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CHALCIDOID PARASITOIDS (HYMENOPTERA: CHALCIDOIDEA) ASSOCIATED WITH *SIBINIA SUBELLIPTICA* (DESBROCHERS, 1873) (COLEOPTERA: CURCULIONIDAE) IN BULGARIA

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ABSTRACT

The weevil *Sibinia subelliptica* (Desbrochers, 1873) and a rich complex of associated chalcidoid parasitoids were reared from inflorescences of *Dianthus giganteus* d'Urv. (Caryophyllaceae) in Bulgaria. The following taxa were identified: *Aprostocetus venustus* (Gahan) and *Baryscapus* sp. near *garganus* (Domenichini, 1958) (Eulophidae); *Eupelmus barai* Fusu and *E. microzonus* Förster (Eupelmidae); *Aximopsis augasmae* (Zerova), *A. collina* (Zerova), *Eurytoma coleophorae* Zerova and *E. coleopterae* Zerova (Eurytomidae); *Catolaccus crassiceps* (Masi) and *Pteromalus ochrocerus* (Thomson) (Pteromalidae); and *Exopristus trigonomerus* (Masi) (Torymidae). Two of these, *E. coleophorae* and *E. coleopterae*, are new records for Bulgaria. All parasitoid-host relationships are documented here for the first time. New plant associations with *D. giganteus* are recorded here for all reared parasitoids except *E. barai* and *E. microzonus*. The new trophic association of *S. subelliptica* with *D. giganteus* is established here.

Key words: *Dianthus giganteus*, parasitoid complex, new records, new host, new associations.

RESUMEN

Parasitoides calcidoides (Hymenoptera: Chalcidoidea) asociados con *Sibinia subelliptica* (Desbrochers, 1873) (Coleoptera: Curculionidae) en Bulgaria

El gorgojo *Sibinia subelliptica* (Desbrochers, 1873) y un rico complejo de parasitoides calcidoides asociados se criaron a partir de inflorescencias de *Dianthus giganteus* d'Urv. (Caryophyllaceae) en Bulgaria. Se identificaron los siguientes taxones: *Aprostocetus venustus* (Gahan) y *Baryscapus* sp. cercana a *garganus* (Domenichini, 1958) (Eulophidae); *Eupelmus barai* Fusu y *E. microzonus* Förster (Eupelmidae); *Aximopsis augasmae* (Zerova), *A. collina* (Zerova), *Eurytoma coleophorae* Zerova y *E. coleopterae* Zerova (Eurytomidae); *Catolaccus crassiceps* (Masi) y *Pteromalus ochrocerus* (Thomson) (Pteromalidae); y *Exopristus trigonomerus* (Masi) (Torymidae). Dos de éstos, *E. coleophorae* y *E. coleopterae*, son registrados como nuevos para Bulgaria. Todas las mencionadas relaciones parasitoide-hospedador se documentan por primera vez, así como se registra por primera vez la asociación de la planta *D. giganteus* con todos los parasitoides obtenidos, excepto en los casos de *E. barai* y *E. microzonus*. Se establece la nueva asociación trófica de *S. subelliptica* con *D. giganteus*.

Palabras clave: *Dianthus giganteus*, complejo parasitoide, nuevos registros, nuevo hospedador, nuevas asociaciones.

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Introduction

The genus *Sibinia* Germar, 1817 (Coleoptera: Curculionidae: Curculioninae) includes three subgenera: *Dichotychius* Bedel, 1885, *Microtychius* Casey, 1910 and *Sibinia* s. str. (Skuhrovec *et al*., 2015). The Palaearctic *S.* (*Sibinia*) species develop in plant representatives of different genera belonging to family Caryophyllaceae [*Arenaria* L., *Cerastium* L., *Dianthus* L., *Eremogone* Fenzl, *Gypsophila* L., *Lychnis* L., *Melandrium* Röhl., *Minuartia* L., *Petrorhagia* (Ser.) Link, *Psammophiliella* Ikonn., *Saponaria* L., *Silene* L., *Spergula* L., *Spergularia* (Pers.) J. Presl & C. Presl] (Szénási, 2013; Yunakov *et al*., 2018). Species in subgenus *Dichotychius* live on Plumbaginaceae (Skuhrovec *et al*., 2015) and those in *Microtychius* have hosts belonging to different genera of family Fabaceae (Clark, 1978). Species of *Sibinia* are trophically associated with narrow host ranges. Some of them are monophagous whereas other are restricted to two or more plant species of a single genus. The larvae of most species feed mainly on the seeds of their hosts (Clark, 1978). *Sibinia* species are found in different types of habitats: salty lands, sands, xerothermic grasslands and meadows, steppes, dry and stony slopes, forest edges, agricultural habitats and salt marshes (Yunakov *et al*., 2018). Some species of the genus are considered as bioindicators of natural sandy habitats (Košťálová & Szénási, 2015).

