



Parasitoid Wasps (Hymenoptera: Chalcidoidea) Associated with *Bruchophagus astragali* Fedoseeva (Hymenoptera: Eurytomidae) and *Bruchidius marginalis* (Fabricius) (Coleoptera: Chrysomelidae) on *Astragalus* *glycyphyllos* L. (Fabaceae) in Bulgaria

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Abstract: The seed-eater wasp *Bruchophagus astragali* Fedoseeva, 1954, seed beetle *Bruchidius marginalis* (Fabricius, 1775) and a great diversity of associated parasitoid wasps were reared from fruits of liquorice milkvetch *Astragalus glycyphyllos* L. (Fabaceae) in Bulgaria. The following species of Chalcidoidea were identified: *Baryscapus endemus* (Walker) and *Pediobius bruchicida* (Rondani) (Eulophidae); *Anastatus* sp., *Eupelmus confusus* Al khatib, *E. barai* Fusu, *E. messene* Walker and *E. vesicularis* (Retzius) (Eupelmidae) and *Dinarmus acutus* (Thomson), *Pteromalus sequester* Walker and *Pteromalus* sp. cf. *semotus* (Walker) (Pteromalidae). New associations with both seed-feeding species and also with liquorice milkvetch were found for many of the reared parasitoids.

Key words: liquorice milkvetch, chalcidoid parasitoids, seed wasp, seed beetle, new hosts, new associations.

Introduction

The genus *Bruchophagus* Ashmead, 1888 (Hymenoptera: Chalcidoidea: Eurytomidae) is represented in the world fauna with more than 165 species. Among them, 90 species have a Palaearctic distribution (NOYES 2019). All species are phytophagous (BOUČEK 1988, LA SALLE 2005) and develop mainly as seed-feeders of legume plants of the family Fabaceae (ZEROVA et al. 2021), with a few species known as gall inducers on citrus trees (LA SALLE 2005).

Bruchophagus astragali Fedoseeva, 1954 is a species whose larvae feed inside seeds of different species of *Astragalus* L. (Fabaceae) (POPESCU 2009). It is reported in Bulgaria, Georgia, Germany, Hungary, Iran, Kazakhstan, Mongolia, Romania, Russia, Sweden, Turkey, Turkmenistan and the former USSR (ZEROVA et al. 2021). This seed-eater wasp is associated with five chalcidoid parasitoids belonging to the families Eulophidae, Eurytomidae and Pteromalidae (MITROIU & ANDRIESCU 2003, PERJU 2005, HAGHIGHIAN & SADEGHI 2010, ZEROVA et al. 2021).

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The genus *Bruchidius* Schilsky, 1905 (Coleoptera: Chrysomelidae: Bruchinae) includes about 300 species of seed beetles and is widely distributed in the Old World. Some of them have been introduced to territories outside their native area (KINGSOLVER 2004, STOJANOVA 2010). These beetles attack and damage seeds and pods of some edible, medicinal, and ornamental legumes and wild forest plants (ARORA 1980). Their larvae develop mainly in leguminous plant seeds and less often feed on representatives of the plant families Apiaceae and Asteraceae (BOROWIEC 1987). Other species of the genus are used as biological control agents against some invasive plant species (DERBEL et al. 2007, LÓPEZ-NÚÑEZ et al. 2020).

Bruchidius marginalis (Fabricius, 1775) is found in various countries, from Spain and Latvia to the Caucasus (DELOBEL & DELOBEL 2007). Recently, this beetle was also recorded from the territory of the Republic of Khakassia (LOPATINA & LUKYANTSEV 2023). It belongs to the *Bruchidius astragalii* species group whose larvae live and feed on the seeds of various *Astragalus* spp. (BOROWIEC 1985). The imago is anthophagous (ALEKSEEV & BUKEJS 2011) and flies from April to September (SCHOTT 2004). To date, *B. marginalis* has been recorded as a host of nine hymenopterous parasitoids belong-

ing to the families Eupelmidae and Pteromalidae (DE LUCA 1965, VIDAL 1997, DZHANOKMEN 2017, PÉREZ-BENAVIDES et al. 2019).

