

Eupelmus messene Walker, 1839 and *E. microzonus* Förster, 1860 as parasitoids of *Aulacidea hieracii* (Bouché, 1834) (Hymenoptera, Eupelmidae, Cynipidae)

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Abstract

In the southeast of European Russia, the gall wasp *Aulacidea hieracii* (Bouché, 1834) is attacked by ten parasitoid species, including *Eupelmus* (*Eupelmus*) *microzonus* Förster, 1860 and *E. (Macroneura) messene* Walker, 1839. Although both members of the genus *Eupelmus* Dalman, 1820 are idiobiont ectoparasitoids, they demonstrate different life-history strategies in respect to many bionomic features. Specifically, *E. messene* is represented by brachypterous thelytokous females which lay single eggs directly onto the host body. This species can parasitize both concealed and exposed larvae and pupae of *A. hieracii*, but fails to attack its primary parasitoids. On the contrary, arrhenotokous males and females of *E. microzonus* are fully winged. These parasitoids usually lay several eggs per host which are placed onto the wall of the host chamber and covered with a particular fibrous substance. *E. microzonus* never parasitizes pupae or exposed larvae, although it can readily attack concealed larvae of *A. hieracii* and its primary parasitoids. In addition, hibernating individuals of *E. messene* undergo obligatory larval diapause, but those of *E. microzonus* are able to develop without exposure to subzero temperatures. All these data collectively suggest that the former species is highly specialized to exploit *A. hieracii* as a host, whereas the latter one mostly exhibits the so-called morphotypical specialization. These different strategies allow *E. messene* and *E. microzonus* to coexist on the same host species, as a local specialist and a more or less evenly distributed generalist, respectively.

Keywords

Arrhenotoky, brachyptery, diapause, host range, hyperparasitism, life-history strategy, niche partitioning, thelytoky

Introduction

Aulacidea hieracii (Bouché, 1834) is a widely distributed Holarctic species of gall wasp attacking a number of perennial plants of the genus *Hieracium* Linnaeus (Asteraceae) (Zerova et al. 1988; Melika 2006; Sliva and Shorthouse 2006). As in many other Cynipidae (Askew et al. 2006), multilocular stem galls of *A. hieracii* attract numerous parasitoids, which mostly belong to the superfamily Chalcidoidea (see e.g. Noyes 2019). Studying these parasitic wasps in the southeast of European Russia, we detected two members of the genus *Eupelmus* Dalman, 1820 with poorly studied biological features. Moreover, these species apparently had different life-history strategies. To understand how these related parasitoids could coexist on the same host species, we undertook a detailed investigation of their life histories. The results of this study are given below.

The present paper is dedicated to the 75th anniversary of Prof. Arkady S. Lelej, a world-class expert on taxonomy, zoogeography and biology of the order Hymenoptera.

Material and methods

Galls of *A. hieracii* (Fig. 1A, B) were collected on *Hieracium robustum* Fries by MIN during different seasons of 2016–2021 in the field near the city of Saratov, Russia (51°33'N, 46°04'E). The galls were individually placed into Petri dishes and kept at room temperature. A small number of the galls collected in spring and summer were stored in a fridge at 5 °C for at least three weeks prior to rearing gall wasps and parasitoids from them. Emerging insects were extracted from the dishes, identified and, in the case of *Eupelmus* species, used for further experiments. In addition, some extra galls were collected and dissected. Both fragments of these galls and hymenopteran larvae extracted from them were also used in the experiments.

Emerged parasitic wasps were identified by VEG, identifications of Ichneumonidae, Pteromalidae and Eupelmidae were later checked by Andrey I. Khalaim, Ekaterina V. Tselikh (both from the Zoological Institute of the Russian Academy of Sciences) as well as by Lucian Fusu (Alexandru Ioan Cuza University) and Oksana V. Koschelova (All-Russian Institute of Plant Protection) respectively. Voucher specimens of gall wasps (Fig. 1F) and parasitoids are kept in the collection of the Zoological Museum of Moscow State University. The majority of insect photos were taken using a Canon EOS-6D camera with a Canon MP-E 65 mm lens; some images were also obtained with a Canon S100 camera. In most cases, extended focus technology was used.