Sibinia subelliptica (Desbrochers, 1873) is a Palaearctic curculionid species known from Europe, Anatolia, Siberia, East Asia (Russian Far East, N Korea, Japan, NE China) and N Africa (Algeria) (Balalaikins, 2013). It is a thermophilous oligophagous beetle that develops on plant species from the genus *Dianthus*, mostly in xerothermic habitats (Angelov, 1980). Its known hosts are: *D. acicularis* Fischer ex Ledebour (Dedyukhin & Martynenko, 2020), *D. andrzejowskianus* Kulcz. (Yunakov *et al*., 2018), *D. armeria* L. (Tempère & Péricart, 1989), *D. balbisii* Ser. (Clark, 1978), *D. borbasii* Vandas (Yunakov *et al*., 2018), *D. carthusianorum* L. (Cmoluch *et al*., 1975; Clark, 1978), *D. ferrugineus* Miller (Teodor, 2011), *D. pratensis* Bieb. (Dedyukhin, 2014), *D. sylvestris* Wulfen (Teodor, 2011), *D.* ?*versicolor* Fisch. ex Link (Dedyukhin, 2012). The females lay their eggs at the base of the flowers (Angelov, 1980). The complete development of the species from egg to imago takes place in the flowers of the host plant without the larva to enter the soil to pupate (Alziar, 1976), although pupation in the soil has been reported in the literature (Angelov, 1980; Tempère & Péricart, 1989). The species is common in Bulgaria from May to July from 200 to 1000 m a. s. l. (Angelov, 1980).

The genus *Dianthus* L. (Caryophyllaceae) is represented by annual, biennial and perennial herbaceous plants (Kirillov *et al*., 2016). It is widespread mainly in Europe, the Mediterranean region and in Asia, and a few species are known from Africa and America (Chandra *et al*., 2016). *Dianthus giganteus* d'Urv. is a perennial plant that grows on stony and grassy places in the lowlands and mountains. It occurs throughout Bulgaria up to 2100 m a. s. l. It is also distributed in Albania, former Yugoslavia, Greece, Italy, Romania, Turkey and SW Asia (Stojanov, 1966).

There is no information in the literature about parasitoids of the curculionid beetle *S. subelliptica*. The aim of this study is to provide the first data on a parasitoid complex probably associated with this weevil.

Material and methods

Over 2100 dry inflorescences of *D. giganteus* were collected on 1.VIII.2015 and on 15.VII. 2016 in

Fig. 1.– Dissected flowers of *Dianthus giganteus* infected by *Sibinia subelliptica*. Scale bar = 1 mm.

Fig. 1.– Flores disecadas de *Dianthus giganteus* infectadas por *Sibinia subelliptica*. Escala = 1 mm.

Arbanasi Village near Veliko Tarnovo (43°05'50.1" N 25°40'38.3" E, 417 m). The collected material was stored in laboratory conditions in plastic bottles darkened with an aluminium foil and with an additional transparent tube at temperature 23-26° C. The tops of the bottles were cut out and covered with white cheesecloth. The material was stored for at least one year until all emergences had ceased. Emerged parasitoids were aspirated with a pooter, fixed in ethanol and later examined under a stereo microscope. During the dissection of at least 300 inflorescences, we found 500 imagos of *S. subelliptica* at the base of the flowers (Fig. 1). Only 12 specimens were observed outside the inflorescences.

The weevil and its parasitoids were identified following Graham (1969, 1987, 1991), Angelov (1980), Grissell (1995), Zerova & Seryogina (1999), Zerova (2010), Gibson & Fusu (2016), Fusu (2017) and Fursov *et al*. (2019).

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

Colour photographs were taken with a Canon 5D Mark III digital camera.

Fig. 2.– Inflorescence of *Dianthus giganteus* with emerging holes of the chalcidoid parasitoids associated with *Sibinia subelliptica* (view from the lower side). Scale bar = 1 cm.

Fig. 2.– Inflorescencia de *Dianthus giganteus* con orificios de las emergencias de los parasitoides calcidoideos asociados con *Sibinia subelliptica* (vista desde la parte posterior). Escala = 1 cm.

