

PARASITOID COMPLEX OF *ZYGAENA FILIPENDULAE* L. (LEPIDOPTERA: ZYGAENIDAE)

V. ŽIKIĆ^{1*}, S. S. STANKOVIĆ¹, A. PETROVIĆ², MARIJANA ILIĆ-MILOŠEVIĆ¹ and
KEES VAN ACHTERBERG³

¹ Faculty of Sciences, Department of Biology and Ecology, University of Niš, 18000 Niš, Serbia

² Institute of Zoology, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia

³ Department of Terrestrial Zoology, Naturalis Biodiversity Center, Postbus 9517, 2300 RA Leiden, Netherlands

Abstract - Caterpillars of *Zygaena filipendulae* Linnaeus were sampled during May and June in the Sicevo Gorge in southern Serbia. All parasitized larvae were found on grey elm trees (*Ulmus canescens*). During the short period before metamorphosis of *Z. filipendulae*, we found the whole specter of parasitoid wasps: *Cotesia zygaenarum* Marshall (Braconidae), *Gelis agilis* (Fabricius) and *Mesochorus velox* Holmgren (Ichneumonidae), *Elasmus platyedrae* Ferrière and *Pediobius* sp. (Eulophidae), *Eupelmus vesicularis* (Retzius) (Eupelmidae) and *Brachymeria tibialis* (Walker) (Chalcididae). Beside hymenopteran parasitoids, we found parasitoid flies from the family Tachinidae, *Phryxe nemea* (Meigen) (Diptera). All 46 observed *Z. filipendulae* larvae found on grey elm trees were parasitized, but three pupae were found directly on *Lotus corniculatus*. Two species are newly reported as parasitoids of *Z. filipendulae*: *E. platyedrae* and *Eupelmus vesicularis* and three species (*G. agilis*, *M. velox* and *E. platyedrae*) are new to the fauna of Serbia.

Key words: *Zygaena filipendulae*, parasitoids, hyperparasitoids, trophic associations

INTRODUCTION

The family Zygaenidae (burnet moths) contains day-flying moths that are distributed throughout Europe. More than 45 species of burnet moths are capable of sequestering the cyanogenic glucosides linamarin and lotaustralin from their food plants (Zagrobelny et al., 2007), as well as of biosynthesizing it *de novo* from the parent amino acids valine and isoleucine (Nährstedt, 1988). Biosynthesis and sequestration of these poisonous compounds lower the predation on the larvae and adults (Zagrobelny et al., 2007). *Zygaena filipendulae* Linnaeus 1758 (six-spot burnet moth) is a common European species, usually found in large colonies in grassy areas. Females lay their eggs in irregularly shaped batches on the leaves of food-plants in the late summer (Hofmann and

Kia-Hofmann, 2011). In Serbia, after overwintering the larvae resume feeding in April until the middle of May. The larvae feed mainly on bird's-foot trefoil (*Lotus corniculatus*). The biology of the six-spot burnet is well-studied (Holik, 1938) as well as the biochemistry of biosynthesizing and sequestering of cyanogenic glucosides (Zagrobelny and Moller, 2011). On the other hand, knowledge about the relationships between *Z. filipendulae* and its natural enemies, especially parasitoids, is scarce (Askew and Shaw, 2001; Kaźmierczak and Dabrowski, 2003; Askew et al., 2008; Shaw et al., 2010).

The larvae of many *Zygaena* species represent suitable hosts for various parasitoids from the order Hymenoptera (e.g. Schwarz and Shaw, 1998; Bailevski, 1999; Askew and Shaw, 2001). Kaźmierczak

and Dabrowski (2003) reported as many as 51 species of hymenopteran parasitoids (from seven families) that parasitize Zygaenidae in southern Poland. The most common parasitoids of burnets belong to the superfamilies Ichneumonoidea (Braconidae and Ichneumonidae) and Chalcidoidea (Chalcididae, Elasmidae, Eulophidae, Pteromalidae, Torymidae and Trichogrammatidae); most of them are gregarious or solitary internal koinobionts (Shaw and Askew, 1976; Shaw and Huddleston, 1991; Grisell, 1995; Rubio, 2005).

