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First records of *Glyptapanteles liparidis* (*Braconidae, Microgastrinae*) and *Gelis areator* (*Ichneumonidae, Cryptinae*) in Slovenia

*Vladimir Žikić[1], Tanja Bohinc[2], Christos Athanassiou[3], Paraskevi Agrafioti[3], Matilde Eizaguirre[4], Drago Trajber[5], Stanislav Trdan[2]**

[1]*Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia*
[2]*Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3]*Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Nea Ionia, Greece*
[4]*Department of Crop and Forest Sciences, University of Lleida-Agrotenio Center, Lleida, Spain*
[5]*Slovenia Forest Service, Regional Unit Murska Sobota, Murska Sobota, Slovenia*

**Corresponding author: stanislav.trdan@bf.uni-lj.si*

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Abstract: *Lymantria dispar*, a well-known defoliating pest species, also presents a health challenge due to the allergenic potential of its hairs, making it interesting to study from various perspectives. As chemical control methods decline in popularity, the search for effective natural enemies of this pest, such as predators, parasites and parasitoids, are expanding. On this occasion, we report the discovery of the primary parasitoid *Glyptapanteles liparidis* and the secondary parasitoid *Gelis areator* for the fauna of Slovenia. Illustrations and a short biology are given for both species.

Keywords: antagonists; competitors; hymenopterans; hyperparasitoid; primary parasitoid

The spongy moth, *Lymantria dispar* (L.) (*Lepidoptera*: *Erebidae*), is a polyphagous Eurasian-origin insect, that was introduced to Africa and North and South America. It is a univoltine species with sporadic outbreaks (Hlásny et al. 2016). The caterpillars are voracious eaters that cause complete defoliation in deciduous and coniferous forests and urban areas (Boukouvala et al. 2022). Particularly in urban environments, the caterpillars are known to trigger allergies in humans and domestic animals (Aldrich et al. 1997; Haq et al. 2021). As insecticide use is now discouraged or restricted in many regions

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where this moth is distributed, research into environmentally friendly control methods has increased (El-Sayed et al. 2006; Hajek, Delalibera 2019). Several insect species have already been confirmed to be potential natural enemies of the spongy moth (e.g. Alalouni et al. 2013; Žikić et al. 2017). The success of parasitism/predation can depend on the phase of the outbreak. It has been confirmed that more than 150 species of parasitoids can attack the spongy moth in Europe, i.e. 109 hymenopteran species and 56 dipteran species. Despite this high number of natural enemies, only a few of them have the potential to suppress the spongy moth. Therefore, it is important for researchers to identify new natural enemies of the spongy moth, especially those that are more effective in suppressing the spongy moth than already identified species. Currently, the entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu et Soper, which is nowadays widespread in the Balkans and central Europe, has been used very successfully against the spongy moth (Zúbrik et al. 2018).

The genus *Glyptapanteles* is in the *Microgastrinae* subfamily, one of the largest subfamilies of *Braconidae* with approximately 3 000 species described out of approximately 40 000 that have not yet been described (Fernandez-Triana et al. 2020). To date, 113 species of the genus *Glyptapanteles* have been described. The status of the genus is still not definitive, as some authors consider *Glyptapanteles* to be a synonym of *Protapanteles* Ashmead (e.g. Yu et al. 2012; van Achterberg et al. 2017). On the other hand, Fernandez-Triana et al. (2020) noted that there are specific morphological differences between the two genera, such as the structure of the hypopygium, ovipositor sheath, tergite 1 and others previously observed by Mason (1981). Certainly, many analyses are needed to conclusively define the status of the members of this genus. *Glyptapanteles* parasitize Macrolepidopteran larvae. Many species are gregarious, and a smaller number are solitary. The aim of this paper is to report the discovery of two new parasitoids of *L. dispar* in Slovenia.