Results

During the study we reared 512 specimens of *S. subelliptica*, along with 905 specimens of hymenopteran parasitoids belonging to 11 species (Table 1) of five families of Chalcidoidea (Eulophidae, Eupelmidae, Eurytomidae, Pteromalidae and Torymidae), from inflorescences of *D. giganteus* (Fig. 2).

Since only 1/7 of the inflorescences were dissected, the number of beetles in them can be expected to exceed 3500 individuals. In addition to these, we obtained hundreds of thrips (Thysanoptera) and four specimens of unidentified species of Chrysopidae (Neuroptera). Whilst *S. subelliptica* was not proven to be the host species of all these parasitoids, we believe that it was very probably the host (primary or secondary) of at least the most frequently reared Hymenoptera. Two of the parasitoid species, *Eurytoma*

Table 1.– Parasitoids reared from inflorescences of *Dianthus giganteus* d'Urv. from Arbanasi Village.

Tabla 1.– Parasitoides obtenidos a partir de inflorescencias de *Dianthus giganteus* d'Urv. de Arbanasi Village.

Family	Parasitoid species	Number of individuals			
		01.VIII.2015		15.VII.2016	
		♀♀	♂♂	♀♀	♂♂
Eulophidae	*Aprostocetus venustus* (Gahan, 1914)	11	4	51	25
	Baryscapus sp. near *garganus*	7	1	–	–
Eupelmidae	*Eupelmus barai* Fusu, 2017*	–	–	6	–
	Eupelmus microzonus Förster, 1860**	9	–	6	–
Eurytomidae	*Aximopsis augasmae* (Zerova, 1977)	–	–	2	2
	Aximopsis collina (Zerova, 1984)	63	30	39	42
	Eurytoma coleophorae Zerova, 1977	–	–	3	–
	Eurytoma coleopterae Zerova, 1978	45	7	52	25
Pteromalidae	*Catolaccus crassiceps* (Masi, 1911)	–	–	2	–
	Pteromalus ochrocerus (Thomson, 1878)	51	27	36	14
Torymidae	*Exopristus trigonomerus* (Masi, 1916)	135	54	87	69

* Number of specimens reported by Antov & Stojanova (2020).
** Number of specimens reported by Antov & Stojanova (2015, 2020).

coleophorae and *E. coleopterae*, are recorded for the first time from Bulgaria. All parasitoids are suggested to be associated with *S. subelliptica* for the first time and, with the exception of *E. barai* and *E. microzonus*, all parasitoid species are newly associated with *D. giganteus*. Also, *D. giganteus* is recorded here as a new host plant of *S. subelliptica*.

Although we cannot conclusively confirm that all reared parasitoids are primary parasitoids of *S. subelliptica*, their large number (with the exception of *C. crassiceps* and *E. coleophorae*) suggests a more common than accidental association with this host.

Discussion

The review of the literature shows that there are no data about parasitoids of *S. subelliptica*. However, parasitoids belonging to four families of Chalcidoidea (Eulophidae, Eupelmidae, Eurytomidae and Pteromalidae) and one family of Ichneumonoidea (Braconidae) have been recorded on eight other *Sibinia* spp. (Table 2).

Aprostocetus venustus is a parasitoid of species of *Bruchophagus* Ashmead (Hymenoptera: Eurytomidae) in seeds of Fabaceae (Graham, 1987; Boyadzhiev, 2006; Ribes Escolà & Askew, 2009) as well as of species of *Contarinia* Rondani (Diptera: Cecidomyiidae) in *Sorghum* (Poaceae) [as *Tetrastichus venustus* Gahan] (Wiseman *et al*., 1978) and *Mango* (Anacardiaceae) (Yefremova *et al*., 2007). It has been also reared from *Dasineura oleae* (Angelini) (Diptera: Cecidomyiidae) on *Olea europaea* L. (Oleaceae) (Doganlar, 1992). Recently, *A. venustus* was reared from flower heads of *Xeranthemum annuum* L. (Asteraceae) along with *Larinus sibiricus* Gyllenhal (Coleoptera: Curculionidae) and a rich complex of hymenopteran parasitoids associated with this beetle (Antov *et al*., 2020). We found no information in the literature for previous rearings of this eulophid species from *D. giganteus*.

