Research Article

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Species of the Genus *Ooencyrtus* Ashmead, 1900 (Hymenoptera: Encyrtidae), Egg Parasitoids of *Thaumetopoea solitaria* (Lepidoptera: Notodontidae) in Bulgaria

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Abstract: The study was carried out in 2010-2015 at 6 sites in the Eastern Rhodopes: Madzharovo, Dam Ivailovgrad, Dupkata, Quarry, Liikanen and Meden Buk. A total of 424 *Thaumetopoea solitaria* egg clusters were collected from these sites and kept at room temperature. Out of a total of 52,628 eggs in the samples, 3 species of parasitoids of the genus *Ooencyrtus*, i.e. *O. masii*, *O. pityocampae* and *Ooencyrtus* sp. nr. *indefinitus*, were found. The dominant species was *O. masii*. The least abundant species, represented only by two emerged female individuals, was *O. pityocapae*. In the other two species, individuals of both sexes were recorded. In *O.* sp. nr. *indefinitus*, the ratio ♀♀:♂♂ was almost 1:1, whereas in *O. masii* the female individuals predominated. In laboratory conditions, the period of emergence of parasitoids was 41 days. For both species, *O. masii* and *O.* sp. nr. *indefinitus*, a similarity in the emergence dynamics was observed. In 2014, it was more extended, and shorter in the next year. The peak of emergence of *O. masii* was 20-25 days, earlier than that of the other parasitoids.

Key words: *Thaumetopoea solitaria*, *Ooencyrtus* spp., egg parasitoids, Eastern Rhodopes, Bulgaria

Introduction

Thaumetopoea solitaria (Freyer, 1838) (Lepidoptera: Notodontidae) was reported for the fauna of Bulgaria at the beginning of the XX century (Buresch 1915). The feeding plant is *Pistacia terebinthus* L., 1753, a species of no economic importance. This is one of the reasons for the limited number of studies on this insect species, mostly focussed on its occurrence and, to a lesser extent, on its biology.

Studies of *T. solitaria* parasitoids are few for its entire geographical range. For Israel, there are studies on parasitoids of caterpillars and pupae by Halperin (1983, 1990) and Kugler (1979). The publication of the latter author is on species of the family Tachinidae. Such survey on tachinid parasitoids of *T. solitaria* in Bulgaria was reported by Hubenov (1985).

Mirchev *et al.* (2014a, b) carried out the first studies on egg parasitoids of pistachio processionary moth and recorded species of the families Eupelmidae and Encyrtidae (Hymenoptera).

The aim of the present article is to reveal the species composition of representatives of the genus *Ooencyrtus* Ashmead, 1900 and to describe their phenology. In addition, we analyse their occurrence on the basis of several quantitative indices on the basis of a five-year study on egg parasitoids of *T. solitaria* in the Eastern Rhodopes.

Material and Methods

The study was carried out in 2010-2015 at 6 sites in the Eastern Rhodopes (Table 1). The habitats were mainly in the region of Ivaylovgrad. The air distance

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between the two most remote sites, Madzharovo and Meden Buk, was 33 km. Egg clusters of *T. solitaria* were collected during or immediately after hatching caterpillars of the pistachio processionary moth. They were transported to the entomology laboratory of the Forest Research Institute, Sofia. Each egg cluster was separately placed in a test tube with cotton stopper and kept at room temperature. Daily observations were implemented. Living individuals were placed in Eppendorf test tubes with 95% ethanol, and the dead ones were sampled in dry capsules for subsequent species identification.

The assessment of the parasitoids recorded and the environment where they were found was made on the basis of quantitative indices: index of occurrence (IO), representing the relative share of the parasitoids recorded on a particular site (sample); index of domination (ID), the percentage ratio of a specific parasitoid compared to the total number of parasitoids found; loyalty index (degrees of adherence) (LI), the percentage ratio between the individuals habitats with respect to a particular parasitoid (Beklemischev 1961). As for the latter index, since the collected experimental material considerably varies in individual habitats, the actual values are cited (LI) and, for the sake of comparability, recalculated indices (LIr) were also included that represented a weighted average value to the number of eggs of the host *T. solitaria.*

