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Research Article

The Parasitoid Complex Associated with *Larinus sibiricus* Gyllenhal, 1835 (Coleoptera: Curculionidae) in Bulgaria

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Abstract: The weevil *Larinus sibiricus* Gyllenhal and a rich complex of associated hymenopteran parasitoids were reared from flower heads of *Xeranthemum annuum* L. (Asteraceae) in Bulgaria. As a result, the following taxa were established: *Bracon urinator* (Fabricius) (Braconidae), *Exeristes roborator* (Fabricius) (Ichneumonidae), *Cyrtoptyx* sp., *Norbanus cerasiops* (Masi), *Pteromalus vibulenus* (Walker), *Stenoselma nigrum* Delucchi (Pteromalidae), *Exopristus trigonomerus* (Masi) (Torymidae), *Leptomastix ephyra* Noyes & Hayat (Encyrtidae), *Aprostocetus venustus* (Gahan), *Baryscapus* sp. near *carthami* Graham (Eulophidae), *Eupelmus microzonus* Förster (Eupelmidae) and *Eurytoma curculionum* Mayr (Eurytomidae). Three of them (*E. curculionum*, *L. ephyra* and *N. cerasiops*) are recorded for the first time from Bulgaria and *L. ephyra* is a newly recorded species for the Balkan Peninsula and Europe. New associations with *L. sibiricus* and *X. annuum* were established for all reared parasitoids except *B. urinator*.

Key words: *Xeranthemum annuum*, parasitoids, new records, new host, new associations

Introduction

The family Curculionidae Latreille, 1802 (Coleoptera: Curculionoidea) has a worldwide distribution and consists of about 51,000 described species classified in 4600 genera. This family includes more than 80 % of all weevil species. Their larvae are mainly endophytes and develop inside all parts of plants; there are also ectophytes as well as coprophagous, myrmecophilous and predatory larvae in some groups (Oberprieler et al. 2007).

The genus *Larinus* Dejean, 1821 (Coleoptera: Curculionidae: Lixinae) includes approximately 180 species (Gültekin 2006) widely distributed in the Old World (Gültekin 2006, Hoebeke & Spichiger 2016, Gültekin & Perrin 2018), while only a few species are known to have been introduced in the Australian and Nearctic regions (Woodburn & Briese 1996, Hoebeke & Spichiger 2016). Species of *Larinus* are known with their preferences for different habitats. Some of them dwell in wet meadows, pasture or weedy communities, while others are associated with xerothermic communities (Skuhrovec & Gosik 2011). Larvae feed mainly on various representatives of the plant family Asteraceae such as *Arctium* L., *Carduus* L., *Carlina* L., *Centaurea* L., *Cirsium* Miller, *Cynara* L., *Echinops* L., *Onopordum* L., *Scolymus* L., *Silybum* Adans. (Skuhrovec & Gosik 2011, Gültekin & Podlussány 2012a). Some species of the genus are considered useful as they are used as biological control agents against invasive thistles and knapweeds (Woodburn & Briese 1996, Louda & O'Brien 2002, Gültekin et al. 2008, Minteer et al. 2011, Gültekin & Anderson 2017).

Larinus sibiricus Gyllenhal, 1835 is known from SE Europe and Western Asia (Gültekin &

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Podlussány 2012b). Its biology and host plants were studied in details for the first time in NE Anatolia (Gültekin & Korotyaev 2005). Later, Zotov (2009) described the preimaginal stages of the species. It is associated with xerothermic habitats inhabited by *Xeranthemum annuum* L. that has been recorded as the main host of this weevil (Gültekin & Korotyaev 2005). The species is common in Bulgaria from May to October (Angelov 1978).

The genus *Xeranthemum* L. is known from Southern Europe, North Africa and South-western Asia and includes five annual species used as ornamental flowers (Garnatje & Martín 2007). *Xeranthemum annuum* (immortelle, annual everlasting) is a thermophilic species and grows on arid rocky meadows, sands, dry slopes, roadsides, fields in Southern Europe and Anatolia (Stanković et al. 2011).

So far, only *Bracon urinator* is known to parasitise the larvae of *L. sibiricus* (Gültekin & Korotyaev 2005). The aim of the article is to present new information on the complex of parasitoid species associated with *L. sibiricus*.