The genus *Astragalus* L. is the most diverse in the family Fabaceae and comprises 2300 to 2900 described species (CASTILLÓN et al. 2023). They are annual and perennial plants with a wide range of applications (fodder, food, medicine, ornamental plants, etc.) (AMIRI et al. 2020). The liquorice milkvetch, *Astragalus glycyphyllos* L., is a perennial herbaceous plant widely distributed throughout Europe and the temperate regions of Asia (GNAT et al. 2014). In Bulgaria, it grows mainly in dry grassy places, forests and scrub up to 1800 m a.s.l. in mountainous regions (VALEV 1976, STOYANOV et al. 2021).

The aim of this study is to present a list of parasitoids associated with *B. astragalii* and *B. marginalis* in Bulgaria.

Materials and Methods

This study was undertaken in 2019. Over 850 clusters of dry fruits of *A. glycyphyllos* were collected from two localities in the Kyustendil region [W of Lelintsi vill. (42.22797°N / 22.72602°E, 800 m) and Eremia vill. (42°12'53.8"N / 22°50'11.3"E,

Table 1. Parasitoid wasps reared from the fruits of *Astragalus glycyphyllos* L. in Bulgaria.

Family	Parasitoid species	Number of individuals				Percentage of total
		W of Lelintsi vill. 18.VIII.2019		Eremia vill. 22.VIII.2019		
		♀♀	♂♂	♀♀	♂♂	
Eulophidae	<i>Baryscapus endemus</i> (Walker, 1839)	110	36	-	-	5.47
	<i>Pediobius bruchicida</i> (Rondani, 1872)	16	1	-	-	0.63
Eupelmidae	<i>Anastatus</i> sp.	2	1	-	-	0.11
	<i>Eupelmus confusus</i> Al khatib, 2015	33	11	-	-	1.64
	<i>Eupelmus barai</i> Fusu, 2017	246	48	-	-	11.01
	<i>Eupelmus messene</i> Walker, 1839	3	-	-	-	0.11
	<i>Eupelmus vesicularis</i> (Retzius, 1783)	17	-	-	-	0.63
Pteromalidae	<i>Dinarmus acutus</i> (Thomson, 1878)	41	8	9	1	2.21
	<i>Pteromalus sequester</i> Walker, 1835	312	130	1	-	16.59
	<i>Pteromalus</i> sp. cf <i>semotus</i> (Walker, 1834)	1048	591	3	1	61.55

600 m)]. The plant material containing host insects and their parasitoids was placed in plastic bottles covered with fine white cheesecloth and stored under laboratory conditions at a temperature of 26-27° C until all emergences had ceased. The emerged insects were aspirated with a exhaustor, fixed in ethyl alcohol and later examined under a stereo microscope.

The species were identified using the keys of BOUČEK (1965), GRAHAM (1969, 1991), KALINA (1981), BOROWIEC (1985, 1987), DECELLE (1989), RASPLUS (1989), BOUČEK & RASPLUS (1991), ZEROVA & SERYOGINA (1994), ZEROVA (1995), GIBSON & FUSU (2016) and FUSU (2017).

The examined material is deposited in the authors' collections (University of Plovdiv and Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences).

The photos of the pteromalids were taken using a Canon EOS 200D digital camera attached to a Zeiss Stemi 508 doc stereo microscope. The remaining insects were photographed using a Leica EZ4 W stereo microscope with an integrated 5.0 megapixel CMOS WiFi Camera. The images were processed through Zerene Stacker and Helicon Focus 8.0 software and subsequently edited in Adobe Photoshop to enhance clarity.

Results

During this study, 1198 specimens of *B. astragali* (Fig. 1) and 111 specimens of *B. marginalis* (Fig. 2) were reared, along with 2669 specimens of parasitoid wasps belonging to ten species of three Chalcidoidea families (Eulophidae, Eupelmidae and Pteromalidae) (Table 1, Figs 3-12).