Many parasitoids from the subfamily Microgasterinae (Braconidae) are known as gregarious parasitoids of various caterpillars, including *Zygaena* species, species of the genus *Cotesia*, e.g. *C. ancilla* (Nixon) and *C. zyganae* Marshall, or other members from this subfamily; *Apanteles* Foerster or *Microgaster* Latreille (Barbour, 2009; Güçlüand Özbek, 2011). Beside Microgasterinae, there are other braconid species, e.g. *Conspinaria* spp. (Quicke et al., 2004), as well as *Aleiodes*, e.g. *A. bicolor* (Spinola) attacking zygaenid larvae (Aydogdu and Beyarslan, 2005). *Cotesia zyganae* is a gregarious parasitoid of caterpillars belonging to the families Crambidae, Lymantriidae, Notodontidae, Nymphalidae, Pieridae, Plutellidae, Sphingidae, as well as Zygaenidae. From the genus *Zygaena* seven species are known: *Z. brizae* (Esper), *Z. ephialtes* (Linnaeus), *Z. filipendulae* (Linnaeus), *Z. laeta* (Hübner), *Z. loniceriae* (Scheven), *Z. trifolii* (Esper), and *Z. viciae* (Denis and Schiffermüller) (Yu et al., 2012). Its life cycle from egg to adult is very short, taking approximately 15-30 days, depending on the species and the climatological conditions, especially temperature.

Many ichneumonid species are hyperparasitoids of other parasitoid Hymenoptera, frequently collected from different burnets, e.g. *Agrothereutes* Foerster, *Gelis* Thunberg, *Mesochorus* Gravenhorst (Schwarz and Shaw, 1998) and *Listrognathus* Tschek (Gupta and Kamath, 1967). *Gelis agilis* (Fabricius, 1775) is recorded throughout Europe as a polyphagous species (Yu et al., 2012). There are about 135 known hosts for *G. agilis* inhabiting the Palaearctic region, belonging to the orders Lepidoptera, Dip-

tera, Coleoptera and Hymenoptera. *Gelis agilis* is known as a hyperparasitoid of 37 species of Braconidae belonging to the genera *Aleiodes*, *Bracon*, *Coeloides*, *Cotesia*, *Apanteles*, *Protapanteles*, *Dolcogaster*, *Microgaster* and *Macrocentrus* (Yu et al., 2012). The second important ichneumonid parasitoid is *Mesochorus velox* Holmgren 1860. The biology of *M. velox* has not been well investigated. It is a solitary koinobiont hyperparasitoid of primary parasitoids of ecto- and endoparasitoid Ichneumonoidea with a broad range of hosts. Hyperparasitoids oviposit the first two instars of the primary parasitoid, completing its life cycle in the final (= third) larval instar or pupal stage of the host (Yeargan and Braman, 1989).

The very diverse superfamily Chalcidoidea includes 17 families with about 22,500 described species, mainly cosmopolitan, but poorly investigated (Noyes, 1990). One of the well-studied and important members of the family Chalcididae that attacks the pupae of *Zygaena* spp. is *Brachymeria tibialis* (Walker 1834). It is a primary parasitoid or hyperparasitoid of mainly Lepidoptera, Diptera, Coleoptera and Hymenoptera (Askew and Shaw, 2001; Shaw et al., 2010). The second chalcidoid family of interest here is Eulophidae which contains very tiny parasitoid wasps that are primary internal parasitoids of the eggs, larvae or pupae of Lepidoptera, Coleoptera and Diptera (Goulet and Huber, 1993). *Elasmus* Westwood 1833 is a worldwide genus with about 28 species occurring in Europe. *Elasmus* species are the primary parasitoids of Lepidoptera cocoons and hyperparasitoids of the larvae of parasitoid Diptera and Hymenoptera (Askew et al., 1997). They are reared from Ichneumonidae, Braconidae and sporadically from nests of *Polistes* spp. (Vespidae) (Goulet, and Huber, 1993). *Elasmus platydras* Ferrière 1935 is known as gregarious ectoparasitoid of Gelechiidae, e.g. the diapausing larvae of *Pecrinophora gossypiella* (Saunders) (Hekal, 1991) and of the hollyhock seed moth *Pexicopia malvella* (Hübner) (Döganlar, 1985). Only three species are known for the Serbian fauna: *E. flabellatus* (Fonscolombe 1832), *E. nudus* (Nees 1834) and *E. viridiceps* Thomson 1878.