Unless otherwise stated, all experiments were conducted at room temperature (23–25 °C) and 30–40% relative humidity. Ovipositing parasitoids were provided with water and diluted sugar syrup *ad libitum*. To study oviposition and feeding behavior of parasitic wasps as well as to identify their type of parthenogenesis (arrhenotoky vs. thelytoky), virgin females of both species were offered gall fragments containing larvae and pupae of *A. hieracii* (Fig. 1C–E) and its primary parasitoids in a Petri dish. In a few gall fragments, certain host chambers were left partially open to observe fine details of parasitoid

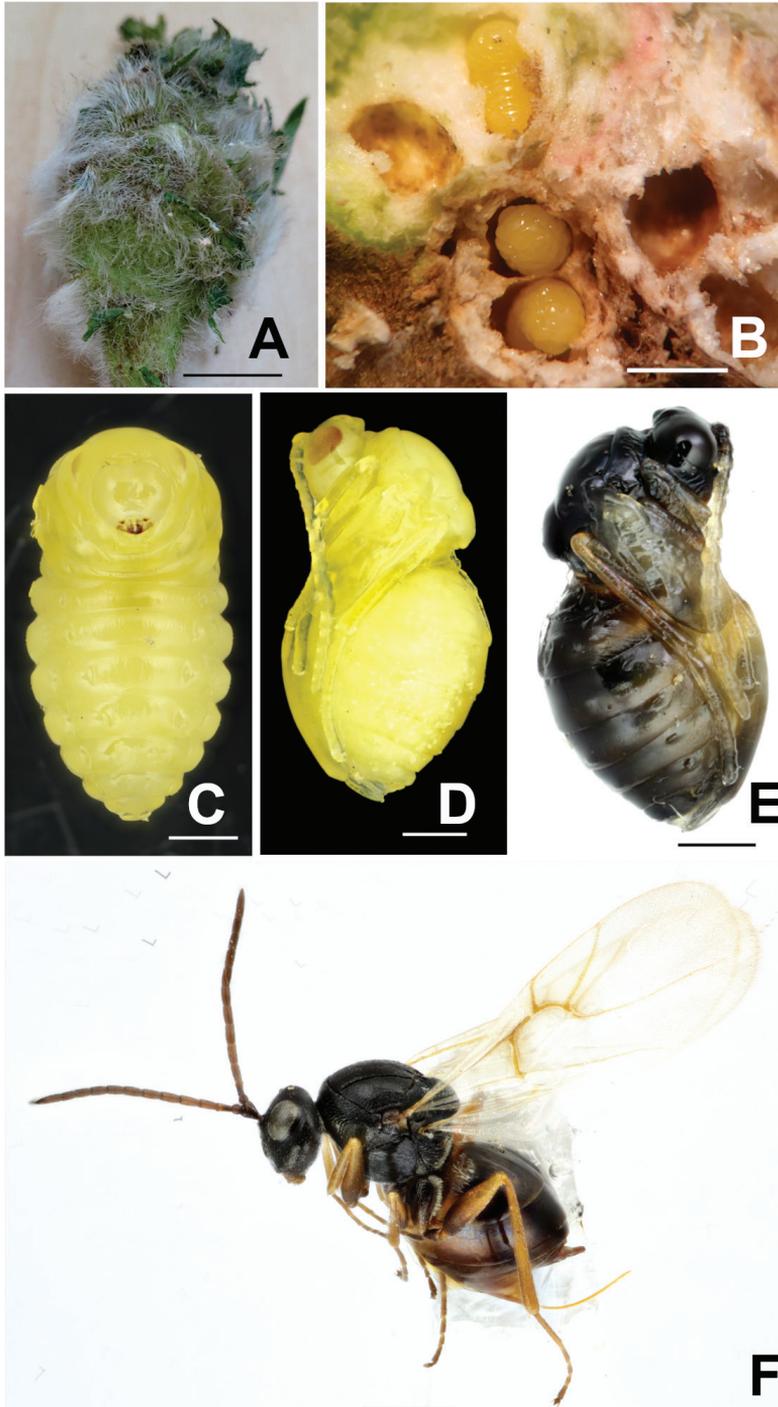


Figure 1. *Aulacidea hieracii*. Gall on *Hieracium robustum* **A** general view **B** cross section with mature larvae **C** final-instar larva **D** early pupa **E** late pupa **F** adult female. Scale bars: 10 mm (**A**); 2 mm (**B**); 0.5 mm (**C-E**); 1.5 mm (**F**).

behavior during oviposition and host feeding. Exposed larvae and pupae of these species were also offered to the ovipositing females, either individually or in choice experiments; in the latter case, various potential hosts were arranged alternately in a circle. After at least 48 h, both exposed hosts and gall fragments were transferred to other Petri dishes and kept until all insects reared from them. The remaining fragments were then dissected in order to identify individuals which were accepted or rejected by the parasitoids. Some extra galls were also dissected after exposing to female wasps, to examine the number and localization of eggs laid by them as well as to describe the morphology and behavior of immature stages of parasitoids. All behavioral experiments were video recorded, and these records were analyzed to determine the sequence and duration of behavioral acts.

