EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **121**: 280–283, 2024
doi: 10.14411/eje.2024.029
SHORT COMMUNICATION

The effect of maternal factors of *Cotesia glomerata* (Braconidae) on its larval competitor *Hyposoter ebeninus* (Ichneumonidae)

Maximilien A.C. Cuny[1], Mitchel E. Bourne[1], Ruben Bos[1], Antonino Cusumano[2] and Erik H. Poelman[1]

[1] Wageningen University, Laboratory of Entomology, Wageningen, The Netherlands; e-mails: maxcuny@hotmail.com, mitchel.bourne@uzh.ch, rubenbos@buiting.nl, erik.poelman@wur.nl

[2] Department of Agricultural, Food, and Forest Sciences, University of Palermo, Palermo, Italy; e-mail: antonino.cusumano@unipa.it

Key words. Hymenoptera, parasitoid, physiological suppression, interspecific competition, maternal factors, *Pieris brassicae*, oviposition fluids

Abstract. Parasitoids of different species frequently develop in the same host, a phenomenon referred to as "multiparasitism". Although the outcomes of multiparasitism have been well-documented in the literature, the underlying mechanisms, particularly the substances injected by a female parasitoid along with her egg(s) into a host during parasitism, remain relatively unexplored. Previous work on parasitoids associated with the cabbage white butterfly, *Pieris brassicae* (Lepidoptera: Pieridae) has shown that the larva of the solitary parasitoid *Hyposoter ebeninus* (Hymenoptera: Ichneumonidae) has a higher survival but a longer development time when competing with the gregarious parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae). In this study, we hypothesize that the maternal factors injected by *C. glomerata* are responsible for the effect on the performance of *H. ebeninus* larvae. This hypothesis was tested using *P. brassicae* caterpillars first parasitized with *H. ebeninus* and then injected with *C. glomerata* maternal factors, or parasitized by both parasitoids. Our results suggest that *C. glomerata* maternal factors are at least partially responsible for the reduction in *H. ebeninus* developmental mortality (likely through effects on the immune response of the host caterpillar), but does not seem to affect its development time. We discuss these results and the current knowledge of maternal-factor-mediated parasitoid interactions.

INTRODUCTION

Parasitic wasps, also known as parasitoids, typically lay one or several eggs on (ectoparasitoid) or inside (endoparasitoid) an herbivorous host where they complete their entire development until pupation, ultimately leading to the host's death (Godfray, 1994). Particularly endoparasitoids have developed, together with their hosts, defensive strategies to win this antagonistic interaction: the host's immune system may kill parasitoid eggs and larvae via encapsulation (i.e. haemocytes that attach to foreign objects), while adult parasitoids inject factors in their host that impair the hosts immune system (Strand & Burke, 2019). The main factors injected by endoparasitoids belonging to the Braconidae and Ichneumonidae families are polydnaviruses (PDVs) and venom (Beckage & Gelman, 2004). Polydnaviruses are endogenous viruses that replicate in the calyx region of the parasitoid ovaries (Volkoff et al., 2010). After being injected in their host during oviposition, PDVs infect the host's cells to express their virulence genes and produce virulence factors that negatively affect haemocytes (Drezen et al., 2017). Parasitoid venom is a complex cocktail that comprises both protein-based and non-protein-based compounds. It plays an important role in the success of development of the endoparasitoid by interfering with the host immune system and/or by playing a synergistic role with PDVs (Moreau & Asgari, 2015). Both PDVs and venom also play an important role in the manipulation of the parasitized host's development (Cuny & Poelman, 2022).

In nature, parasitoids often compete with other parasitoid larvae developing within the same host, a phenomenon known as intrinsic competition (Cusumano et al., 2016). Endoparasitoid larvae can compete through physical attacks using mandibles and/or through physiological suppression, either by releasing factors directly into the host milieu or through injection by adult females along with eggs (Cusumano et al., 2012; Paul et al., 2024). In some cases, parasitoid larvae can even benefit from the suppression of the host's immune system caused by the maternal factors injected by another species, resulting in facilitation (Magdaraog et al., 2016). Past research extensively investigated intrinsic competition, but it primarily concentrated on competition mediated by the parasitoid larvae (Tillman & Powell, 1992; Harvey et al., 2013; Cusumano et al., 2016). As a result, little is known about the mechanisms, particularly when competition is driven by maternal factors injected during oviposition (Pekas et al., 2023). In a study by Poelman et al. (2014), the solitary parasitoid *Hyposoter ebeninus* exhibited higher survival rate but a longer development time when competing with the gregarious parasitoid *Cotesia glomerata* in the herbivorous host *Pieris brassicae*. *Hyposoter ebeninus* is a superior intrinsic competitor, usually winning competitions against *C. glomerata.* However, *C. glomerata* larvae are