Another eulophid parasitoid is *Pediobius* sp. *Pediobius* is a large genus containing almost 200 species with cosmopolitan distribution with extremely wide host range (Noyes, 2002, 2003). Many species are gregarious endoparasitoids such as *P. furvus* (Gahan). There are only a few solitary parasitoids, associated with leafminers (Diptera: Agromyzidae) or thrips (Thysanoptera). In this genus, hyperparasitism occurs, e.g. *P. imbrues* (Walker), which is a hyperparasitoid of *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) – the black-headed caterpillar of coconut (Ghosh and Abdurahiman, 1988). Some species have been used as biological control agents, e.g. *P. foveolatus* (Crawford) against the Mexican bean beetle parasitoid.

The third chalcidoid family is Eupelmidae, representing ecto- or endoparasitoid wasps of various hosts from several insect orders: Orthoptera, Blattodea, Mantodea, Hemiptera, Neuroptera, Diptera, Lepidoptera, Hymenoptera and the wood-inhabiting Coleoptera that attack the immature stages of insects – final larval instar and pupa stages, as well as the eggs of Odonata and spiders (Yu et al., 2012). *Dermatopelte budensis* Erdős and Novicky is a well-studied species and a parasitoid on several *Zygaena* species (Askew et al., 2008). Another Chalcidoidea family attacking burnets is Torymidae, e.g. *Monodontomerus vicicellae* (Walker) (Shaw and Bland, 2007). An important species for this investigation is *Eupelmus vesicularis* (Retzius, 1783), a species primarily parasitizing gall wasps from the family Cynipidae (Hymenoptera) and galls caused by flies from the family Cecidomyiidae (Diptera).

Tachinidae (Diptera) are the primary parasitoids of *Zygaena* larvae (Ford and Shaw, 1991). *Phryxe nemea* (Meigen, 1824) is an endoparasitoid fly of caterpillars belonging to various butterfly families and sporadically is an endoparasitoid of sawflies. The biology of this species is not well investigated, but *Ph. prima* (Brauer and Bergens-tamm), which is a parasitoid of the widely spread *Zygaena carniolica* (Scopoli) or *Ph. magnicornis* (Zetterstedt), a parasitoid of *Z. lonicerae* (Scheven) are better studied.

MATERIALS AND METHODS

The caterpillars and pupae of *Zygaena filipendulae* were collected in the Sićevo Gorge in the southeastern part of Serbia, positioned near the city of Niš (N 43°19'41", E 22°03'56", 750 m a.s.l.), during the period 05.05–01.06.2012. Specimens were collected in four field samplings: 05.05.2012, 15.05.2012, 23.5.2012 and 01.06.2012.

The caterpillars of the later larval instars, with or without visible signs of parasitism, were collected from the underside of grey elm leaves, *Ulmus canescens* Melville. Elm trees were up to 2 m high and the caterpillars were scattered on them and reached the tips of the branches. In some cases, we found only parasitoid cocoons attached directly to the elm leaves grouped in clusters near the caterpillar body. Each finding was counted as parasitoids from one *Z. filipendulae* larva. The pupae of *Z. filipendulae* were collected from herbaceous vegetation under the elm trees. The dominant plant in this vegetation was *Lotus corniculatus* L.

Samples were placed separately into transparent plastic jars with perforated covers and left in the growth cabinet under the following conditions: relative humidity 65%, temperature 22.5°C and a photoperiod: light:dark=16:8h.

For identification, a ZEISS Discovery V8 stereomicroscope was used, and photographs of the specimens were taken by a Canon Powershot G10 digital camera.

During the first collecting period in early May (05.05.2012), we just noted the caterpillars that had already been parasitized. Fourteen larvae were collected (Table 1). On 15.05.2012, we found 16 larvae of the six-spot burnet, all with visible signs of parasitism. During the next appearance on the terrain on 23.05.2012, we collected 10 caterpillars with a smaller number of cocoons spun on their integument compared to the first two samples collected. Finally, in early June (01.06.2012) only six, mostly destroyed caterpillars, were found in the same place.