Materials and Methods

This study was conducted in 2016 in Arbanasi Village, Veliko Tarnovo (N43°05′50.1″, E025°40′38.3″). A total of 1021 flower heads of *X. annuum* were collected by the first author. The material has been stored under laboratory conditions in plastic boxes darkened with an aluminium foil and with an additional transparent tube for the removal of the imagoes. Emerged insects were fixed in ethanol and studied latter under stereo microscope.

Identification of the weevil and its parasitoids was based on keys by Graham (1969, 1987, 1991), Angelov (1978), Trjapitzin (1989), Dzhanokmen & Herthevtzian (1990), Bouček & Rasplus (1991), Wahl & Sharkey (1993), Noyes & Hayat (1994), Tobias (1995), Kolarov (1997), Zerova & Seryogina (1999), Papp (2008), Zerova (2010), Gibson & Fusu (2016), Varga (2017), Gültekin & Perrin (2018) and Klimmek & Baur (2018).

The specimens are preserved as card-mounted specimens in the authors' collection (University of Plovdiv and Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences).

Results

During the study, we reared 119 specimens of *L. sibiricus* from flower heads of *X. annuum* along with 12 species (Table 1) of hymenopterans belonging to six families of Chalcidoidea (Encyrtidae, Eulophidae, Eupelmidae, Eurytomidae, Pteromalidae and Torymidae) and two families of Ichneumonoidea (Braconidae and Ichneumonidae). In this study, *Leptomastix ephyra* was recorded as a new species for the Balkan Peninsula and Europe. Three species, *Eurytoma curculionum*, *L. ephyra* and *Norbanus cerasiops*, were recorded for first time from Bulgaria. With the exception of one species (*Bracon urinator*), all other parasitoid species were established for the first time from this host.

Discussion

Bracon urinator is a polyphagous parasitoid whose host range includes various insects belonging to Coleoptera and Diptera (Beyarslan et al. 2017). In Bulgaria, it has been recorded for the first time in the region of Marikostino Village by Atanassov (1962). So far, it is the only known species parasitising the larvae of *L. sibiricus* in flower heads of *X. annuum* (Gültekin & Korotyaev 2005).

Exeristes roborator is a polyphagous ectoparasitoid, attacking the larvae of a wide range of insect hosts belonging to Coleoptera, Hymenoptera and Lepidoptera (Thompson & Parker 1928). It is known also as a hyperparasitoid of *Gregopimpla malacosomae* (Seyrig) (Ichneumonidae) (Kolarov 1997, 2012, Aliyev & Maharramova 2009). Fifteen *Larinus* species are known as hosts of the species (Volovnik 1994, Kasparyan & Gültekin 2002, Perju 2005, Gültekin et al. 2008, Aliyev & Maharramova 2009, Daneshvar et al. 2009, Pisică & Popescu 2009, Kolarov 2012). Of these, it has been recorded as a parasitoid of *Larinus pollinis* Laicharting (= *brevis* Herbst) in Bulgaria (Kolarov 2012). Considering the wide variety of hosts of this ichneumonid species, its appearance in our reared material is not surprising. Although this parasitoid is associated with high number of plants of the family Asteraceae, we found no information for its rearing from immortelle. Thus, our study added *X. annuum* as a new associated plant of *E. roborator*.

Cyrtoptyx Delucchi, 1956 is a small pteromalid genus of parasitic wasps consisting of 11 species (Noyes 2019). Only one species is known from Bulgaria – *C. latipes* (Rondani) (= *C. dacicida* Masi), recorded by Thuróczy (1990). The hosts of the species of *Cyrtoptyx* include various holometabolous insects belonging to four orders: Coleoptera, Diptera, Hymenoptera and Lepidoptera (Noyes 2019). Species of the genus are associated with plants belonging to Amaranthaceae, Anacardiaceae, Apiaceae, Cannabidaceae, Capparidaceae, Caryophyllaceae, Chenopodiaceae, Fabaceae, Fagaceae, Oleaceae, Scrophulariaceae, Tamaricaceae and Zygophyllace-

Table 1. Parasitoids reared from flower heads of *Xeranthemum annuum* L. from Arbanasi Village.