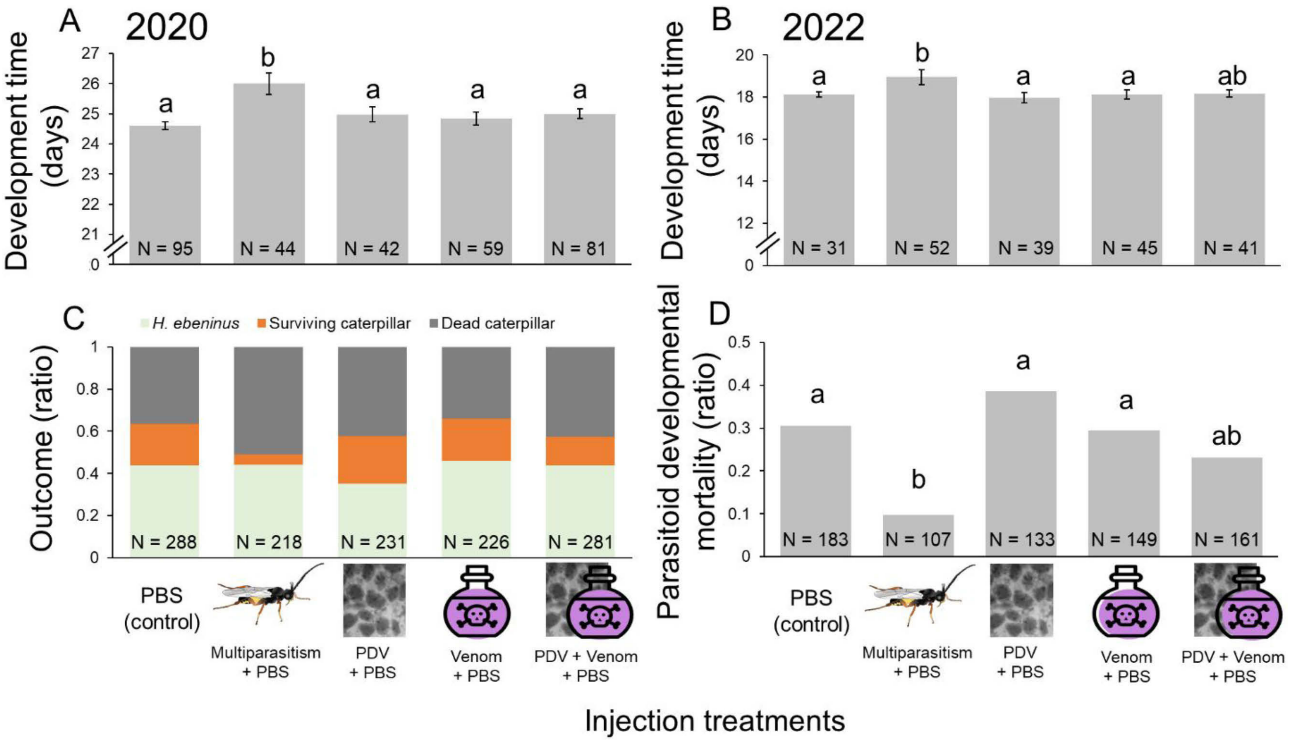

Fig. 1. (A) Mean (± SEM) *Hyposoter ebeninus* development time from parasitism to adult emergence (data from the first two blocks in 2020). (B) Mean (± SEM) *H. ebeninus* development time from parasitism to adult emergence (data from the last two blocks in 2022). (C) Mean ratio of emerged *Hyposoter ebeninus* parasitoids, surviving caterpillars and caterpillar mortality. (D) Mean ratio of surviving caterpillars, which corresponds to *H. ebeninus* mortality during its development (dead caterpillars were excluded). All caterpillars were first parasitized by *H. ebeninus*, and then injected by one among five injection and/or parasitism treatments. Different letters indicate statistically significant differences ($P < 0.05$) among treatments.