Results

Parasitoids of *A. hieracii*

During this study, ten parasitoid species were constantly reared from the galls of *A. hieracii*, including *E. microzonus* and *E. messene* (Table 1; Fig. 2). For example, 349 specimens of different parasitic wasps, together with 417 individuals of the host species, were reared from 63 galls collected in 2018, and the percentage of parasitism therefore constituted about 46% (Table 1).

Certain features of adult morphology and parthenogenesis

Females of *E. messene* are brachypterous (Fig. 2A). When they emerge from the galls, their shortened wings otherwise look normal, but then the distal part of the forewing thickens, turns upright and gets brownish. On the contrary, females of *E. microzonus* have fully developed transparent wings (Fig. 2B).

During the laboratory experiments conducted from 2018 to 2021, five and twelve ovipositing females of *E. microzonus* and *E. messene* produced 22 males and 20 females,

Table 1. Gall wasps and parasitoids reared from galls of *A. hieracii* in 2018.

Species	Males	Females
<i>Aulacidea hieracii</i> (Bouché, 1834) (Cynipidae)	191	226
<i>Exeristes roborator</i> (Fabricius, 1793) (Ichneumonidae)	5	0
<i>Eurytoma cynipsea</i> (Boheman, 1836) (Eurytomidae)	80	98
<i>E. hybrida</i> Zerova, 1978 (Eurytomidae)	0	2
<i>Eurytoma</i> sp. aff. <i>strigifrons</i> Thomson, 1876 (Eurytomidae)	3	2
<i>Sycophila submutica</i> (Thomson, 1876) (Eurytomidae)	24	34
<i>Torymus chloromerus</i> (Walker, 1833) (Torymidae)	0	4
<i>Ormyrus discolor</i> Zerova, 2005 (Ormyridae)	12	10
<i>Pteromalus vibulenus</i> (Walker, 1839) (Pteromalidae)	22	45
<i>Eupelmus</i> (<i>Macroneura</i>) <i>messene</i> Walker, 1839 (Eupelmidae)	0	6
<i>E. (Eupelmus) microzonus</i> Förster, 1860 (Eupelmidae)	1	1



Figure 2. Adult females of *Eupelmus* **A** *E. messene* **B** *E. microzonus*. Scale bars: 0.5 mm.

respectively. We therefore were able to breed the latter species under laboratory conditions for at least four generations. As for *E. microzonus*, its reproduction in the lab terminated every time when all reared virgin females of this species produced exclusively male progeny.

Host-attacking behavior

For newly hatched females of *E. microzonus* and *E. messene*, it takes about 20–25 and 25–30 days, respectively, to start ovipositing. Although the latter species, apart from *E. microzonus*, can successfully utilize exposed hosts in the lab (see below), the basic scheme of attacking concealed hosts appears to be virtually the same in females of both members of the genus *Eupelmus*. Nevertheless, this scheme can be substantially modified depending on the parasitoid species. The following observations are based on the experiments with eight and twelve females of *E. microzonus* and *E. messene* respectively.

1. Initial examination. The female walks along the surface of the substrate, holding her head down and tapping the gall with the tips of her antennae. The examination is sometimes interrupted by grooming of antennae, metasoma, legs, and wings (in the case of *E. microzonus*).

2. Drilling. When the female finds an appropriate site for inserting the ovipositor, she stops and taps the substrate with an increased frequency, holding her head closer to the surface. After the tapping, the female raises up on her legs and bends her metasoma down, touching the selected point with its tip. The parasitoid then installs its ovipositor on the surface of the substrate, moving its metasoma backwards, almost up to its normal position, and begins to press the ovipositor down, twisting its metasoma along the longitudinal axis and pushing the whole body in an up-and-down fashion. During this process, the antennae remain more or less immobile until the end of oviposition, but *E. messene* occasionally taps the substrate with just one antenna. If the concealed host is out of reach, the female withdraws the ovipositor and drills the substrate in another place. The average duration of the drilling stage is about 2 to 3 min.

3. Host immobilization and probing. At the beginning of this stage, the female touches and stings the host several times with her ovipositor for about 40 sec. After the first paralyzing injections, the parasitoid usually takes its ovipositor away from the host and then remains motionless for about 2 min, awaiting an onset of host paralysis.

Sometimes the female continues host probing for a longer time (up to 6 min), holding her metasoma closer to the substrate and rotating it slowly. In this case, the parasitoid does not make ovipositor thrusts characteristic of the drilling stage. The ovipositor is usually inserted into the substrate up to the base, and it can bend almost at the right angle while examining the host chamber.

