Short Communication

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First Report of Tritrophic Relationships among Soft Scale *Physokermes hemicryphus* (Dalman, 1826) (Hemiptera), Polyembryonic Parasitoid *Pseudorhopus testaceus* (Ratzeburg, 1848) (Hymenoptera) and the Predator *Anthribus nebulosus* Forster, 1770 (Coleoptera)

***Marina Dervišević*[1*], *Aleksandar Stojanović*[2], *Marija Simonović*[1] & *Draga Graora*[1]**

[1] Faculty of Agriculture, University of Belgrade, 11080 Belgrade, Serbia
[2] Natural History Museum, 11000 Belgrade, Serbia

Abstract: The tritrophic relationships among the soft scale *Physokermes hemicryphus* (Dalman, 1826) (Hemiptera), the polyembryonic parasitoid *Pseudorhopus testaceus* (Ratzeburg, 1848) (Hymenoptera) and the predator *Anthribus nebulosus* Forster, 1770 (Coleoptera) is documented and reported for the first time. In Belgrade region (Serbia), in scale insects *P. hemicryphus*, the simultaneous presence of *P. testaceus* parasitoid larvae and *A. nebulosus* larvae has been registered. Larvae of *A. nebulosus*, apart from scales' eggs, also feed on larvae of parasitoids *P. testaceus.* Competitive interactions between these two species, which are natural enemies of *P. hemicryphus*, have not been known until now.

Key words: tritrophic relationships, Coccidae, Encyrtidae, Anthribidae

Introduction

Small spruce scale *Physokermes hemicryphus* (Dalman, 1826) (Hemiptera: Coccidae) is a Holarctic oligophagous species developing on the plants of the genera *Picea* Mill. and *Abies* Mill. (Pinaceae). Numerous colonies are developed on infested plants, causing needles to dry out and fall off, and due to long-lasting infestation, even branches to dry out. Young and physiologically weakened plants are particularly endangered (Schmutterer 1965, Kosztarab & Kozár 1988), which has also been registered in Belgrade (Kozarževskaja & Vlainić 1982, Mihajlović & Kozarževskaja 1983).

The high abundance of populations of this pest is regulated by a relatively large number of natural enemies. Until now, 18 parasitoid species have been registered on *P. hemicryphus*, belonging to the families Aphelinidae, Encyrtidae, Eulophidae and Pteromalidae (Noyes 2018). This scale host is significantly parasitised by *Pseudorhopus testaceus* (Ratzeburg, 1848) (Hymenoptera: Encyrtidae) (Schmutterer 1965, Santas 1988, Stathas et al. 2011). *Pseudorhopus testaceus* is a polyembryonic parasitoid exhibiting a development cycle synchronised with the cycle of *P. hemicryphus.* Parasitoid females lay eggs into host's first instar larvae, not disturbing their further growth and metamorphosis, so adult wasps eclose from the females of the scales (Vojnovich & Sugonyaev 1993). There are not enough data on the parasitoids of *P. hemicryphus* in Serbia. Three species were registered in Belgrade area in the 1980's: *Microterys lunatus* (Dalman, 1820), *Coccophagus lycimnia* (Walker, 1839) and *Pseudorhopus testaceus*, the latter one registered by the same study for the first time for Serbia (Mihajlović & Kozarževskaja 1983).

[*]Corresponding author: dervisevic.marina1989@gmail.com

Among predators, *Anthribus nebulosus* Forster, 1770 (Coleoptera: Anthribidae) is considered the most efficient species in reducing the numbers of *P. hemicryphus* (Klausnitzer & Förster 1976, Kosztarab & Kozár 1983, Santas 1988). It has also been registered as a predator of 14 scale insect species in Europe and Central Asia. Life cycle of *A. nebulosus* is synchronised with that of the host. Overall development of the predator takes place under the scale of the insect, where larvae feed on the eggs of the host (Kosztarab & Kozár 1983). This species has been introduced from Hungary to Virginia for the purpose of biological control of *Physokermes inopinatus* Danzig & Kozár, 1973 (Kosztarab & Rhoades 1983). In Serbia, *A. nebulosus* has been registered as a significant predator of *P. hemicryphus* and *P. piceae* (Schrank, 1801) (Mihajlović & Kozarževskaja 1983, Graora et al. 2012).

Pseudorhopus testaceus and *A. nebulosus* represent serious natural enemies of *P. hemicryphus*; however, their interspecific interactions with one another have not been studied. In this paper, for the first time, feeding of the predatory larvae of *A. nebulosus* on polyembryonic parasitoid *P. testaceus* in the scale *P. hemicryphus* has been described.

Materials and Methods

In Serbia, Belgrade region (44°46'57"N, 20°30'50"E), on May 3rd, 2017, five specimens of *P. hemicryphus* were sampled from one tree of *Picea abies* L. and a relatively large number of parasitoid larvae were observed. A detailed inspection and dissection using a stereomicroscope were performed. Sampling was repeated on May 11th, 2017, at the same locality, when 11 spruce branches were collected with 145 scales on them; of these, 42 scales exhibited the presence of polyembryonic larvae. Since predatory larvae are not visible through scales' cuticle, these scales were reared in the laboratory, each into a separate glass tube (8 x 4.5 mm, stoppered with tulle). Then the presence and number of parasitoids and predators were determined. After the development had terminated, the numbers of reared specimens of both species were determined. Spruce branches with remaining scales were put into glass cylinders in order to monitor the development of scales. The determination of parasitoid wasp species was completed by the second author.