Results

We analysed 424 egg clusters containing 52,628 eggs. Three parasitoid species of the genus *Ooencyrtus* were reared: *O. masii* (Mercet, 1921), *O. pityocapae* (Mercet, 1921) and *Ooencyrtus* sp. nr. *indefinitus* Myartseva, 1982 (Table 2). The dominant species was *O. masii*, with index of domination (ID) 84.7; it was found in all six habitats studied. The second most abundant species, *O.* sp. nr. *indefinitus* (ID 14.6) was absent in the samples from Meden Buk, Dam and Liikanen. *O. pityocampae* was represented by only 2 female individuals emerged from the samples collected in 2010 and 2011 from the protected area Dupkata near Ivaylovgrad. From all 16 samples, in no sample were all the three species recorded to co-occur. In nine samples, only one species was found, in seven samples, two species were recorded, with clearly expressed domination of one of them. An exception is the sample from Quarry collected in 2014, where the participation of *O. masii* and *O.* sp. nr. *indefinitus* is almost equal, IO 52.4 and 47.6, respectively.

The data from the recalculated values of loyalty index (degrees of adherence) (IL) (Table 3) showed that the highest share was that of *O. masii* in Dam. This concentration is even stronger with *O.* sp. nr. *indefinitus*, which is concentrated almost entirely in Quarry.

Of the two species of the genus *Ooencyrtus* in the samples from 2014 and 2015, the ratio between males and females of *O.* sp. nr. *indefinitus* is almost 1:1 whereas, for *O. masii*, the female individuals considerably predominated (Table 4). As it has been noted above, out of the 2010 and 2011 samples, 2 ♀♀ individuals of *O. pityocampae* emerged.

The 2014 and 2015 samples were collected in almost the same calendar time, with a difference of about a week. These samples were kept in a laboratory under completely comparable conditions. In 2014, the period of emergence of *O. masii* and O. sp. nr. *indefinitus* lasted 41 days (from 25 April to 4 June) (Fig. 1) and, in 2015, 35 days (from 29 April to 2 June) (Fig. 2). In these two species, a similarity in the trend of the emergence dynamics was observed. In 2014 it was more extended and in 2015 it was shorter. In the first year, *O. masii* emerged in 37 days and in the second year in 19 days, i.e. roughly half the time; for *O.* sp. nr. *indefinitus,* these values were 27 (2014) and 18 (2015) days. There were differences in their phenology. The peak of *O. masii* emergence was 20-25 days before that of *O.* sp. nr. *indefinitus*.

Discussion

The genus *Ooencyrtus* comprises 312 species (Noyes 2016). *O. masii* is distributed in Europe and Asia as

Table 1. Main characteristics of studied areas

Sample plot	Locality	Geographical coordinates	Altitude, m
Madzharovo	Madzharovo town	41º38’50.1’’ N; 25º52’54.3’’ E	200
Dam Ivailovgrad	Ivaylovgrad town	41º35’03.2’’ N; 26º06’33.7’’ E	140
Dupkata	Ivaylovgrad town	41º31’41.7’’ N; 26º06’57.1’’ E	320
Quarry	Ivaylovgrad town	41º31’51.2’’ N; 26º05’32.1’’ E	330
Liikanen	Belopolyane village	41º26’51.4’’ N; 26º08’38.3’’ E	200
Meden Buk	Meden Buk village	41º22’10.3’’ N; 26º03’37.6’’ E	120

Table2. Egg parasitoids of the genus *Ooencyrtus* on *Thaumetopoea solitaria* in Bulgaria

Site of collection			M. Buk	Dupkata					Madzharovo				Dam		Liikanen		Quarry		Total number	Index domination
T. solitaria	Date of collection		8.4.14	27.3.10	13.4.11	27.3.12	8.4.14	16.4.15	12.4.11	27.3.12	7.4.14	16.4.15	27.3.10	16.4.15	27.3.12	8.4.14	8.4.14	16.4.15		
	Eeg clusters, n		16	33	34	39	44	29	23	20	18	8	5	8	48	35	26	38		
	Total number of eggs		1 921	4 076	4 228	4 833	5 656	3 566	2 970	2 832	2 523	1 118	526	1 231	5 447	4 081	3 158	4 462		
Parasitoids	*O. masii*	n	14	27	14	7	10	8	15	5	6	11	12	7	30	21	11	40	238	84.7
		*	100.0	96.4	93.3	100.0	76.9	88.9	100.0	100.0	100	78.6	100.0	100.0	100.0	100.0	52.4	62.5		
	O. sp. nr. *indefinitus*	n	-	-	-	-	3	1	-	-	-	3	-	-	-	-	10	24	41	14.6
		*	-	-	-	-	23.1	11.1	-	-	-	21.4	-	-	-	-	47.6	37.5		
	O. pityocampae	n	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.7
		*	-	3.6	6.7	-	-	-	-	-	-	-	-	-	-	-	-	-		