Baryscapus Förster, 1856 is a large and biologically diverse genus in the subfamily Tetrastichinae (Eulophidae) (Graham, 1991) comprising 128 species with a cosmopolitan distribution (Noyes, 2019). Species of the genus develop mostly as gregarious or more rarely as solitary endoparasitoids of larvae or pupae of a wide variety of insect hosts belonging to Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Neuroptera. Some species of *Baryscapus* are secondary parasitoids of species of the families Braconidae and Ichneumonidae and of the superfamilies Chalcidoidea and Cynipoidea (Graham, 1991). We assume that *Baryscapus* sp. near *garganus* is an undescribed species and differs from females of *B. garganus* mainly in structure of antennae and thorax as well as the longer abdomen. Its taxonomic characterization will be a subject of further studies.

Eupelmus barai is a polyphagous parasitoid whose host range include various insects belonging to Coleoptera (Curculionidae), Diptera (Cecidomyiidae and Tephritidae), Hymenoptera (Cynipidae, Diprionidae and Eurytomidae) and Lepidoptera (Coleophoridae, Millieridae, Tortricidae and Yponomeutidae). In the family Curculionidae, only *Scolytus rugulosus* (Müller) has been recorded as its host from Moldova (Fusu, 2017).

Eupelmus microzonus is a polyphagous parasitoid attacking the larvae and pupae of a wide range of insect hosts that are usually concealed in galls, flower heads, seeds, grass stems or cocoons and belong to Coleoptera, Diptera, Hymenoptera and Lepidoptera

Table 2.– List of the known hymenopterous parasitoids and their *Sibinia* hosts obtained from: 1 – Rogers *et al*. (1975), 2 – Clark (1978), 3 – Gibson (2013), 4 – Beyarslan & Şahin (2019), 5 – Fursov *et al*. (2019).

Tabla 2.– Lista de los parasitoides himenópteros conocidos y de sus hospedadores en *Sibinia* obtenidos a partir de: 1 – Rogers *et al*. (1975), 2 – Clark (1978), 3 – Gibson (2013), 4 – Beyarslan & Şahin (2019), 5 – Fursov *et al*. (2019).

***Sibinia* spp.**	**Parasitoids**
S. femoralis Germar	*Bracon intercessor* Nees (Braconidae)[4] *Eurytoma coleopterae* Zerova (Eurytomidae)[5]
S. inermis (Casey)	*Eutrichosoma mirabile* Ashmead (Pteromalidae)[2]
S. pallida (Schaeffer)	*Lyrcus* sp. [= *Zatropis* sp.] (Pteromalidae)[2]
S. seminicola Clark	*Horismenus* sp. (Eulophidae)[2] *Eupelmus* sp. (Eupelmidae)[2] Pteromalinae (Pteromalidae)[2]
S. setosa (LeConte) [= *S. sulcatula* (Casey)]	*Eurydinoteloides perdubia* (Girault) [= *Zatropis perdubius* (Girault)] (Pteromalidae)[1] *Lyrcus capitis* (Burks) [= *Zatropis capitis* Burks] (Pteromalidae)[1] *Eutrichosoma mirabile* Ashmead (Pteromalidae)[2] *Trimeromicrus maculatus* Gahan (Pteromalidae)[3]
S. simplex (Casey)	*Eutrichosoma mirabile* Ashmead (Pteromalidae)[2] *Tetrastichus* sp. (Eulophidae)[2]
S. variegata (Casey)	*Urosigalphus breviovipositorus* Gibson (Braconidae)[2]
S. viscariae (Linnaeus)	*Bracon variator* Nees (Braconidae)[4]

(Bouček, 1977; Antov & Stojanova, 2020). The following curculionid beetles are known as its hosts: *Anthonomus pomorum* (Linnaeus) (Gibson & Fusu, 2016), *Ceutorhynchus obstrictus* (Marsham) (Haye *et al*., 2018), *Curculio glandium* Marsham (Kostjukov *et al*., 2018), *Microlarinus lareynii* (Jacquelin du Val) (Soydanbay-Tunçyürek, 1976), *Pachytychius hordei* (Brullé) (Gözüaçık & Şimşek, 2015), *Tychius aureolus* Kiesenwetter [= *T. femoralis* Brisout] and *T. flavus* Becker (Nikolskaya, 1952). Recently, the species was also associated with *L. sibiricus* (Antov *et al*., 2020). It has been recorded as a parasitoid of three weevil species of the family Brentidae: *Catapion seniculus* (Kirby) [= *Apion seniculus* Kirby] (Mambetova, 1975, as cited by Noyes, 2019), *Hemitrichapion reflexum* (Gyllenhal) (Muhtarova *et al*., 2009) and *Oxystoma ochropus* (Germar) (Lotfalizadeh & Hashemi, 2015).