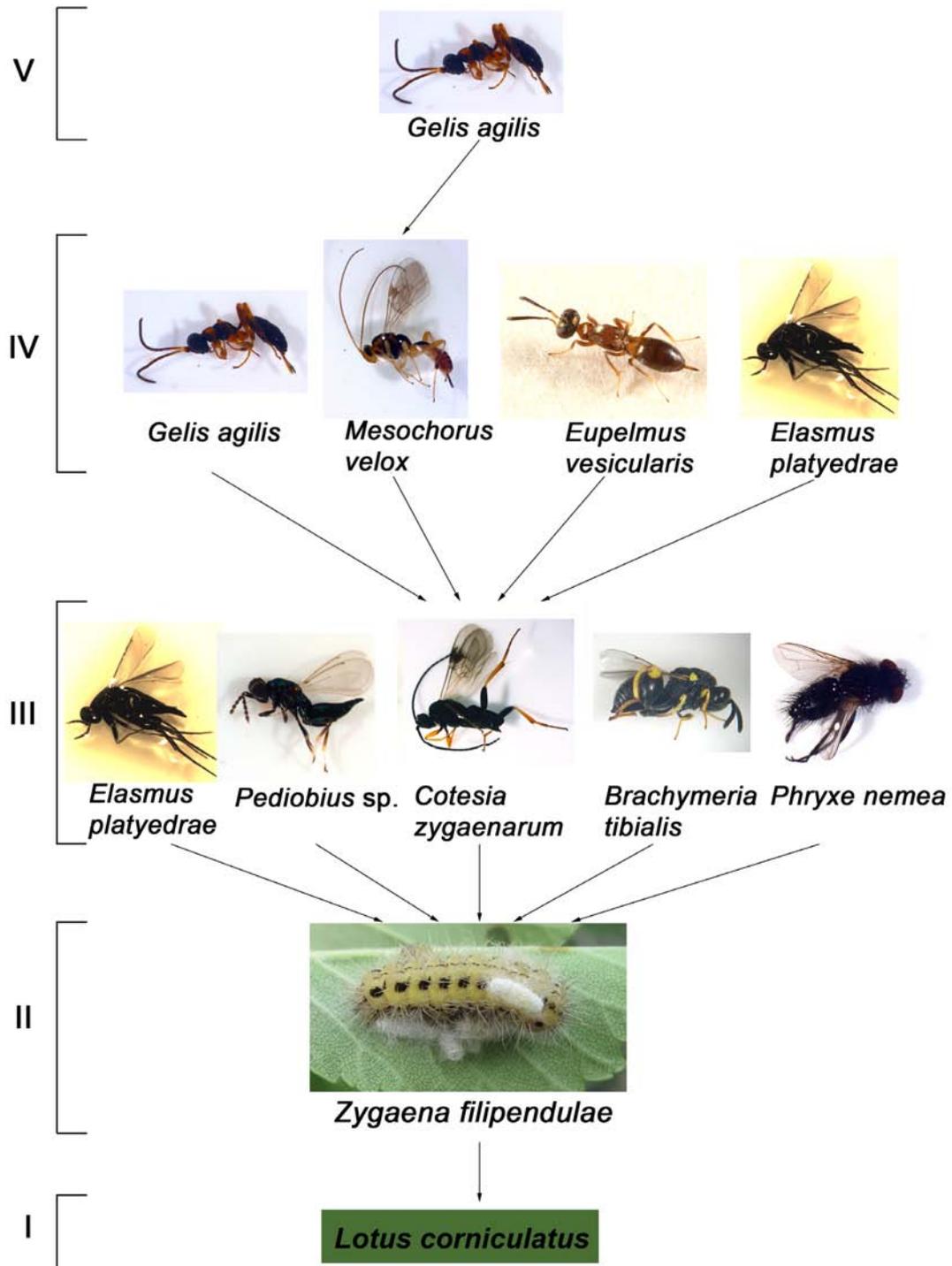


Fig. 1. Multitrophic associations of parasitoids of *Zygaena filipendulae*. I – host plant, II – herbivorous host, III – primary parasitoid, IV – secondary parasitoid, V – tertiary parasitoid.