Results

In the scales of *P. hemicryphus* sampled from *Picea abies* in Belgrade area, a simultaneous presence of parasitoid larvae of *P. testaceus* and predatory larvae of *A. nebulosus* was registered. The co-occurrence was associated with specific feeding of coleopteran larvae predating on parasitoid larvae. When there were no scale eggs as basic food for the predatory larva, it was feeding on polyembrionic parasitoid *P. testaceus* (Figs. 1 and 2). Among the infested 42 individuals of scales, a simultaneous presence of parasitoids and a predatory larva was recorded in 39 scales (92.9%). In these scales, the predatory larva had eaten all parasitoid larvae during its development, thus 39 adults of *A. nebulosus* eclosed.

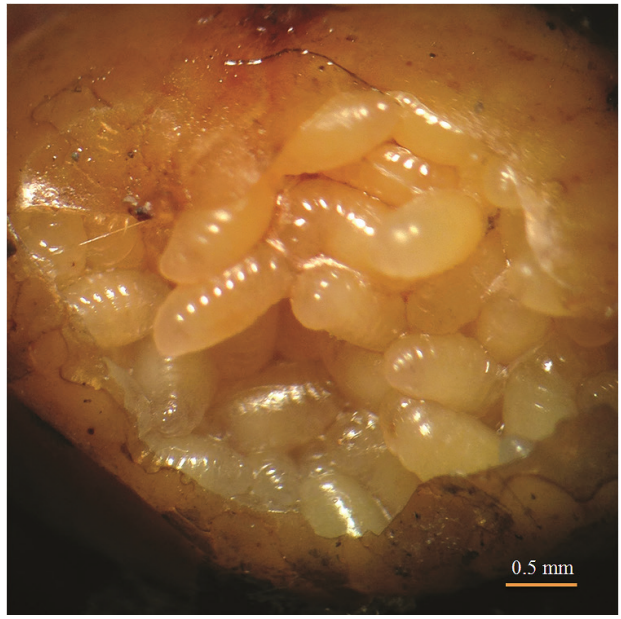

Fig. 1. Larvae of *Pseudorhopus testaceus* (Ratzeburg, 1848).

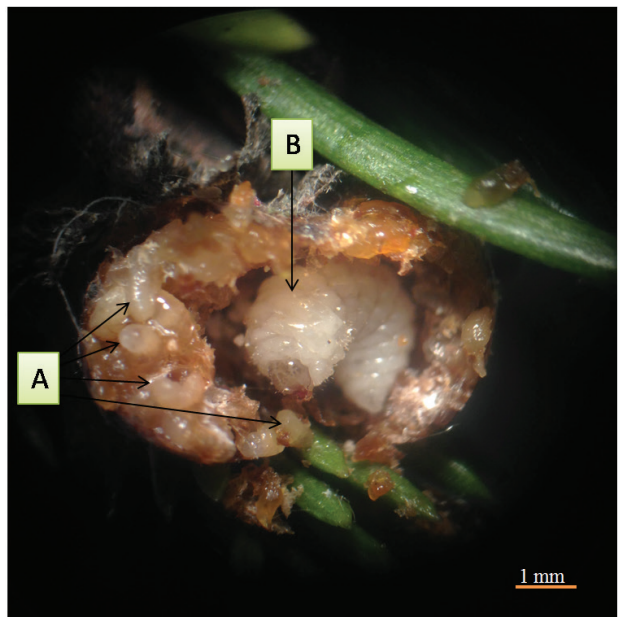

Fig. 2. Co-occurrence of larvae of *Pseudorhopus testaceus* (Ratzeburg, 1848) (**A**) and *Anthribus nebulosus* Forster, 1770 (**B**) associated with the same scale individual.

The eclosion was recorded on May 23rd, 2017, when 35 adults were recorded, while the remaining four adults eclosed on May 25th, 2017.

Predatory larvae were not present in three scales of *P. hemicryphus* (7.1%) and the larvae of *P. testaceus* developed to adults. The beginning of eclosion was recorded on May 23rd, 2017. From one scale, parasitoids were flying out for 2-3 days. Mass flying out of adults of *P. testaceus* was recorded on May 25th, in the period of first-stage *P. hemicryphus* larvae hatching, into which the parasitoid laid eggs.

Discussion

The high level of life cycle synchronisation between the parasitoid and the host was studied in detail in the area of Saint Petersburg (Russia). In this area, the eclosion of adults and the scale larvae hatching were registered at the beginning of August (Vojnovich & Sugonyaev 1993). The eclosed parasitoid adults from one scale are of the same sex, which is characteristic for the polyembryony. Thus, among the three scales of *P. hemicryphus* observed by us, 127 ♂ have eclosed from one scale, 131 ♀ from the second scale and 155 ♀ from the third scale. In Saint Petersburg area, av. 27 adult parasitoids per scale (Vojnovich & Sugonyaev 1993); in Germany, av. 39.54 adults per scale (Klausnitzer & Förster 1976) have been registered. Males and females eclose at the same time and their life span is 2-3 days.

We describe the first record of tritrophic relations among *P. hemicryphus*, *P. testaceus* and *A. nebulosus* – the larva of *A. nebulosus* feeds on larvae of *P. testaceus* inside the scale *P. hemicryphus*. *Anthribus nebulosus* is a successful competitor utilising 92.9% of infested scales compared to *P. testaceus* utilising only 7.1% of them in our study. Further investigations will determine the frequency of this phenomenon in nature and the negative impact of the feeding of *A. nebulosus* on the abundance of populations of *P. testaceus*.

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