3a. Host feeding. Before ovipositing (see below), the female can use the host for her own feeding. Moreover, she often abandons the host just after feeding without laying eggs onto it. For example, females of *E. microzonus* oviposited only onto 19 out of 56 larvae of *A. hieracii* in our experiments; the remaining larvae subsequently died due to feeding by adult wasps. If the parasitoid attacks the exposed host, as *E. messene* does under laboratory conditions (see above), the female turns around after stinging the host, finds the wound and then begins to feed on the host's hemolymph which always comes out from the puncture wound. However, if the host is concealed, *E. microzonus* starts to construct a feeding tube. In the beginning of this process, the female does not fully remove her ovipositor after stinging the host. A drop of transparent secretion then appears at the apical part of the slightly vibrating ovipositor; the secretion is gradually becoming dull as the tube grows. When the tube reaches the very base of the ovipositor, the female dips the tube into the wound and withdraws the ovipositor slowly. Construction of the feeding tube normally lasts approximately 5 min. After constructing the tube, the female turns around, finds the upper end of the capillary, and begins to feed on the host's hemolymph. The process of host feeding usually takes about 2 to 3 min. To be precise, we sometimes observed females of *E. messene* apparently feeding on liquids coming from the puncture on the surface of the gall, but whether this species also constructs a feeding tube, remains an open question.

4. Oviposition. The beginning of this stage can be easily recognized by weak vibrating movements of the ovipositor. After 20 to 30 sec, the vibration turns into infrequent thrusts, a longish-oval egg coming out during the longest of them. When the egg reaches the end of the ovipositor, the latter resumes its vibrating movements. The egg is then attached either to the dorsal surface of the host's body (*E. messene*) or to the wall of the host chamber (*E. microzonus*). Moreover, a single female of the former species always lays only one egg onto the host, whereas that of *E. microzonus* often deposits several eggs (up to nine) into the host chamber. The process of egg-laying therefore usually lasts about 3 to 7 min. In the case of *E. microzonus*, the parasitoid often attaches freshly laid eggs to the wall of the host chamber with a characteristic fibrous network using its ovipositor. When the eggs are laid and covered with the protective substance, the female withdraws the ovipositor.

5. Final examination. At this stage, the female examines the substrate on the walk. She often stops, raises her head and makes vibrational antennal movements, apparently looking for new hosts.

The described scheme is subject to modification, since parasitoids can either repeat certain acts or terminate the whole sequence at almost every stage. The female usually abandons the host after the initial examination, unsuccessful drilling attempts,

host immobilization or probing. Anyway, our preliminary study did not reveal any significant difference regarding duration of main behavioral acts between the two species. In addition, *E. messene* can successfully attack both exposed and concealed hosts, whereas *E. microzonus* never lays eggs on the former ones. Moreover, *E. messene* acts as a strictly primary parasitoid of *A. hieracii* and can develop on both its larvae and pupae. On the other hand, *E. microzonus* often attacks larvae of *Eurytoma* spp. and *Sycophila submutica*, thus displaying facultative hyperparasitism, but this parasitoid does not find host pupae suitable for oviposition. Specifically, females of *E. messene* laid eggs on twenty-nine exposed immature individuals of *A. hieracii* in our experiments (including eleven pupae), but failed to oviposit onto five larvae of *Eurytoma* as well as onto two larvae and ten pupae of *Sycophila*. On the other hand, females of *E. microzonus* rejected seven and three exposed larvae and pupae of *A. hieracii*, respectively, as well as seven concealed pupae of the same host species that were offered to ovipositing parasitoids. Individuals of *E. microzonus* could also develop on at least two concealed larvae of *Eurytoma* spp., in addition to three larvae of *S. submutica*.

Life cycle

A. hieracii, the principal host of *E. microzonus* and *E. messene* in the studied habitat, is a univoltine species, with adults emerging in May and mature larvae overwintering in galls (Zerova et al. 1988; Melika 2006). Consequently, both *E. messene* and *E. microzonus* are univoltine as well, with their adults also emerging in May. These species usually overwinter as partly grown larvae and complete feeding after hibernation. However, larvae of *E. messene* have an obligatory winter diapause and therefore must be exposed to subzero temperatures to complete their development.