With the exception of *P. sequester*, all parasitoids are newly associated with *B. astragali*. New associations with *B. marginalis* were established for all parasitoids except *E. vesicularis*, *D. acutus*, and *P. sequester*. The plant associations with *A. glycyphyllos* are newly recorded here for *B. endemus*, *P. bruchicida*, *Anastatus* sp., *E. confusus* and *Pteromalus* sp. cf *semotus*.

Discussion

The review of the literature sources shows that five chalcidoid parasitoids in Eulophidae, Eurytomidae and Pteromalidae have been recorded as associated with *B. astragali*, and nine in Eupelmidae and Pteromalidae with *B. marginalis* (Tables 2, 3).

All recorded parasitoids, except *D. acutus*, are polyphagous and have a wide range of hosts.

Baryscapus endemus (Fig. 3) attacks many species of Coleoptera (Curculionidae), Hemiptera (Coccidae and Kermesidae), Hymenoptera (Eurytomidae), Lepidoptera (Coleophoridae, Geometridae, Gracillariidae, Heterogynidae, Noctuidae, Notodontidae, Oecophoridae, Tortricidae and Yponomeutidae). Moreover, it can develop as a hyperparasitoid of some Hymenoptera species belonging to Braconidae, Encyrtidae, Eulophidae, Ichneumonidae and Pteromalidae (GRAHAM 1991, NOYES 2019). In the order Coleoptera, it has been associated only with hosts in the family Curculionidae (NOYES 2019). In Eurytomidae, its known hosts are *Bruchophagus gibbus* (Boheman), *Bruchophagus platypterus* (Walker), *Bruchophagus roddi* Gussakovskiy and *Eurytoma onobrychidis* Nikolskaya (GRAHAM 1991, PERJU 2005).

Pediobius bruchicida (Fig. 4) has been recorded as a primary parasitoid of numerous phytophagous insects belonging to Coleoptera (Chrysomelidae and Curculionidae), Diptera (Cecidomyiidae), Hymenoptera (Cynipidae) and Lepidoptera (Arctiidae, Gelechiidae, Geometridae, Lymantriidae, Noctuidae, Notodontidae, Pieridae, Pyralidae, Tortricidae and Yponomeutidae), but also is known to acts as a secondary parasitoid of Diptera (Tachinidae) and Hymenoptera (Braconidae, Encyrtidae, Eulophidae and Ichneumonidae) (NOYES 2019). The only known seed beetle host of *P. bruchicida* is *Bruchus* sp. (BOUČEK & ASKEW 1968). The species is reported as a parasitoid of *Bruchophagus robiniae* Zerova in seed pods of *Robinia pseudoacacia* L. (Fabaceae) in Romania (LAKATOS et al. 2018).

Anastatus Motschulsky, 1859 is a large and speciose genus in the subfamily Eupelminae (Eupelmidae) consisting of about 150 species with cosmopolitan distribution (CHEN et al. 2019, PENG et al. 2020). The species are mostly primary egg parasitoids of a wide diversity of insect orders, and a few are known to be secondary parasitoids. Some have been reared from Coleoptera larvae and Diptera puparia (GIBSON 1995). In our study, we reared two females (Fig. 5) and one male of an *Anastatus* species. Its appearance in our material is probably due to the presence of eggs of some other insects that have been laid on the liquorice milkvetch pods and collected together with them. The small number of reared specimens, combined with the fact that *Anastatus* are mostly endoparasitoids of insect eggs, suggests that its association with *Bruchophagus* or *Bruchidius* is accidental.