Parasitoids	Number of parasitoids		Collection date of flower heads
	♀♀	♂♂	
Braconidae Latreille, 1829			
Bracon urinator (Fabricius, 1798)	2	2	15.VII.2016
Ichneumonidae Latreille, 1802			
Exeristes roborator (Fabricius, 1793)	5	8	15.VII.2016
Pteromalidae Dalman, 1820			
Cyrtoptyx sp.	1	2	15.VII.2016
Norbanus cerasiops (Masi, 1922)	1		15.VII.2016
Pteromalus vibulenus (Walker, 1839)	2		15.VII.2016
Stenoselma nigrum Delucchi, 1956	2		15.VII.2016
Torymidae Walker, 1833			
Exopristus trigonomerus (Masi, 1916)	13	15	15.VII.2016
Encyrtidae Walker, 1837			
Leptomastix ephyra Noyes & Hayat, 1994	1		15.VII.2016
Eulophidae Westwood, 1829			
Aprostocetus venustus (Gahan, 1914)	1		15.VII.2016
Baryscapus sp. near *carthami* Graham, 1991	10	7	15.VII.2016
Eupelmidae Walker, 1833			
Eupelmus microzonus **Förster, 1860**	1		15.VII.2016
Eurytomidae Walker, 1832			
Eurytoma curculionum Mayr, 1878	4	3	15.VII.2016

ae (Noyes 2019). We added the family Asteraceae in the list of the plant associations of the genus.

Norbanus cerasiops is known as a primary parasitoid of the larvae of various curculionid beetles belonging to the genera *Larinus* and *Lixus* Fabricius (Graham 1969, Bouček 1977, Rizzo & Mitroiu 2010, Dzhanokmen 2017). It may be a hyperparasitoid through *Bracon intercessor* Nees (Hymenoptera: Braconidae) or *Eurytoma* sp. (Hymenoptera: Eurytomidae) (Graham 1969). Detailed information about its distribution and hosts is provided by Rizzo & Mitroiu (2010) and Dzhanokmen (2017). In this study, an association of *N. cerasiops* with *L. sibiricus* on *X. annuum* is recorded for the first time, but a secondary parasitism via *B. urinator* and *E. curculionum*, emerged from the same flower heads, cannot be excluded.

Pteromalus vibulenus is known as a primary parasitoid attacking various hosts belonging to eight families of three insect orders: Coleoptera, Hymenoptera and Lepidoptera (Noyes 2019). In Bulgaria, it has been recorded by Thuróczy (1990) and Todorov (2013). Among species of *Larinus*, only *L. sturnus* (Schaller) has been recorded as a primary host of *P. vibulenus* and its plant associations mainly include plants of the family Asteraceae (Noyes 2019).

Stenoselma nigrum is recorded as a primary parasitoid of Buprestidae (Coleoptera), Cynipidae (Hymenoptera) and Sesiidae (Lepidoptera); it has been known as associated with six plant species belonging to Asteraceae, among which *X. annuum* is absent (Noyes 2019).

According to Zerova & Seryogina (1999), *Exopristus trigonomerus* is a parasitoid of species of Coleoptera and Diptera developing in stems and galls of herbaceous plants. The species has been recorded as a parasitoid of *Pachytychius hordei* (Brullé) (Coleoptera: Curculionidae) (Altinayar 1981) and later reared from seeds of *Scrophularia* sp. (Scrophulariaceae) along with *Gymnetron bipunctulatum* Rossi (Coleoptera: Curculionidae) (Zerova & Seryogina 1999). Bolu (2006) obtained it from larvae of the apple fruit weevil *Tatianaerhynchites aequatus* (L.) (Coleoptera: Rhynchitidae) collected in Turkey. Recently, Cobo et al. (2014) recorded it as a parasitoid of *Larinus canescens* Gyllenhal developing inside the capitulum of *Centaurea ornata* Willd. (Asteraceae) in Spain. In Bulgaria, *E. trigonomerus* has been reported by Angelov (1970), Stojanova (2014) and Stojanova & Antov (2018). Noyes (2019) listed it as a hyperparasitoid through species of the genus *Bracon*. *Exopristus trigonomerus* is a primary, solitary parasitoid of *L. sibiricus*, but secondary parasitism via *B. urinator* emerged from the same flower heads cannot be excluded.