In *E. microzonus*, the egg is longish-oval (about 0.05×0.2 mm) and semi-transparent, with a very short anterior process and a longer posterior one (Fig. 3A). The first-instar larva that hatches from the egg is approximately 0.25 mm long. It has 13-segmented spindle-shaped body, with approximately first or second metasomal segment being the widest, a chitinized head capsule and stout spines on most segments. The feeding larva actively moves along the surface of the host at the speed of 6 to 10 mm/min, apparently looking for potential competitors. This behavior is characteristic of some other ectoparasitoid chalcids, e.g. Eurytomidae (Fisher 1970). Moreover, since several eggs are usually laid into the host camera even by a single female parasitoid, cannibalism often takes place between the larvae of *E. microzonus*, at least in the lab. Eventually, only one larva usually survives. However, if too many eggs are laid on the same host individual, all parasitoids can die. The subsequent instars are becoming substantially less active and therefore more grub-like. After overwintering, the larva continues to feed and grows into its final-instar (about 2.0 mm long; Fig. 3B). The larva then turns into a prepupa, and after that it molts into the pupal stage. The early pupa is whitish (Fig. 3C), then it eventually darkens and begins to resemble the adult insect. Duration of developmental stages of *E. microzonus* is shown in Table 2.

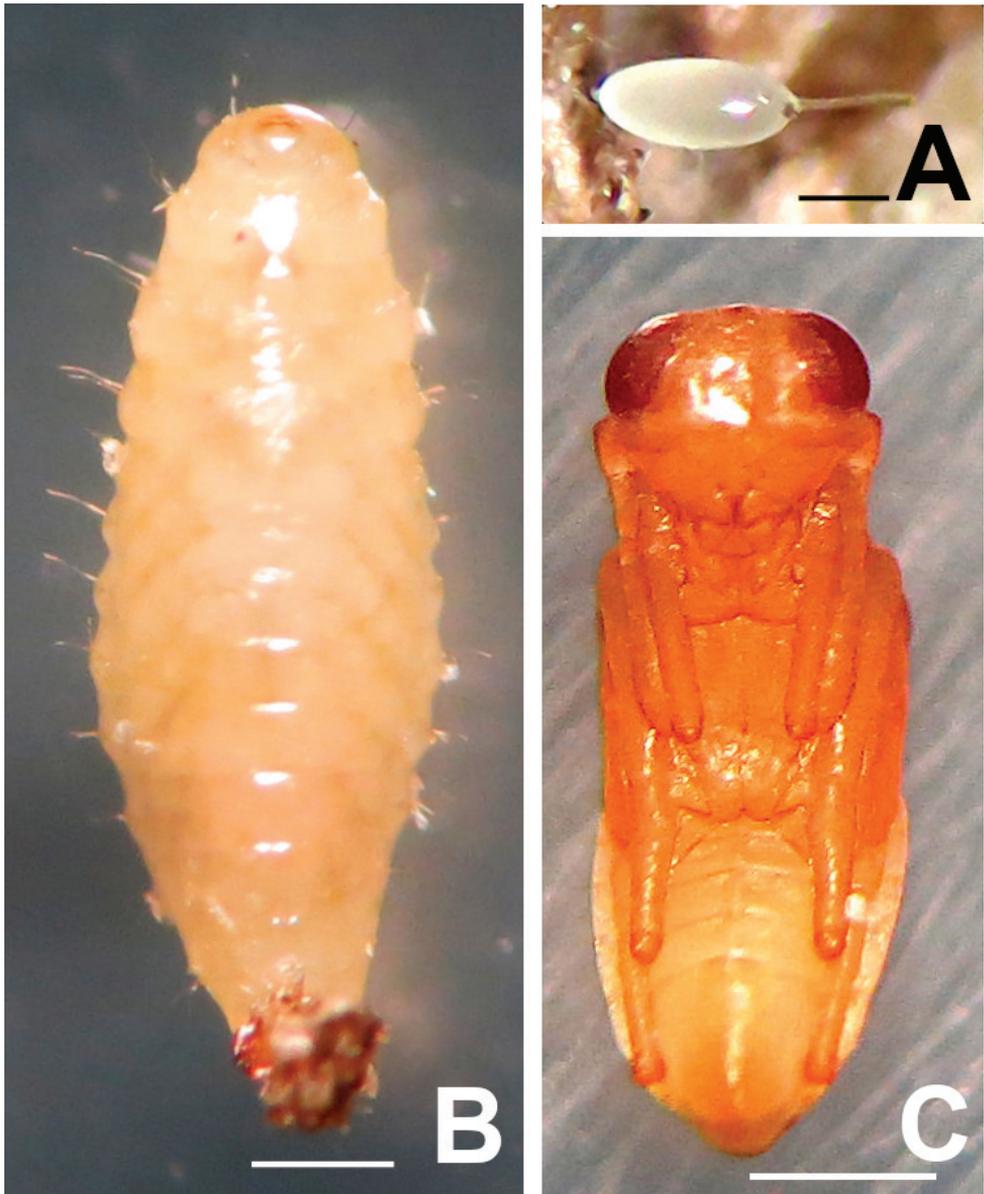


Figure 3. Immature stages of *E. microzonus* **A** egg **B** final-instar larva **C** male pupa. Scale bars: 0.1 mm (**A**); 0.4 mm (**B**); 0.5 mm (**C**).