After the emerging of adult parasitoids, we counted the number of cocoons and parasitoids. Calculating the level of parasitism by counting the number of cocoons per caterpillar was not necessary because all analyzed caterpillars were parasitized.

RESULTS

In total 46 caterpillars and 10 pupae of *Zygaena filipendulae* were collected (Table 1). All collected caterpillars were parasitized. The number of observed parasitoid cocoons per caterpillar was between 8 and 24. In total, there were 735 cocoons from which 608 hymenopteran specimens and 2 flies emerged. We found seven species of hymenopteran parasitoids belonging to five families: *Cotesia zyganaerum* Marshall (Braconidae), *Gelis agilis* (Fabricius) and *Mesochorus velox* Holmgren (Ichneumonidae), *Elasmus platyedrae* (Ferrière) and *Pediobius* sp. (Eulophidae), *Eupelmus vesicularis* (Retzius) (Eupelmidae) and *Brachymeria tibialis* (Walker) (Chalcididae). In addition to the hymenopteran parasitoids we found the parasitoid fly *Phryxe nemea* (Meigen) (Tachinidae: Diptera) (Table 1). Three out of the ten collected pupae were parasitized, each with one parasitoid specimen (Table 1). Five primary parasitoids were identified, *C. zyganaerum*, *B. tibialis*, *E. platyedrae* and *Pediobius* sp. and *P. nemea*, and four secondary parasitoids, *M. velox*, *G. agilis*, *E. vesicularis* and *E. platyedrae*. Eight trophic associations on different levels of parasitism were found. In Fig. 1, we marked the place of each member in the trophic chain by Roman numerals: I – host plant, II – herbivorous host, III – primary parasitoid, IV – secondary parasitoid and V – tertiary parasitoid.

Analyzing the first sample (early May), we found only the primary parasitoid *Cotesia zyganaerum*, with both sexes present. *Cotesia* cocoons had been spun separately from each other. In the second sample, collected in the middle of May, beside the specimens of *C. zyganaerum* two ichneumonid species emerged as hyperparasitoids of *C. zyganaerum*: *Mesochorus velox* and *Gelis agilis*. In the third sample (late May), in addition to the primary braconid parasitoid and two ichneumonid hyperparasitoids, one more para-

sitoid emerged – *Pediobius* sp. Morphologically *Pediobius* sp. is very close to *P. cassidae* Erdős. The fourth sample (early June) was very diverse: only two specimens of *C. zyganaerum* and a few specimens of *G. agilis* were found, but several primary and secondary parasitoids emerged. From the family Eulophidae we obtained two specimens of *Elasmus platyedrae*, male and female, as the primary parasitoid of *Z. filipendulae*, and as the secondary parasitoid of *C. zyganaerum*, one male of *Phryxe nemea* (Tachinidae) as another primary parasitoid of *Z. filipendulae*.

In addition, we found another parasitoid wasp, *Eupelmus vesicularis* (Eupelmidae), as a secondary parasitoid of *Cotesia zyganaerum*, thus presenting a new trophic association. During the last sampling, we collected 10 pupae of *Z. filipendulae* and 2 larvae more directly from the leaves of *L. corniculatus*. After 25 days, we found that three pupae were parasitized: two by *Brachymeria tibialis* (Chalcididae) and one by *Phryxe nemea* (Tachinidae). Seven non-infected six-spot burnet were collected as pupae and resulted in adult moths.

The trophic association between *Z. filipendulae* and its parasitoids is shown in Fig. 1. *Zygaena filipendulae* is for the first time recorded as a host for the parasitoids *Elasmus platyedrae* and *Eupelmus vesicularis*. Three parasitoid species (*G. agilis*, *M. velox* and *E. platyedrae*) are new to the fauna of Serbia.

DISCUSSION

Zygaena filipendulae larvae feed on various leguminous plants, but the main host plant is *Lotus corniculatus*. Regardless the plant species, larvae are usually found on vegetation with a height less than 30 cm where pupation also takes place (Sarin and Bergman, 2010). All larvae investigated in this study were collected on the underside of grey elm leaves (*Ulmus canescens*) up to 2 m high. This is most likely the consequence of changes in behavior induced by the primary parasitoids. Our data indicates that *Cotesia zyganaerum* induces caterpillars to move 1-2 m up the trees. This is the first published record of such an influence of *C. zyganaerum* on its host. Brodeur

Table 1. The list of collected parasitoid species and the number of males and females.