Eupelmus confusus (Fig. 6) is known to be associated with a wide spectrum of hosts belonging to Coleoptera (Chrysomelidae: Bruchinae) (PIN-



Figs 1-12. Seed-feeding and parasitoid species reared from fruits of *A. glycyphyllos* in Bulgaria: 1 – *Bruchophagus astragali*; 2 – *Bruchidius marginalis*; 3 – *Baryscapus endemus*; 4 – *Pediobius bruchicida*; 5 – *Anastatus* sp.; 6 – *Eupelmus confusus*; 7 – *E. barai*; 8 – *E. messene*; 9 – *E. vesicularis*; 10 – *Dinarmus acutus*; 11 – *Pteromalus sequester*; 12 – *Pteromalus* sp. cf. *semotus*. Scale bar = 0.5 mm.

Table 2. List of known parasitoids associated with *B. astragali*.

Family	Parasitoid species	References
Eulophidae	<i>Elasmus nudus</i> (Nees, 1834)	PERJU (2005)
	<i>Tetrastichus</i> sp.	HAGHIGHIAN & SADEGHI (2010); ZEROVA et al. (2021)
Eurytomidae	<i>Eurytoma aspila</i> (Walker, 1836)	PERJU (2005)
Pteromalidae	<i>Pteromalus sequester</i> Walker, 1835	PERJU (2005)
	<i>Spintherus dubius</i> (Nees, 1834)	MITROIU & ANDRIESCU (2003)

Table 3. List of known parasitoids associated with *B. marginalis*.

Family	Parasitoid species	References
Eupelmidae	<i>Anastatus bifasciatus</i> (Geoffroy, 1785) as <i>Eupelmus bifasciatus</i> Förster	DE LUCA (1965)
	<i>Eupelmus urozonus</i> Dalman, 1820	VIDAL (1997); PÉREZ-BENAVIDES et al. (2019)
	<i>Eupelmus vesicularis</i> (Retzius, 1783) as <i>Eupelmella vesicularis</i> Retzius	DE LUCA (1965)
Pteromalidae	<i>Cyrtogaster vulgaris</i> Walker, 1833	DE LUCA (1965); DZHANOKMEN (2017)
	<i>Dinarmus acutus</i> (Thomson, 1878)	DZHANOKMEN (2017); PÉREZ-BENAVIDES et al. (2019)
	<i>Pteromalus sequester</i> Walker, 1835 as <i>Pteromalus varius</i> Walker (in DE LUCA 1965)	DE LUCA (1965); VIDAL (1997); DZHANOKMEN (2017); PÉREZ-BENAVIDES et al. (2019)
	<i>Stenomalina micans</i> (Olivier, 1813) as <i>Pteromalus micans</i> Nees	DE LUCA (1965)
	<i>Trichomalus inscitus</i> (Walker, 1835) as ? <i>Pteromalus affinis</i> Walker	DE LUCA (1965)
	<i>Trichomalus statutus</i> (Förster, 1841) as <i>Pteromalus statutus</i> Förster	DE LUCA (1965)

TILIOAIE et al. 2018, RĂDAC et al. 2021), Diptera (Cecidomyiidae, Perisclidae and Tephritidae), Hymenoptera (Braconidae, Cynipidae, Eulophidae and Eurytomidae) and Lepidoptera (Gelechiidae, Perisclidae, Pyralidae and Tineidae) (AL KHATIB et al. 2014, GIBSON & FUSU 2016). It was reared from the seeds of *Albizia julibrissin* Durazz. and *Cercis siliquastrum* L. (Fabaceae) infested with *Bruchidius terrenus* (Sharp) and *Bruchidius siliquastrum* Delobel larvae in Romania (PINTILIOAIE et al. 2018). In Romania, this parasitoid was also obtained from the seed pods of *Gleditsia triacanthos* L. (Fabaceae) infested with *Amblycerus robiniae* (Fabricius), *Megabruchidius dorsalis* (Fåhraeus) and *Megabruchidius tonkineus* (Pic) larvae (RĂDAC et al. 2021). In Spain, the species has been reared from fruits of *Asphodelus ramosus* L. (Asphodelaceae) with a *Bruchophagus* sp. (ASKEW & NIEVES-ALDREY 2017).