The life cycle of *E. messene* generally resembles that of the previous species. In addition, immature stages of *E. messene* are also similar in size and morphology to those of *E. microzonus* (Fig. 4). On the other hand, larval development of *E. messene* is interrupted by an obligatory larval diapause, apart from the former species. Duration of developmental stages of *E. messene* is also shown in Table 2.

Table 2. Duration of developmental stages of two *Eupelmus* species (mean \pm standard deviation, days).

Stage	<i>E. messene</i>	<i>E. microzonus</i>
Egg	4 \pm 1 (9 females)	3 \pm 1 (8 males)
Larva	14 \pm 0 (9 females)	12 \pm 1 (3 males)
Pupa	10 \pm 2 (9 females)	12 \pm 0 (9 males)
Adult	55 \pm 5 (9 females)	18 \pm 4 (8 males), 53 \pm 9 (4 females)

**Figure 4.** Immature stages of *E. messene* **A** egg **B** final-instar larva **C** female pupa. Scale bars: 0.1 mm (**A**); 0.3 mm (**B**); 0.5 mm (**C**).

Discussion

General remarks

Among the parasitic wasps reared during the present study, certain genera and species were already associated with *A. hieracii* (Askew et al. 2006; Noyes 2019). However, both *Eupelmus* species as well as members of the genera *Exeristes* Förster, 1869 and *Ormyrus* Westwood, 1832 are recorded for the first time as parasitoids of this gall wasp (Table 1). In fact, a few years ago we detected a particular *Eupelmus* species which attacked *A. hieracii* in the Saratov Province of Russia (Anikin and Nikelshparg 2017), but this parasitoid is now identified as *E. messene*.

There was a number of historical reports on the ecology and behavior of Eupelmidae and of the genus *Eupelmus* in particular (Packard 1916; McConnell 1918; Caffrey 1921; Phillips and Poos 1921, 1927; Clausen 1927; Gahan 1933; Taylor 1937; Morris 1938; Askew 1961; Delanoue and Arambourg 1965 etc.), although they were generally scattered among the existing literature. Most these reports were summarized in an excellent review compiled by Clausen (1940). This author already noted extensive bionomic differences between various members of the taxa mentioned above, including their host ranges and the ability to act as facultative hyperparasitoids. Recent research generally confirms this pattern, showing, for example, that groups of closely related idiobionts of the genus *Eupelmus* can include both strict specialists and broad generalists, as well as some intermediate forms (Al Khatib et al. 2016; Gibson and Fusu 2016; Fusu 2017). However, we are unaware of any previous comparative study explicitly showing how different members of the family Eupelmidae can coexist in sympatry while exploiting the same host. Nevertheless, analogous interactions between a particular gall-making hymenopteran species and its ectoparasitoids can apparently be found in North America, where chalcid wasps of the genus *Tetramesa* Walker, 1848 (Eurytomidae), including *T. tritici* (Fitch, 1859), are also attacked by at least two members of the family Eupelmidae, i.e., the fully winged arrhenotokous species, *Brasema allynii* (French, 1882), and the brachypterous thelytokous one identified as *Eupelmus vesicularis* (Retzius, 1783) (Phillips and Poos 1921, 1927, also see below).

Previous observations on the biology of *E. microzonus* and *E. messene* are scarce. Specifically, *E. microzonus* mostly attacks Cynipidae and Eurytomidae on herbaceous plants and certain shrubs (Gibson and Fusu 2016), but many details of its life history remained unknown up to now. As for *E. messene*, its species status was restored just a few years ago from a synonym of *E. vesicularis* (Fusu 2017), and therefore any data on its ecology and behavior are of special value. Fusu (2017) also suggests that the species studied in North America by McConnell (1918) and Phillips and Poos (1927) under the name of *Eupelminus saltator* (Lindeman, 1887) which was later synonymized by Gahan (1933) with *E. vesicularis*, was in fact *E. messene*. Nevertheless, individuals of the latter species which we reared from *A. hieracii* (Anikin and Nikelshparg 2017), always placed their eggs directly onto the host and never acted as facultative hyperparasitoids, as opposed to the parasitic wasps observed by Phillips and Poos (1927).