MATERIAL AND METHODS

Material collected. A visibly parasitized fourth instar (L4) *L. dispar* larva (Figure 1) was collected on the bark of the common hornbeam, *Carpinus betulus* L. approximately 0.5 m above the ground on May 25, 2023 in northeastern Slovenia, Ginjevec forest, Nedelica, Turnišče (46°37'28.2"N, 16°20'58.4"E, 170 m a.s.l.; see Figures 2, 3). We re-

Figure 1. Larva of *Lymantria dispar*, surrounded by the cocoons of *Glyptapanteles liparidis* in the locality of the Ginjevec forest

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Figure 2. Locality where the wasps *Glypanteles liparidis* and *Gelis areator* were collected in Slovenia (MapCustomizer 2023)

Figure 3. The Ginjevec forest with indication of the place from where the wasps *Glypanteles liparidis* and *Gelis areator* were collected (Google Maps 2023)

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moved the still-alive caterpillar surrounded by parasitoid cocoons from the bark using tweezers and transferred them to the Laboratory for Phytomedicine (Biotechnical Faculty, University of Ljubljana, Slovenia) in a plastic jar with a lid. In the laboratory, we left the jar containing the cocoons in the shade at room temperature. After one week, the first adults emerged from the cocoons. From about twenty cocoons distributed around *L. dispar* larva, 5 male *Glyptapanteles liparidis* (Bouché, 1834), and 2 male *Gelis areator* emerged (Panzer, 1804; Figures 4, 5). Parasitoid adults were transferred to 60% ethanol and stored in a refrigerator until they were sent in an Eppendorf tube to the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš (Serbia) for morphological determination. To identify specimens of *Glyptapanteles*, we used the key provided by Tobias et al. (1986), and for *Gelis* we used Schwarz and Shaw (1999).

Investigated site. The Ginjevec forest covers an area of 380 ha. Among the most common tree species found in the forest are pedunculate oak (*Quercus robur* L.) (60%), hornbeam (25%) and Scots pine (*Pinus sylverstris* L.) (10%). Tree species such as black alder (*Alnus glutinosa* L.) and European ash (*Fraxinus excelsior* L.) can also be found in the Ginjevec forest, but they make up less than 5% of the total. The Ginjevec forest is surrounded by agricultural land, dominated by the cultivation of winter wheat and maize, the Dobrovnik gravel pit, and six villages: Turnišče, Nedelica, Radmožanci, Kamovci, Žitkovci, and Dobrovnik (Figure 3). The eastern edge of the Ginjevec forest is approximately 3 km from the Slovenian-Hungarian border.