more effective at suppressing the host's immune system, leading to lower mortality by encapsulation compared to *H. ebeninus.* The authors hypothesized that *H. ebeninus* larvae benefitted from the impairment of the host immune system caused by *C. glomerata* PDV and venom. Here, we tested this hypothesis measuring the performance of *H. ebeninus* developing in caterpillars that were first parasitized with *H. ebeninus* and then injected with *C. glomerata* maternal factors, or parasitized by both parasitoids.

MATERIAL AND METHODS

Plants and insects

Pieris brassicae caterpillars and their parasitoids (*Cotesia glomerata* and *Hyposoter ebeninus*) were obtained from our rearing facilities at Wageningen University (22 ± 1°C, 50–70% RH,16L : 8D photoperiod). Caterpillars were fed with wild cabbage plants (*Brassica oleracea*) of the population "Kimmeridge" (Gols et al., 2008), while parasitoids were provided with water and honey. Plants were grown in 2-L pots placed in a glasshouse compartment (22 ± 4°C, 50–70% RH) under artificial light (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 16L : 8D photoperiod) in addition to natural daylight.

Parasitism of the caterpillars

We individually parasitized all the caterpillars (early 3rd instar) with either *H. ebeninus* or both *H. ebeninus* and *C. glomerata* (Poelman et al., 2014). In the case of multiparasitism, caterpillars were first parasitized by *H. ebeninus* and then parasitized by *C. glomerata* less than 2 h later, minimizing the advantage provided by the order of parasitism. In each repetition of the entire experiment, we parasitized about 300 caterpillars (~60 caterpillars per treatment).

Isolation of polydnavirus particles and venom

We isolated calyx fluid (containing the PDV particles) and venom from female adult *C. glomerata* and stored them in a phosphate buffered saline (PBS) solution (as described in Cusumano et al., 2018). Considering that *C. glomerata* wasps deplete their egg resources after about 10 parasitism events (Zhu et al., 2015), we assumed that 1/10 of the total amount of calyx fluid and venom per parasitoid individual is injected into the host during each parasitism event. Therefore, we prepared solutions in 250 µL microcentrifuge tubes filled with PBS at one wasp equivalent per µL (i.e. one parasitoid venom gland or ovaries per µL) to inject 0.1 µl of solution per caterpillar, which corresponds to 0.1 wasp equivalent.

Caterpillar injections and parasitoid performance

Caterpillars were first anaesthetised with CO_2 and then injected using a FemtoJet® 4i (Eppendorf) with 0.1 µL of one among the following treatments: (i) PBS (negative control), (ii) PBS (positive control, using multiparasitized caterpillars), (iii) calyx fluid (with PDVs), (iv) venom, and (v) calyx fluid (with PDVs) + venom. All these caterpillars were parasitized by *H. ebeninus* in the morning of the same day, but caterpillars from treatment (ii) were parasitized with both *H. ebeninus* and *C. glomerata*.

After microinjections, caterpillars were placed on 6-week-old mesh-covered *B. oleracea* plants in a greenhouse compartment (22 ± 4°C, 50–70% RH) under artificial light (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 16L : 8D photoperiod) in addition to natural daylight. Ten caterpillars from the same treatment were placed on each plant (about 6 plants per injection treatment and per repetition) until parasitoid emergence. Parasitoid cocoons were collected and individually placed in 1.5 ml microcentrifuge tubes closed with cotton. Adult emergence was checked three times per day to measure the de-