Fully winged vs. brachypterous individuals

Both male and female *E. microzonus* have fully developed wings, whereas these organs are obviously reduced and non-functional in *E. messene*. Since many members of the genus *Hieracium*, including *H. robustum*, usually form more or less dense patches (see e.g. Tupitsyna 2004), we therefore can assume that ovipositing females of *E. messene* are generally confined to a particular patch of the hawkweed species hosting *A. hieracii*. In contrast with that, individuals of *E. microzonus* are apparently able to freely move within certain habitats and perhaps even between them.

Type of parthenogenesis

Virgin females of *E. microzonus* and *E. messene* produced only male and female progeny, respectively, thus confirming arrhenotoky in the former species and thelytoky in the latter (Gibson and Fusu 2016; Fusu 2017). Although the precise mechanism of thelytokous parthenogenesis in *E. messene*, e.g. automixis vs. apomixis (see Gokhman and Kuznetsova 2018), is unknown, this feature is obviously an adaptation to the low population density and decreased motility of *E. messene* whose individuals do not need to find a mate to complete their life cycle. To be precise, brachypterous females do produce males by arrhenotoky in certain other species of the subgenus *Macroneura* Walker, 1837 (see e.g. Fusu 2017), but these males are always fully winged.

Exposed vs. concealed hosts; facultative hyperparasitism; suitable host stages

Both studied *Eupelmus* species normally attacked immature stages of *A. hieracii* inside the galls. However, *E. messene* could also oviposit onto the exposed larvae and pupae of this gall wasp, but *E. microzonus* completely failed to attack them in our experiments. On the other hand, the latter species readily oviposited not only on *A. hieracii*, but also on *Eurytoma* spp. and *S. submutica*, i.e., it displayed facultative hyperparasitism. In contrast to *E. microzonus*, *E. messene* was a strictly primary parasitoid and never demonstrated hyperparasitic behavior. Finally, *E. messene* could attack and successfully develop either on larvae or pupae of *A. hieracii*, whereas *E. microzonus* always rejected pupae of this gall wasp and of its parasitoids.

Host feeding

Before the female of *E. microzonus* begins to oviposit, it has to feed on the host's hemolymph. To get access to it, the parasitoid first pierces the host's skin with its ovipositor, and then constructs a feeding tube (see above). As for *E. messene*, it can feed on host's hemolymph in a direct contact with the hosts, but construction of the feeding tube was never reported for this species (McConnell 1918; Phillips and Poos 1927; Gahan 1933). The widespread nature of host feeding in the Eupelmidae was already noted by Clausen (1940). This is apparently not surprising, since this trait is especially necessary

for ectoparasitoids which have to provide their eggs with all nutrients until the larva hatches from the egg (Quicke 1997). In addition, most parasitoids cannot synthesize lipids *de novo* (Visser et al. 2010), and therefore have to obtain them (e.g., for vitellogenesis) only via host feeding. In the comprehensive review on the subject, Jervis and Kidd (1986) listed several members of Eupelmidae which demonstrated this trait, both with or without constructing the feeding tube (see also Delanoue and Arambourg 1965). According to the classification used in this review, host feeding in the two *Eupelmus* species can be termed both concurrent (= followed by oviposition onto the same host specimen) and non-concurrent. In addition, it can be both destructive and non-destructive (Jervis and Kidd 1986), since hosts either die or survive after feeding by adult parasitoids.

Number and placement of eggs

E. microzonus usually deposits several eggs into the host chamber, thus exhibiting superparasitism (Quicke 1997); these eggs are often fastened to the wall of the chamber using characteristic fibrous substance. This fibrous attachment of the eggs by certain members of the family Eupelmidae, including *Eupelmus urozonus* Dalman, 1820, was mentioned by Packard (1916), Taylor (1937), Askew (1961) and Delanoue and Arambourg (1965). Superparasitism leads to almost inevitable larval cannibalism in *E. microzonus*, but it is an apparent artifact resulting from the lack of suitable hosts under experimental conditions. A similar situation was described for some other Eupelmidae by Phillips and Poos (1927) and Morris (1938). On the contrary, in our experiments *E. messene* always laid a single egg on the dorsal side of the host and never covered the eggs with any fibrous material.

Winter diapause

Both studied *Eupelmus* species were strictly univoltine in our experiments, like most other members of the family (Clausen 1940). Specifically, these parasitoids overwintered as partly grown larvae and completed feeding in spring. However, larvae of *E. messene* could not pupate without being exposed to subzero temperatures for at least several weeks, whereas this was not necessary for those of *E. microzonus*. In other words, *E. messene*, apart from the latter species, has an obligatory winter diapause, which provides phenological synchronization between the parasitoid and its host (Quicke 1997). In some obviously extreme cases found in other Eupelmidae, larval diapause can last up to two years (Caffrey 1921). On the other hand, lack of diapause in *E. microzonus* apparently indicates that this species can utilize hosts with different phenologies.