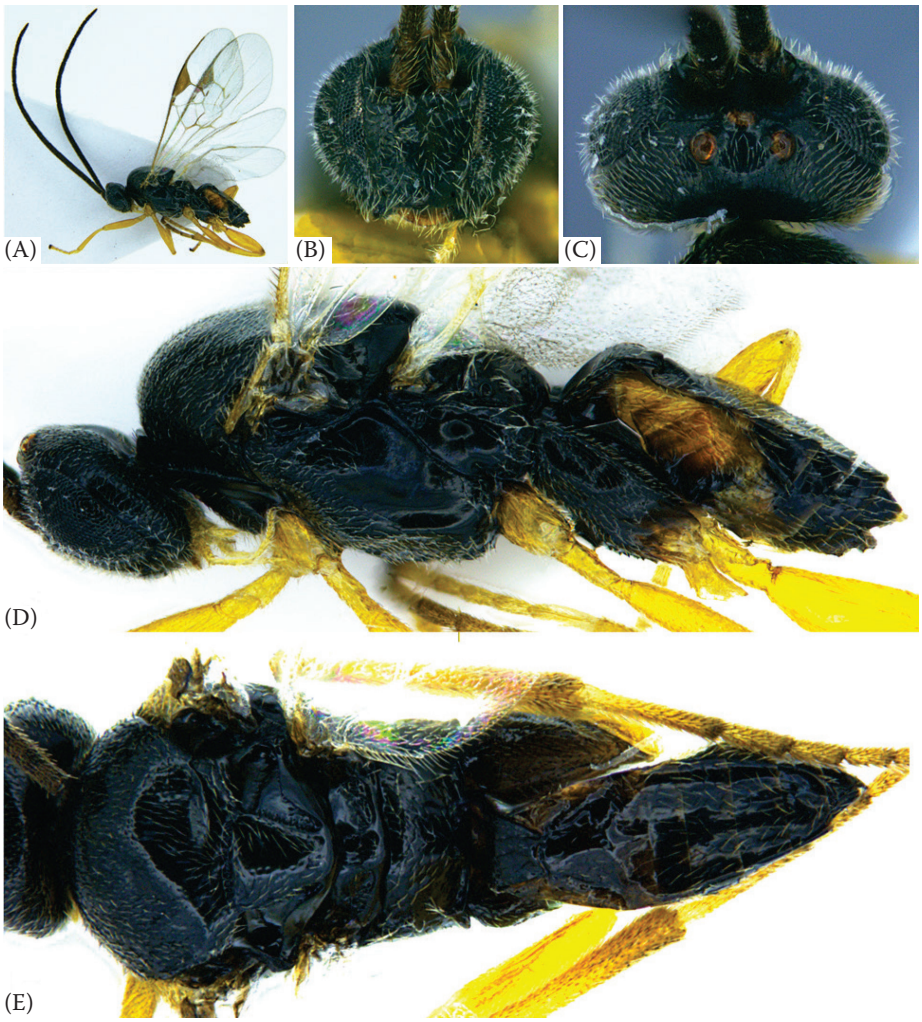

Figure 4. Male of *Glyptapanteles liparidis*: (A) habitus, (B) head (frontal view), (C) head (dorsal view), (D) body (lateral view), (E) body (dorsal view)

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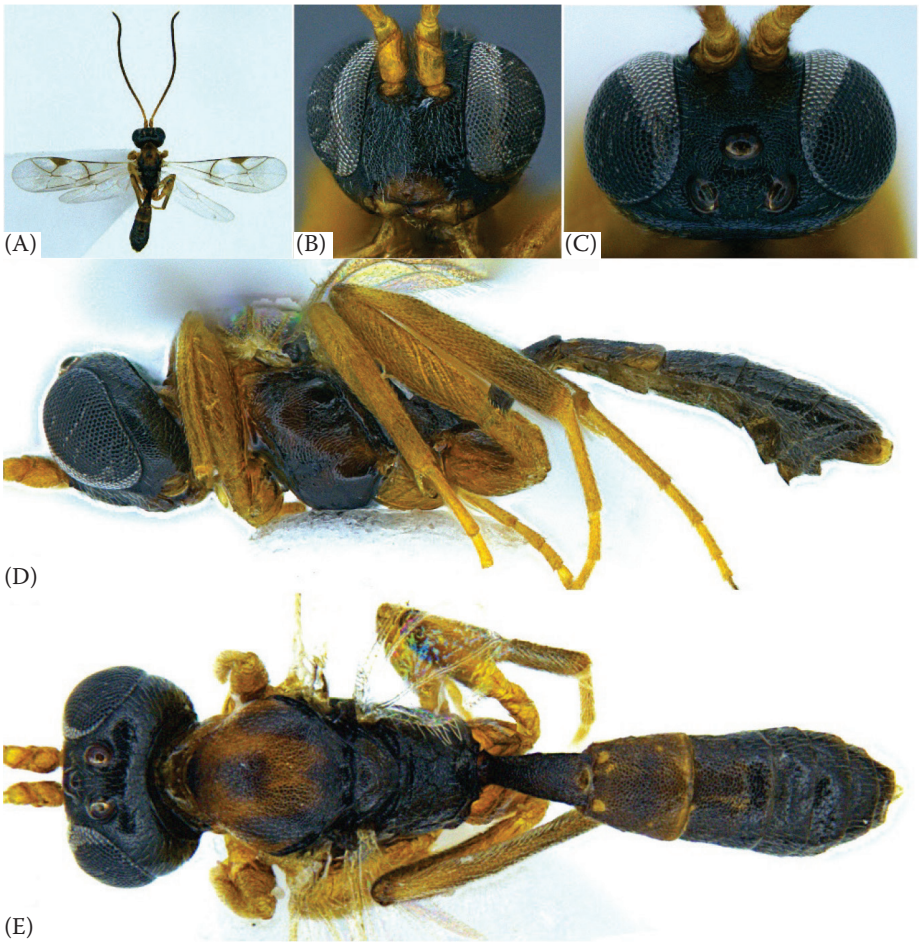