Collecting date	cocoons/ zygaenid larva	Braconidae		Ichneumonidae		Chalcidiidae	Eulophidae		Eupelmidae	Tachinidae				
		<i>Cotesia zygaenarum</i>		<i>Mesochorus velox</i>		<i>Gelis agilis</i>	<i>Brachymeria tibialis</i>	<i>Elasmus platyedrae</i>	<i>Pediobius</i> sp.	<i>Eupelmus vesicularis</i>	<i>Phryxe nemea</i>			
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
05.05.2012	12	11	-	-	-	-	-	-	-	-	-			
	14	8	2	-	-	-	-	-	-	-	-			
	15	10	2	-	-	-	-	-	-	-	-			
	15	4	6	-	-	-	-	-	-	-	-			
	17	8	9	-	-	-	-	-	-	-	-			
	18	7	8	-	-	-	-	-	-	-	-			
	19	10	9	-	-	-	-	-	-	-	-			
	20	10	10	-	-	-	-	-	-	-	-			
	20	8	7	-	-	-	-	-	-	-	-			
	20	7	5	-	-	-	-	-	-	-	-			
	21	8	8	-	-	-	-	-	-	-	-			
	22	10	10	-	-	-	-	-	-	-	-			
	23	12	5	-	-	-	-	-	-	-	-			
24	12	8	-	-	-	-	-	-	-	-				
Σ	260	125	89											
15.05.2012	13	11	-	-	-	-	-	-	-	-	-			
	14	4	3	-	1	-	-	-	-	-	-			
	14	8	3	1	2	-	-	-	-	-	-			
	14	1	1	-	-	8	-	-	-	-	-			
	15	2	3	1	1	-	-	-	-	-	-			
	15	3	3	-	-	-	-	-	-	-	-			
	17	5	5	3	4	-	-	-	-	-	-			
	17	6	-	4	4	-	-	-	-	-	-			
	17	11	6	-	-	-	-	-	-	-	-			
	18	7	8	3	3	-	-	-	-	-	-			
	19	6	6	3	2	-	-	-	-	-	-			
	19	6	6	6	-	-	-	-	-	-	-			
	20	4	5	3	3	-	-	-	-	-	-			
20	5	6	7	1	-	-	-	-	-	-				
20	-	-	-	-	11	8	-	-	-	-				
22	10	8	1	1	-	-	-	-	-	-				
Σ	274	102	69	32	21	19	8							
23.05.2012	10	-	-	4	6	-	-	-	-	-	-			
	12	-	-	-	-	-	-	27	18	-	-			
	12	-	-	4	7	-	-	-	-	-	-			
	12	2	-	2	2	-	-	-	-	-	-			
	12	-	-	-	-	6	6	-	-	-	-			
	13	-	2	-	-	-	-	-	-	-	-			
	15	1	-	-	-	-	-	-	-	-	-			
	15	2	1	-	-	-	-	-	-	-	-			
	15	-	-	-	-	5	9	-	-	-	-			
18	-	-	-	-	10	5	-	-	-	-				
Σ	134	5	3	10	15	21	20	27	18					
01.06.2012	8	-	-	-	-	-	-	-	-	1	-			
	10	-	-	-	-	6	2	-	-	-	-			
	10	-	-	-	-	-	-	1	-	-	-			
	12	-	2	-	-	-	-	-	-	-	1			
	13	-	-	-	-	-	-	1	-	-	-			
	14	-	-	-	-	6	5	-	-	-	-			
	pupa	-	-	-	-	-	-	-	-	-	1			
pupa	-	-	-	-	-	-	-	-	-	-				
pupa	-	-	-	-	-	1	-	-	-	-				
Σ	67		2		12	7	2	1	1	1	1			
Total number ♂♀		395		78		87		2		45		1		2

