of Pteromalidae and shows our incomplete knowledge on these parasitoids.

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