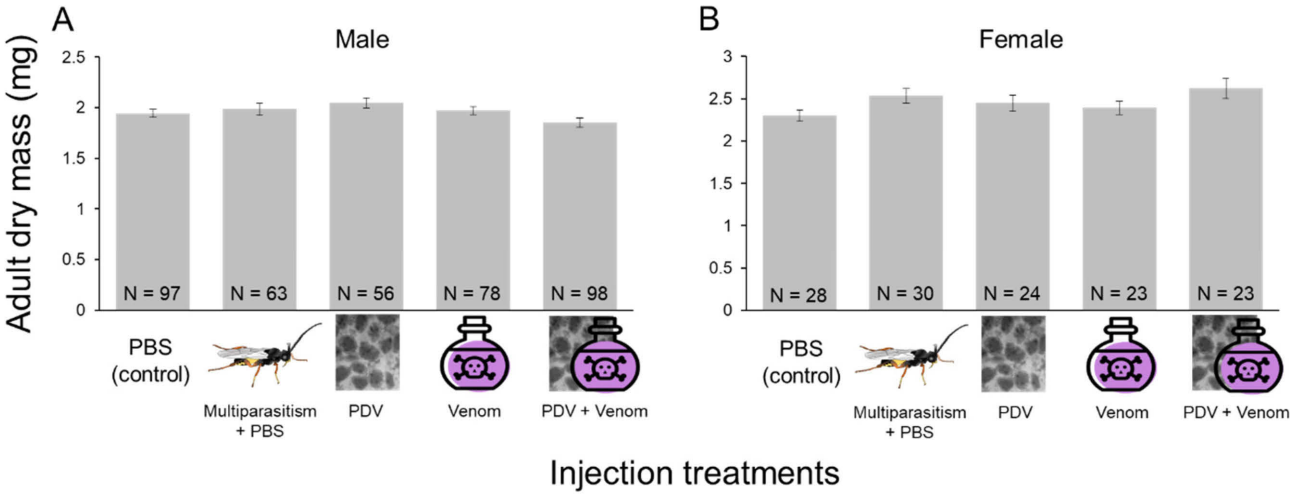

Fig. 2. Mean (± SEM) dry weight of (A) male and (B) female *Hyposoter ebeninus* adults that developed from caterpillars that received one among five injection and/or parasitism treatments.

velopment time (in days). We also noted whether the caterpillars were alive at the end of the experiment, which is likely to be caused by parasitoid encapsulation. However, this could not be verified as traces of parasitoid encapsulation are no longer visible in two-week-old caterpillars. When all parasitoids had emerged, they were killed in a –20°C freezer and placed in an oven at 75°C for 24 h. Then, we weighed them individually on a Sartorius®-CP2P-Analytical Balance (accuracy 0.001 mg) to obtain the dry weight. The entire experiment was replicated four times, referred to as 'blocks': the first two blocks were performed during winter 2020, followed by two subsequent blocks during summer 2022. Despite climate control, greenhouse temperatures varied between the two years and season (± 4°C).

Statistical analysis

Due to the different temperatures between blocks performed in winter 2020 and those performed in summer 2022, we obtained a bimodal distribution of the development time data. Thus, we decided to analyse the blocks from the two years separately. No bimodal distribution was observed for caterpillar and parasitoid mortality as well as parasitoid adult dry mass; we thus analysed the blocks jointly. Prior to each model, residuals normality and homoscedasticity were checked. For all the models, caterpillar injection treatments were used as explanatory fixed factors and the plant identity nested in the blocks were used as random factors. When models showed a significant effect, we used Tukey's post hoc tests. All statistical analyses were performed in R version 4.2.2 (R Core Team, 2021). For the development time, the blocks from 2020 were analysed with a linear mixed model. For the blocks from 2022, because residuals were heterogeneously distributed, we used a generalized least squares model that allowed us to specify a variance structure with the different treatments (VarIdent variance structure from the nlme package in R) (Zuur et al., 2009). To analyse the total and developmental mortality (binary), we used generalized linear mixed models with a binomial distribution. Finally, we analysed the weight of adult parasitoids separately for males and females, using two linear mixed models.

RESULTS AND DISCUSSION

We hypothesized that the strong capacity of *Cotesia glomerata* to supress its host immune system through the injection of maternal factors would favour its intrinsic superior competitor *Hyposoter ebeninus* by reducing its encapsulation, at the expense of a longer development time.