and Vet (1994) described the usurpation of host behavior of one congeneric species, *Cotesia glomerata* (L.). They demonstrated that *C. glomerata* induces the caterpillars of *Pieris brassicae* (L.) to spin a protective web over the parasitoid cocoons, guarding them aggressively from hyperparasitoids, and CvA (author) has observed caterpillars containing this species climbing up to 6 m in height for pupation (unpublished data). Adamo et al. (1997) reported that *C. glomerata* induces behavioral changes in the tobacco hornworm, *Manduca sexta* (L.). There are two conflicting possibilities that can explain the reasons of induction of the host behavior changed by a parasitoid presence in the host body. The first is that the parasitoids are capable of changing the host's behavior, causing the host to move to a place where it will be somehow protected from its natural enemies, such as various predators or hyperparasitoids. The second possibility is adaptive host suicide, i.e. that the host changes its behavior, increasing the likelihood of being eaten by natural enemies (summarized in Quicke, 1997). According to our field data (Table 1) for the *Z. filipendulae*/*C. zygaenarum* system, the first possibility is more probable, because the hyperparasitoids were not collected on the earliest sampling date and their numbers increased in time.

The presence of other parasitoids connected with *Z. filipendulae* tells us that this protection system is not always efficient. The range of parasitoids found beside *Cotesia zygaenarum* confirms this. The most interesting behavior is shown in *E. platydrae*. Although it is known that *Elasmus* species attack lepidopteran larvae, in this case it could be a primary parasitoid of *Z. filipendulae*, but its presence only in the later-sampled material where other parasitoids emerged describes it as a secondary parasitoid on *Cotesia zygaenarum* from Braconidae or *Gelis agilis* from Ichneumonidae. Another possibility is that it is a tertiary parasitoid of the *Mesochorus velox* species, which is also found at the same time. The limited material (only two specimens, a male and a female) does not allow us to understand the connection of this parasitoid wasp with other members in this very complicated trophic chain. In addition, the species

M. velox has been recorded as a parasitoid of other parasitic Hymenoptera, often from the same family – Ichneumonidae, less frequently members from the subfamily Mesochorinae attack tachinid flies (Goulet and Huber, 1993), so a clear explanation of its behavior is not possible.

Despite the fact that *Cotesia zygaenarum* had many competitors to complete its life cycle, with 395 counted specimens, *C. zygaenarum* was very abundant in this investigation. Two ichneumonid wasps, *Mesochorus velox* with 78 specimens and *Gelis agilis* with 87, were in direct competition with hyperparasitizing *C. zygaenarum* and the *G. agilis* hyperparasitizing *M. velox*. The smallest and gregarious wasp *Pediobius* sp. accounted for 45 specimens. The rest of the trophic chain members found in *Z. filipendulae* is presented by only a few specimens of *Brachymeria tibialis*, *Elasmus platydrae* and *Phryxe nemea*, and one of *Eupelmus vesicularis* (Fig. 1). It is evident that females were the most numerous in comparison to the males of *G. agilis*, *M. velox* and *Pediobius* sp., especially in *C. zygaenarum*, (Table 1). Hyperparasitism had reached its maximum at the end of the life cycle of *Z. filipendulae* during the fourth sampling; therefore, we collected only two *C. zygaenarum* specimens. Because we did not find any *Mesochorus* species at that time, 19 specimens of *G. agilis* appear to be the tertiary parasitoid, parasitizing directly *M. velox*.

Eupelmus vesicularis is known from *Agathis calcarata* (Cresson), *Apanteles laricellae* Mason, *A. melanoscelus* (Ratzeburg), *A. solitaries* (Ratzeburg), *Bracon cephi* (Gahan), *B. intercessor* Nees, *Macrocentrus peroneae* Muesebeck, *Rhogas dendrolimi* Matsuda and *Triaspis thoracicus* (Curtis) (Noyes, 2002).

Obviously, the most important species in this trophic chain are *Z. filipendulae* and *C. zygaenarum* (Fig. 1) with many connections to the other members identified here. In this study, three species, *G. agilis*, *M. velox* and *E. platydrae*, are newly recorded for the fauna of Serbia and two new host/parasitoid associations were registered for *C. zygaenarum*/*E. platydrae* and *C. zygaenarum*/*Eupelmus vesicularis*.

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