*- Index of occurrence

Table 3. Percentage of parasitoids of the genus *Ooencyrtus* in studies habitats

Parasitoids	**Σn**	**Habitats**											
		M. buk		**Dupkata**		**Madzharovo**		**Dam**		**Liikanen**		**Quarry**	
		*	**	*	**	*	**	*	**	*	**	*	**
O. massi	238	5.9	19.7	27.8	8.1	15.5	10.6	8.0	29.2	21.4	14.4	21.4	18.0
O. sp. nr. *indefinitus*	41	-	-	9.8	3.7	7.3	6.4	-	-	-	-	82.9	89.9
O. pityocampae	2	-	-	100.0	100.0	-	-	-	-	-	-	-	-

* LI - loyalty index, actually calculated

** LIr - loyalty index recalculated as weighted average value to the number of the host eggs

Table 4. Sex ratio of parasitoids of *Ooencyrtus* genus collected in 2014 and 2015

Parasitoids	♀♀		♂♂		Σ	
	n	%	n	%	n	%
O. masii	97	75.8	31	24.2	128	100.0
O. sp. nr. *indefinitus*	23	56.1	18	43.9	41	100.0

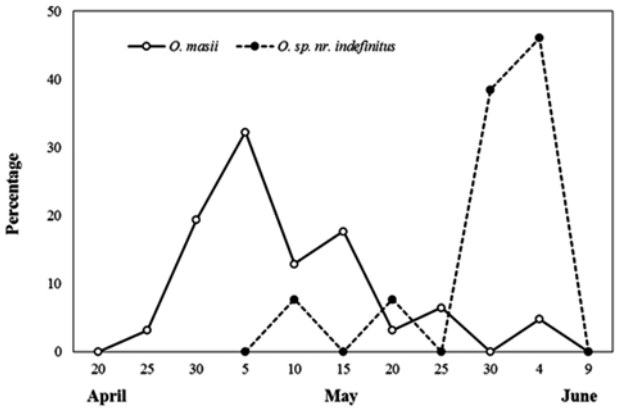

Fig. 1. Emergence dynamics of the parasitoids in 2014: *O. masii* (N=62); *O.* sp. nr. *indefinitus* (N=13)

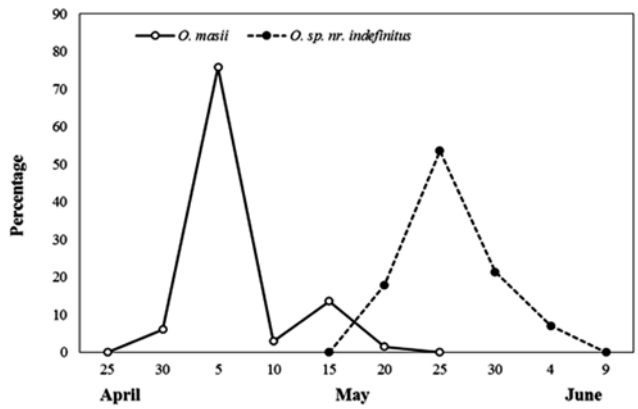

Fig. 2. Emergence dynamics of the parasitoids in 2015: *O. masii* (N=66); *O.* sp. nr. *indefinitus* (N=28)

a parasitoid of lepidopterans *Malacosoma neustria* (L., 1758) (Lasiocampidae), *Lymantria dispar* (L., 1758) (Erebidae), *Thaumetopoea processionea* (L., 1758) (Notodontidae), *Traumatocampa ispartaensis* (Doğanlar & Avci, 2001) (Notodontidae), *Ocneria terebynthina* Staudiger, 1895 (Erebidae) (Noyes 2016) and *T. solitaria* (Mirchev *et al.* 2014a). The gypsy moth (*L. dispar*), lackey moth (*M. neustria*) and oak processionary moth (*T. processionea*) are among the most large-scale pests for deciduous forests in Bulgaria (Mirchev *et al.* 2003). The presence of these alternative hosts is a pre-condition for its high numbers and its role as dominant among the representatives of *Ooencyrtus* as parasitoids on pistachio processionary moth in the habitats under study. For Bulgaria, the species was found as an egg parasitoid of *M. neustria*. The intensity of parasitism was the highest with freshly deposited eggs of this host located on the lower branches of the eastern side of the tree crown (Zanaty 1978).