Life-history strategies of the two *Eupelmus* species

Taken together, our observations and experimental data clearly demonstrate that *E. microzonus* and *E. messene* exhibit different life-history strategies as parasitoids

Table 3. Main differences between life-history strategies of two *Eupelmus* species attacking *A. hieracii*.

Character	<i>E. messene</i>	<i>E. microzonus</i>
Motility	Fully winged	Brachypterous
Type of parthenogenesis	Thelytoky	Arrhenotoky
Ability to oviposit onto exposed hosts	Present	Absent
Facultative hyperparasitism	Absent	Present
Ability to oviposit onto host pupae	Present	Absent
Number of eggs laid by single female onto host	Single	Usually several
Placement of eggs	Directly onto host	Into host chamber
Fibrous attachment of eggs	Absent	Usually present
Construction of feeding tube	Not observed	Present
Obligatory winter diapause	Present	Absent

of *A. hieracii* within the same habitat (Table 3). In particular, *E. messene* is highly specialized to exploit this host species. It exclusively acts as a primary parasitoid of *A. hieracii*, attacking either larvae or pupae of this gall wasp and laying a single egg on the dorsal side of the host. *E. messene* can specifically recognize both concealed and exposed immature stages of *A. hieracii*. In addition, the life cycle of this parasitoid is synchronized with that of the host due to the obligatory larval diapause. Finally, flightless thelytokous females of *E. messene* apparently have restricted motility, being predominantly confined to patches of hawkweed infested by the gall wasp.

We also noted that biological characters of *E. messene* (often referred to as “thelytokous *E. vesicularis*” in the previous literature) studied in the present work often substantially differ from those described by preceding authors. Although some of these discrepancies (e.g., the obligatory winter diapause) can be more or less reliably attributed to intraspecific variation (Quicke 1997), other characteristic features of these parasitoids suggest an apparent involvement of several cryptic lineages. Presence of cryptic biological species in Eupelmidae and in the *E. vesicularis* species group in particular was suspected long ago (see e.g. Morris 1938 and Askew 1961), but now this is further corroborated by recent molecular studies (Al Khatib et al. 2016; Fusu 2017 etc.).

Apart from *E. messene*, *E. microzonus* is adapted to exploit larvae of various host species inside the galls of *A. hieracii*. In other words, this parasitoid exhibits the so-called morphotypical specialization (Kozlov 1970). For example, it cannot oviposit onto exposed hosts of any kind, but readily parasitizes concealed larvae of *Eurytoma* spp. and *S. submutica* in addition to those of *A. hieracii*, thus demonstrating facultative hyperparasitism. However, *E. microzonus* is also unable to attack pupae of the gall wasp, probably because the weakly moving pupa cannot stimulate the respective behavioral reaction of the female parasitoid. To perform host feeding on concealed host larvae, *E. microzonus* always constructs a feeding tube. This parasitoid usually places its eggs onto the wall of the host chamber, using characteristic fibrous substance both to attach the eggs to the wall and to protect them from being destroyed by the host. Moreover, we assume that frequent superparasitism showed by *E. microzonus* can be explained by insufficient number of hosts available in our experiments, because fully winged females of this species can easily move between patches of different hosts in the

field. Higher motility of *E. microzonus* also ensures reliable mating in this arrhenotokous species.

All observations and experimental data described and summarized in the present study therefore collectively suggest that different life-history strategies exhibited by the two *Eupelmus* species allow them to coexist on *A. hieracii*. Although both members of the genus *Eupelmus* utilize this species as an important host, their ecological strategies significantly differ due to apparent niche partitioning (Finke and Snyder 2008). Specifically, *E. messene* and *E. microzonus* can be respectively considered as a local specialist and a more or less evenly distributed generalist.

Conclusions

1. In the southeast of European Russia, *A. hieracii* is attacked by ten parasitoid species, including two members of the genus *Eupelmus*, *E. microzonus* and *E. messene*.

2. Although both *Eupelmus* species are idiobiont ectoparasitoids, they demonstrate different life-history strategies in respect to their flight ability, type of parthenogenesis, potential hyperparasitism, range of suitable host stages, mode of hibernation, and other traits.

3. *E. messene* is highly specialized to exploit *A. hieracii* as a host, whereas *E. microzonus* mostly exhibits morphotypical specialization.

4. These different strategies allow *E. messene* and *E. microzonus* to coexist on the same host species, as a local specialist and a more or less evenly distributed generalist, respectively.

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