The development time of *H. ebeninus* larvae was significantly longer when competing with *Cotesia glomerata* larvae (blocks from 2020: $\chi^2_{(4)} = 22.65$, $P < 0.001$ (Fig. 1A); blocks from 2022: $\chi^2_{(4)} = 14.1$, $P = 0.007$ (Fig. 1B)). These findings align with those reported by Poelman et al. (2014). Additionally, our results demonstrate that the prolongation of *H. ebeninus* development time was not attributed to the PDV and venom injected by *C. glomerata* (Tukey's post-hoc tests for pairwise comparisons between control and 'PDV+Venom+PBS' treatments: $P = 0.957$ and $P = 0.999$, for 2020 and 2022, respectively). Alternatively, we hypothesize that a longer developmental time could be caused by the time spent by *H. ebeninus* larvae swimming in the haemolymph, searching for *C. glomerata* larvae to kill. Contrary to the findings of Poelman et al. (2014), *H. ebeninus* larvae developing in competition with *C. glomerata* larvae did not exhibit a lower total mortality compared to *H. ebeninus* larvae developing alone ($\chi^2_{(4)} = 4.78$, $P = 0.31$) (Fig. 1C). One possible reason for this discrepancy is that in our experiment the larvae were microinjected, which is an invasive manipulation that increases the mortality of caterpillars, particularly in the multiparasitism treatment. However, when we excluded caterpillars that died during the experiment (probably due to the invasive manipulation), we found that the presence of *C. glomerata* significantly reduced *H. ebeninus* developmental mortality (i.e. when wasps die during their development and caterpillars survive), except when caterpillars were injected with both PDV and venom ($\chi^2_{(4)} = 23$, $P < 0.001$) (Fig. 1D). This result is likely to be caused by a reduction in *H. ebeninus* encapsulation thanks to the presence of *C. glomerata* PDVs and venom. However, we cannot exclude the possibility that *C. glomerata* improves the host nutritional milieu or another host trait that has a positive effect on *H. ebeninus* mortality. Only a handful of studies have examined the influence of maternal factors on the outcome of competition among parasitoids (Magdaraog et al., 2016). Contrary to our results, Paul et al. (2024) found no effect of *C. glomerata* maternal factors on the encapsulation of the solitary parasitoid *Cotesia rubecula*, but they killed the caterpillars two days after injection, potentially missing later encapsulations. Interestingly, they showed that injection of *C. rubecula* maternal factors in hosts parasitized by *C. glomerata* has a significant negative effect on their survival. It can be hypothesized that maternal factors of solitary parasitoids are more likely to play a role in the suppression of intrinsic competitors than those of gregarious parasitoids. Finally, similar to Poelman et al. (2014), we found no effect of multiparasitism and parasitoid factors on the dry weight

of adult *H. ebeninus* (females: $\chi^2_{(4)} = 4.79$, P = 0.31; males: $\chi^2_{(4)} = 5.86$, P = 0.21) (Fig. 2A, B).

Although research into microbe-mediated competition such as by polydnaviruses in insect parasitoids is relatively new, recent studies have demonstrated that microbes can influence the outcome of competition. This influence operates directly on competitive parasitoid larvae and indirectly by altering the environment, particularly the physiology of parasitized hosts. As recently noted in a review by Pekas et al. (2023), "The role of PDVs in modulating competition among parasitoids has not received the attention it deserves". In this context, our findings suggest that the reduction of *H. ebeninus* developmental mortality in presence of its intrinsic competitor *C. glomerata* is at least partially mediated by maternal factors. Our results also suggest that the negative effect of multiparasitism on *H. ebeninus* development time is not due to maternal factors, but rather to the time spent chasing competing parasitoid larvae. Future studies should investigate whether the negative effects of maternal factors on intrinsic competitors are a trait that has been lost in gregarious parasitoids due to their scramble competition strategy. Understanding the mechanisms mediating the intrinsic competition between parasitoids is crucial for gaining deeper insights into the role of competition in parasitoid ecology.

ACKNOWLEDGEMENTS. This research was financially supported by Early Postdoc Mobility fellowship from the Swiss National Science Foundation to M.A.C.C. and Open Competition grants from the Netherlands Organization for Scientific Research (NWO): ALWOP.343 to A.C. and ALWOP.368 to E.H.P.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

REFERENCES

Beckage N.E. & Gelman D.B. 2004: Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. — *Annu. Rev. Entomol.* **49**: 299–330.

Cuny M.A.C. & Poelman E.H. 2022: Evolution of koinobiont parasitoid host regulation and consequences for indirect plant defence. — *Evol. Ecol.* **36**: 299–319.