O. indefinitus is described as a parasitoid on *Dolycoris penicillatus* Horváth, 1904 (Hemiptera: Pentatomidae) in Turkmenistan (Myartseva 1982, Trjapitzin 1989). The genus *Dolycoris* includes five Palaearctic species and only one of them, *D. baccarum* (L., 1758), occurs in Bulgaria and the Balkan Peninsula (Josifov 1981).

Concerning *O.* sp. nr. *indefinitus*, the sites where the experimental materials were collected are within a small perimeter and we could hardly expect substantial differences in the structure-defining ecological factors, at least with respect to the climatic component. The conditions for the presence of *O.* sp. nr. *indefinitus* are to be searched primarily in the plant communities of the individual sites. The experimental areas Dupkata and Quarry are indicative in that respect. The air distance between them is less than 2 km and both are karst habitats. On the first site, the numbers of *O.* sp. nr. *indefinitus* are low and, on the second site, it is a large-scale species. The terrain of Dupkata is mainly covered by plantations of *Pinus nigra* Arnold, 1785 and of the second site by sprout deciduous trees and bush vegetation. The egg clusters were collected at the beginning of April. For the April-May, it can be assumed that at least temperature conditions in the laboratory and in the field are not drastically different. The emergence of the species in May, when there are no newly-laid eggs of *T. solitaria* in nature, is evidence of the presence of one or several alternative hosts to ensure the existence of the species. The different floristic composition of the individual habitats determines, in varying degrees, the conditions for the presence of such alternative hosts. A corroboration of this thesis can be found in the results of Mirchev *et al.* (2014a). In the egg clusters of *T. solitaria* collected in the habitat Liikanen on 12 July 2010, all parasitoids had already emerged by that date.

O. pityocampae has an extensive geographical range (Europe, North Africa and Central America) and a wide host range that includes more than 28 species (Noyes 2016). In the habitats studied by us, no fau-

nistic research has been carried out to establish what part of those alternative hosts inhabit them. However, for one of the sites, Dupkata, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) is present with high population numbers, at least in the last decade (Mirchev *et al.* 2012). *O. pityocapae* is a major parasitoid on the pine processionary moth, and only female individuals emerge from the eggs of this host while the appearance of male individuals is extremely rare (Mirchev 2005). The co-occurrence of *T. solitaria* and *T. pityocampa* in this habitat is the prerequisite this highly plastic polyphagous parasitoid to parasitize eggs of the pistachio processionary moth, although in isolated cases and only in some years.

Some ecological factors also influence the sex determination of parasitoids. For *O. pityocampae,* Halperin (1990) has found that this is the temperature during the development of the larval stage; at certain values of temperature, only males, only females, or both sexes emerge. The determining role of temperature for the sex structure of another representative of this genus, *O. submetallicus* (Howard, 1897), has been reported by Wilson & Woolcock (1960). Another factor determining the sex of some polyphagous parasitoids is the host. Bellin *et al.* (1990) report that only males of *Anastatus bifasciatus* (Geoffroy, 1785) (Hymenoptera: Eupelmidae) develop in the eggs of *T. pityocampa*. Mirchev (2005), after analysing a large number of samples, reports that in this host-parasitoid association, the appearance of females is extremely rare. Only male individuals of *Eupelmus vesicularis* (Retzius, 1783) (Hymenoptera: Eupelmidae) develop in eggs of *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera: Notodontidae) (Halperin 1990). The sex ratio of the three representatives of the genus *Ooencyrtus* recorded in this study is different; they developed on one and the same host, under the same temperature conditions.

The emergence dynamics in the two years of our study (2014 and 2015) and for the two parasitoids have different character. In the first year, it is more extended, and in the second one, it is explosive. For *L. dispar*, it has been observed that such dynamics depends on the temperature conditions during the hibernation of the eggs in natural conditions (Mirchev & Popova 1984).

The results obtained serve as grounds for the following inferences:

- The term of emergence indicates that, in addition to *O. masii* and *O. pityocampae* (for which this has been reported), *Ooencyrtus* sp. nr. *indefinitus* is also a polyphagous species.

- The ecological factor determining differences in the species composition and abundance in studied habitats is their floristic diversity ensuring appropriate conditions for the development of alternative hosts. The site Dupkata is indicative in that respect – along with the autochthonous vegetation, there is also introduced *Pinus nigra*, ensuring the constant presence of the pine processionary moth, being a host of *O. pityocampae.* That is why all three parasitoids were established in this habitat.

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