Leptomastix ephyra has been reported as a parasitoid of the solanum mealybug *Phenacoccus solani* Ferris (Hemiptera: Pseudococcidae) in Israel

(Protasov & Mendel 2018). It is known from India (Noyes & Hayat 1994), Israel (Protasov & Mendel 2018) and Turkey (Japoshvili 2012). The establishment of this parasitoid in our material is surprising because the species of *Leptomastix* Förster are primary, solitary endoparasitoids of many Pseudococcidae and most of them are associated with mealybugs on trees or shrubs and less often on grasses (Noyes & Hayat 1994). With this in mind as well as having only one reared specimen, we suppose that this may be an accidental association.

Aprostocetus venustus is considered as a parasitoid of species of *Bruchophagus* Ashmead (Hymenoptera: Eurytomidae) in seeds of *Medicago sativa* L. and *Onobrychis* Miller (Fabaceae) (Graham 1987, LaSalle 1994, Yefremova et al. 2007) as well as a parasitoid of species of *Contarinia* Rondani (Diptera: Cecidomyiidae) in *Sorghum* (Poaceae) and *Mango* (Anacardiaceae) (Yefremova et al. 2007). In Bulgaria this species was reared from seeds of *Robinia pseudoacacia* L. (Fabaceae), along with *Bruchophagus robiniae* Zerova (Hymenoptera: Eurytomidae) (Boyadzhiev 2006).

We think that *Baryscapus* sp. near *carthami* is an undescribed species and differs from females of *B. carthami* mainly in structure of antennae (with slender clava and comparatively long terminal spine), length of propodeum and ovipositor sheaths. Its taxonomic characterisation will be a subject of further studies.

Noyes (2019) recorded *Eupelmus microzonus* as a primary parasitoid of Apionidae, Chrysomelidae (=Bruchidae) and Curculionidae (Coleoptera), Cecidomyiidae, Chloropidae and Tephritidae (Diptera), Cynipidae and Eurytomidae (Hymenoptera), Lasiocampidae, Psychidae and Pyralidae (Lepidoptera) as well as a hyperparasitoid through *Aleiodes esenbeckii* (Hartig) (= *Rhogas dendrolimi* (Matsumura)) (Hymenoptera: Braconidae). The plant associations include species of Asteraceae, Fabaceae, Fagaceae, Lamiaceae, Papaveraceae, Poaceae, Rosaceae, Zygophyllaceae (Noyes 2019) and Caryophyllaceae (Antov & Stojanova 2015). Considering the broad trophic relationships of the species, its establishment in our reared material is not unexpected. Here we cannot firmly confirm that this is a primary parasitoid of *L. sibiricus* due to the presence of other possible primary hosts. Although it is associated with high number of species of Asteraceae, we found no information for its rearing from immortelle. Since we have only one reared specimen, we assume that this may be an accidental association.

Eurytoma curculionum is a polyphagous parasitoid attacking the larvae of a number of species of Coleoptera (Claridge & Askew 1960) and less often those of other insects that feed on plant material (Zerova & Proshchalykin 2012). Muller et al. (2011a) reported it for the first time as a parasitoid of *Ceutorhynchus turbatus* Schultze (Coleoptera: Curculionidae) on *Cardaria draba* (L.) Desv. (Brassicaceae). Muller et al. (2011b) summarised the published data on the known hosts of this species and reported it from larvae of *Microplontus edentulus* (Schultze) (Coleoptera: Curculionidae). It was also bred from *Rhinusa pilosa* (Gyllenhal) (Coleoptera: Curculionidae) galls on *Linaria vulgaris* Mill. (Plantaginaceae) in Serbia (Gassmann et al. 2014). According to records by Noyes (2019), *Bracon intercessor* and *Norbanus cerasiops* (= *Picroscytoides cerasiops*) are hosts of *E. curculionum*. Published records indicate that the majority of the known hosts of this parasitoid belong to the family Curculionidae, so it is not surprising to find it associated with *L. sibiricus.* In our study, the host-parasitod association *L. sibiricus – E. curculionum* is newly recorded, but secondary parasitism via *B. urinator* and *N. cerasiops* that emerged from the same flower heads cannot be excluded. The review of the literature revealed that *E. curculionum* is associated with plants of Asteraceae, Brassicaceae, Campanulaceae, Malvaceae, Plantaginaceae and Polygonaceae (Mayr 1878, Claridge & Askew 1960, Bouček 1977, Zerova 2010, Muller et al. 2011a, b, Gassmann et al. 2014, Kovács et al. 2017). The plant association with *X. annuum* is mentioned here for the first time. The species is widespread in Europe but previously it has not been recorded from Bulgaria (new record).

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