Aximopsis augasmae has been recorded as a parasitoid of the larvae of *Augasma atraphaxidellum* Kuznetzov (Lepidoptera: Coleophoridae) on *Atraphaxis spinosa* L. (Polygonaceae) and some other moth species on *Zygophyllum* L. (Zygophyllaceae) (Zerova & Seryogina, 2006). It was also reported as a parasitoid of *Etiella zinckenella* (Treitschke) (Lepidoptera: Pyralidae) in pods of *Sophora alopecuroides* L. (Fabaceae) (Lotfalizadeh & Hosseini, 2014). There is no data for its rearing from *D. giganteus*.

Aximopsis collina has been recorded as a parasitoid in *Diplolepis* galls on *Rosa* spp. (Lotfalizadeh *et al*., 2006; Zerova, 2010; Mete & Mergen, 2017). Recently, it was associated with seeds of three *Asphodelus* spp. (Delvare *et al*., 2019). There is no data for its rearing from *D. giganteus*.

Eurytoma coleophorae has been reared from galls of *Coleophora serinipennella* Christoph (Lepidoptera: Coleophoridae) on *Atriplex* sp. (Amaranthaceae) and *Ascalenia kabulella* Kasy (Lepidoptera: Cosmopterigidae) on *Myricaria alopecuroides* Schrenk (Tamaricaceae) (Zerova, 2010). There is no data for its rearing from *D. giganteus*. The species is distributed in SE Europe and Kyrgyzstan (Zerova, 2010). This is the first record from Bulgaria.

Eurytoma coleopterae is a parasitoid attacking the larvae of small beetles whose development takes place in stems, galls and seeds of herbaceous plants (Fursov *et al*., 2019). The species has been reared from larvae of *Lixus salsolae* Faust. and *Sibinia femoralis* Germar (Coleoptera: Curculionidae) in galls on *Silene wolgensis* (Hornem.) Otth [= *Otites wolgensis* (Hornem.) Grossh.] (Caryophyllaceae) (Zerova, 1978). Recently, it was obtained from larvae of *Metapion* sp. (Coleoptera: Curculionidae) developing in seeds of *Ruta* sp. (Rutaceae) (Fursov *et al*., 2019). In the family Caryophyllaceae, this species is associated with *S. wolgensis*, but not with *D. giganteus*. It is known from Turkey, Turkmenistan and Ukraine (Fursov *et al*., 2019), but it has not been recorded from Bulgaria so far.

Catolaccus crassiceps is a polyphagous species that develops as a primary or secondary parasitoid on a wide variety of insect hosts belonging to Coleoptera (Curculionidae), Hymenoptera (Braconidae and Cynipidae), Lepidoptera (Coleophoridae, Crambidae, Gelechiidae, Noctuidae, Nolidae and Pieridae) and Neuroptera (Chrysopidae) (Dzhanokmen, 2017). It has been reared from cocoons of *Coniatus indicus* Marshall (Coleoptera: Curculionidae) on *Tamarix dioica* Roxb. ex Roth (Tamaricaceae) (Boućek *et al*., 1978). In the family Caryophyllaceae, this species is associated with *Gypsophila struthium* Loefl. (Askew *et al*., 2001), but not with *D. giganteus*.

Pteromalus ochrocerus is known as a parasitoid on species of Curculionidae (Coleoptera), Cynipidae (Hymenoptera) and Tephritidae (Diptera) (Cobo *et al*., 2016). Its host among the curculionid beetles is *A. pomorum* (Thompson, 1958). Plant associations include Asteraceae and Lamiaceae (Askew *et al*., 2006; Cobo *et al*., 2016), but not Caryophyllaceae.

Exopristus trigonomerus is a parasitoid of species of Coleoptera and probably Diptera concealed in galls and stems of herbaceous plants. Its known hosts among the curculionid beetles are: *Rhinusa bipustulata* (Rossi) [= *Gymnetron bipustulatum* (Rossi)] (Zerova & Seryogina, 1999), *Larinus canescens* Gyllenhal (Cobo *et al*., 2014), *L. sibiricus* (Antov *et al*., 2020) and *P. hordei* (Altınayar, 1981). It has been also obtained from larvae of the apple fruit weevil *Tatianaerhynchites aequatus* (L.) (Coleoptera: Rhynchitidae) in Turkey (Bolu, 2006). Plant associations include Asteraceae, Cistaceae, Fabaceae, Gnetaceae, Papaveraceae, Scrophulariaceae (Noyes, 2019) and Plantaginaceae (Bouček, 1977), but not Caryophyllaceae. In our study, *E. trigonomerus* is the most numerous parasitoid with 189 specimens reared in 2015 and 156 specimens in 2016.

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