Cusumano A., Peri E., Vinson S.B. & Colazza S. 2012: The ovipositing female of *Ooencyrtus telenomicida* relies on physiological mechanisms to mediate intrinsic competition with *Trissolcus basalis*: Physiological suppression in egg parasitoids. — *Entomol. Exp. Appl.* **143**: 155–163.

Cusumano A., Peri E. & Colazza S. 2016: Interspecific competition/facilitation among insect parasitoids. — *Curr. Opin. Insect Sci*. **14**: 12–16.

Cusumano A., Zhu F., Volkoff A.-N., Verbaarschot P., Bloem J., Vogel H., Dicke M. & Poelman E.H. 2018: Parasitic wasp-associated symbiont affects plant-mediated species interactions between herbivores. — *Ecol. Lett.* **21**: 957–967.

Drezen J.-M., Leobold M., Bézier A., Huguet E., Volkoff A.-N. & Herniou E.A. 2017: Endogenous viruses of parasitic wasps: variations on a common theme. — *Curr. Opin. Virol.* **25**: 41–48.

Godfray H.C.J. 1994: *Parasitoids: Behavioral and Evolutionary Ecology.* Princeton University Press, Princeton, NJ, 488 pp.

Gols R., Wagenaar R., Bukovinszky T., Dam N.M. van, Dicke M., Bullock J.M. & Harvey J.A. 2008: Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. — *Ecology* **89**: 1616–1626.

Harvey J.A., Poelman E.H. & Tanaka T. 2013: Intrinsic inter- and intraspecific competition in parasitoid wasps. — *Annu. Rev. Entomol*. **58**: 333–351.

Magdaraog P.M., Tanaka T. & Harvey J.A. 2016: Wasp-associated factors act in interspecies competition during multiparasitism. — *Arch. Insect Biochem. Physiol*. **92**: 87–107.

Moreau S. & Asgari S. 2015: Venom proteins from parasitoid wasps and their biological functions. — *Toxins* **7**: 2385–2412.

Paul R.L., Vyas D.K. & Ode P.J. 2024: Oviposition fluids from adult wasps mediate interspecific competition between parasitoid larvae. — *Ecol. Entomol*. **49**: 31–40.

Pekas A., Alejandro T., Ezio P., Stefano C. & Cusumano A. 2023: Competitive interactions in insect parasitoids: effects of microbial symbionts across tri-trophic levels. — *Curr. Opin. Insect Sci.* **55**: 101001, 7 pp.

Poelman E.H., Gols R., Gumovsky A.V., Cortesero A.M., Dicke M. & Harvey J.A. 2014: Food plant and herbivore host species affect the outcome of intrinsic competition among parasitoid larvae. — *Ecol. Entomol.* **39**: 693–702.

R Core Team 2021: *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, URL: https://www.R-project.org/

Strand M.R. & Burke G.R. 2019: Polydnaviruses: Evolution and function. In Bonning B.C. (ed.): *Insect Molecular Virology: Advances and Emerging Trends*. Caister Academic Press, Poole, pp. 163–181.

Tillman P.G. & Powell J.E. 1992: Interspecific discrimination and larval competition among *Microplitis croceipes*, *Microplitis demolitor*, *Cotesia kazak* (Hym.: Braconidae), and *Hyposoter didymator* (Hym.: Ichneumonidae), parasitoids of *Heliothis virescens* (Lep.: Noctuidae). — *Entomophaga* **37**: 439–451.

Volkoff A.-N., Jouan V., Urbach S., Samain S., Bergoin M., Wincker P., Demettre E., Cousserans F., Provost B., Coulibaly F. et al. 2010: Analysis of virion structural components reveals vestiges of the ancestral ichnovirus genome. — *PLoS Pathog.* **6**: e1000923, 10 pp.

Zhu F., Broekgaarden C., Weldegergis B.T., Harvey J.A., Vosman B., Dicke M. & Poelman E.H. 2015: Parasitism overrides herbivore identity allowing hyperparasitoids to locate their parasitoid host using herbivore-induced plant volatiles. — *Mol. Ecol*. **24**: 2886–2899.

Zuur A.F., Ieno E.N., Walker N., Saveliev A.A. & Smith G.M. 2009: *Mixed Effects Models and Extensions in Ecology with R.* Springer, New York, NY.

Received March 29, 2024; revised and accepted June 17, 2024
Published online July 17, 2024