Figure 5. Male of *Gelis areator*: (A) habitus, (B) head (frontal view), (C) head (dorsal view), (D) body (lateral view), (E) body (dorsal view)

RESULTS AND DISCUSSION

Glyptapanteles liparidis (Figure 4) is a gregarious koinobiont endoparasitoid, primarily known as a natural enemy of *L. dispar* but also recorded to parasitize some lasiocampids, such as many species from the genus *Dendrolimus* (e.g. Papp 1984) as well as *Malacosoma neustria* L. (e.g. Balevski 1999), some erebids, such as *Orgyia* (Chou 1979) or *Euproctis* (e.g. Balevski 1999), and other species. These data should be interpreted cautiously, as past instances of misidentification of parasitoids have resulted in substantial errors that needed later correction. Unlike the spongy moth, *G. liparidis* is a multivoltine species (Cho et al. 2006), the females prefer to lay eggs in early larval instars, L1–L3, but oviposition in L4 instar has also been recorded (Schopf, Hoch 1997). As a rule, the caterpillars are superparasitized, so a smaller or larger number of parasitoids may emerge from them. The emerging caterpillar instar is usually L4. *G. liparidis* is widely distributed across Europe, Russia, China, India, Iran, Japan, and the Korean peninsula (Yu et al. 2016). In the context of parasitism on *Lymantria dispar*, a notable competitor of *G. liparidis* is *G. porthetriae* (Muesebeck, 1928), as mentioned by Marktl et al. (2002). The second parasitoid, *G. porthetriae*, solely parasitizes early larval instars of *L. dispar* (L1 and L2). *G. porthetriae* quickly completes its life cycle inside the host body. A single parasitoid larva emerges from the second larval instar start to spin the cocoon under the host. Hidden under the body of the host, the parasitoid in the cocoon is physically protected from natural enemies for a short time.

Another species associated with *L. dispar* identified in the same sample is *Gelis areator* (Figure 5). Considering its extensive distribution across Eurasia (van Achterberg et al. 2017), it was only a matter of time until this species appeared in Slovenia.

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This ichneumonid is a solitary parasitoid that lays its eggs in the larvae or pupae of *Lepidoptera* and *Hymenoptera*. It acts as both a primary and secondary parasitoid. In its role as a primary parasitoid, *G. areator* primarily targets 'microlepidopterans' from the *Coleophoridae* family (e.g. Baeschlin 1974; Lampe 1984; Parkinson 2016), *Choreutidae* (Shaw, 1984), and *Gracillaridae* (Górska-Drabik, Napiórkowska-Kowalik 2009), as well as many other lepidopterans (Yu et al. 2016) and hymenopterans, e.g. *Cimbicidae* (Vikberg, Viitasaari 1991). As a hyperparasitoid, *G. areator* targets many hymenopterans, including *Ichneumonidae* and *Braconidae* (He et al. 1996). It has also been suggested that it parasitizes certain *Diptera*, *Coleoptera*, and *Neuroptera* (Yu et al. 2016). Considering that *G. areator* regulates the population of the primary parasitoid *G. liparidis*, this has a direct impact on the effectiveness of spongy moth control.

CONCLUSION

The first report of the parasitoids *Glyptapanteles liparidis* and *Gelis areator* in Slovenia is a significant entomological event. Prior to this finding, in-depth research on the distribution of native natural enemies of the spongy moth in Slovenia and its neighbouring countries was lacking.

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