Eupelmus barai (Fig. 7) has been recorded as a parasitoid of many species belonging to ten families (FUSU 2017). There are no data regarding the association of this parasitoid with Bruchinae species. FUSU (2017) examined a specimen of this species reared from *B. roddi* in *Medicago sativa* L. (Fabaceae) seed from France.

Eupelmus messene (Fig. 8) hosts are mainly endophytes developing in plant stems or galls (FUSU 2017, ASKEW et al. 2021). Among those listed by FUSU (2017), there is no data about the association of this species with seed beetles and seed-eating wasps.

Eupelmus vesicularis (Fig. 9) attacks a wide variety of holometabolous insects that usually are concealed within protected habitats such as galls, grass stems, seeds or cocoons (BOUČEK 1977, FUSU 2017). It has been reared from *B. marginalis* larvae in fruits of *A. glycyphyllos* (RUSCHKA 1921). This eupelmid

is associated with the following *Bruchophagus* species: *B. gibbus* (NIKOLSKAYA 1952), *B. platypterus* (PETERSON et al. 1991) and *B. roddi* (PERJU 2005). In addition to these, NOYES (2019) lists *Bruchophagus caraganae* (Nikolskaya) and *B. kolobovae* Fedoseeva (synonyms of *Eurytoma caraganae* Nikolskaya and *Bruchophagus platypterus* (Walker)). Some of the hosts listed for *E. vesicularis* possibly refer to *E. barai* or *E. messene*, species prior to FUSU (2017) being included under *E. vesicularis*.

Dinarmus acutus (Fig. 10) is a primary parasitoid of a wide range of seed beetles in pods of Fabaceae (BOUČEK 1977) belonging to the following genera: *Acanthoscelides* Schilsky, *Bruchidius* Schilsky, *Bruchus* L., *Callosobruchus* Pic and *Paleoacanthoscelides* Borowiec (Chrysomelidae) (DZHANOKMEN 2017, PÉREZ-BENAVIDES et al. 2019). It is also known to be a secondary parasitoid of other *Dinarmus* spp. (TSELIKH & KOSTJUKOV 2017).

Pteromalus sequester (Fig. 11) is a primary parasitoid of various insect hosts belonging to Coleoptera (Apionidae, Chrysomelidae (Bruchinae) and Curculionidae), Diptera (Cecidomyiidae and Tephritidae), Hymenoptera (Eurytomidae) and Lepidoptera (Pyralidae) (TSELIKH 2020). It has been reported as a secondary parasitoid of *Dinarmus italicus* (Masi) [= *Oedaule italica* Masi] in pods of *Calicotome spinosa* (L.) Link (Fabaceae) (GRAHAM 1969).

The following set of morphological characters of females of *Pteromalus* sp. cf. *semotus* (Fig. 12) suggests that they may be specifically distinct from those of *Pteromalus semotus* (Walker): Antennae and legs with paler colouration – scape pale testaceous, flagellum brown above and pale testaceous below (in *semotus* the scape is infuscated and the flagellum entirely brown); legs stout, with the femur, infuscate, tibiae slightly infuscate with pale testaceous distal half (in *semotus* the legs are more slender, with tibiae dark proximally but whitish or yellowish in distal third); head in dorsal view 2.3x as broad as long (2.1x in *semotus*); malar space distinctly longer, about 0.6x length of the eye (in *semotus* only 0.45-0.48x length of the eye); pronotal collar in profile forming more or less obtuse angle with the neck (in *semotus* the pronotal collar has a slightly abrupt front edge and forms a right angle with the neck); thorax in dorsal view more squat, at most 1.5x as long as broad (in *semotus* 1.7x). Based on these differences, we assume that this *Pteromalus* sp. is probably undescribed. In our study, it is the most numerous parasitoid (1643 specimens or 61.55%) and will be subject of a future research.

Conclusion

This study reveals a diverse insect community based upon two phytophagous species using the same food source and their ten chalcidoid parasitoids. Considering the host ranges of these parasitoids, they are most likely primary parasitoids of the seed-eating wasp and/or seed beetle, but secondary parasitism cannot be excluded.

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