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RESEARCH ARTICLE

Egg parasitoids of *Thaumetopoea pityocampa* in the region of Gyumyurdzhinski Snezhnik in Eastern Rhodopes, Bulgaria

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Abstract

The region of Gyumyurdzhinski Snezhnik in the Eastern Rhodopes is the closest in Bulgaria to the Aegean Sea. However, the climate is characterized by specific parameters that are determined by its relief. It is poorly protected from the invasion of cold air masses from the north. From the south, the Gyumyurdzhinski Snezhnik hill restrains the Mediterranean influence. The orography of the area favors the retention of cold air masses and a further drop in temperatures. The experimental material for the study includes 5 generations of *Thaumetopoea pityocampa* (2016, 2017, 2018, 2019, and 2022), collected in 31 locations of four State Forestry Enterprises: Kirkovo, Ardino, Momchilgrad, and Zlatograd. The sample for analysis included 693 egg batches with 148420 eggs in them. Seven primary egg parasitoids were established in this region: *Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Pediobius bruchicida*, *Anastatus bifasciatus*, *Eupelmus vesicularis*, *E. vladimiri*, *Trichogramma* sp. and one hyperparasitoid (*B. transversalis*). Dominant parasitoids were *B. servadeii* and *O. pityocampae*, and *E. vladimiri* and *P. bruchicida* – occasional parasitoids. The hyperparasitoid *B. transversalis* participated in the complex with a relatively low share. The survival of the egg parasitoids in the laboratory conditions, in which the samples were kept, was low. The total mortality of the parasitoids in larval and adult stages was 47.8%. After collecting the samples, in laboratory conditions, a total of 442 individuals of the hyperparasitoid *B. transversalis* emerged, of which 56.3% were females and 43.7% were males. The average number of pine processionary moth eggs in a batch was 214.2. 70.8% of all the eggs in the samples hatched successfully. The egg parasitoids are a very serious natural factor, regulating the density of the pine processionary moth, but their impact varied from 2.1% to 30.3%. The natural characteristics of the area, the air temperature during the stages of eggs and young larvae, are favorable for the development of the pine processionary moth. Unhatched larvae without the influence of entomophages were 7.2%.

Keywords
Thaumetopoea pityocampa, ecology, parasitoids, Eastern Rhodopes, Bulgaria

Introduction

The pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermuller, 1775) (Lepidoptera: Notodontidae) is the most dangerous defoliator in pine forests in the Mediterranean region (Demolin, 1969b; Schmidt et al., 1990), including Bulgaria (Mirchev et al., 2003). The larvae attack the needles of different pine species (Devkota, Schmidt, 1990). Their hairs contain urticarial substances, causing dermatitis (Lamy, 1990).

The egg parasitoids are the most significant biological factor, regulating the numbers of the pine processionary moth (Mirchev, 2005; Schmidt et al., 1999; Tsankov, 1990).

The diversity of egg parasitoids of *T. pityocampa* in different areas is explained by the influence of ecological factors: temperature (Masutti, 1964), altitude (Tiberi, 1990), the floristic richness of the habitats is a prerequisite for the high parasitoid numbers, due to the favorable conditions for the development of alternative hosts (Mirchev, 2005).

Environmental factors influence the survival and other stages of the ontogenetic development of *T. pityocampa*. High summer temperatures are critical for the survival of eggs and young larvae (Huchon, Demolin, 1970; Santos et al., 2011; Robinet et al., 2013). Cold temperatures could be lethal for overwintering larvae (Radchenko, 1927; Russkoff, 1930; Zankov, 1960; Demolin, 1969a; Roques et al., 2015).

The Eastern Rhodope study area is the closest in Bulgaria to the Aegean Sea. However, the climate is characterized by specific parameters that are determined by its relief. It is poorly protected from the invasion of cold air masses from the north. From the south, the Gyumyurdzhinski Snezhnik hill restrains the Mediterranean influence. The orography of the area favors the retention of cold air masses and a further drop in temperatures. Under such conditions, minimum temperatures in the low parts of the region are sometimes from -10 to -12°C and in some cases -24 to -26°C (Georgieva et al., 2018).

The aim of the present study is: (i) to give a geographical and climatic characterization of the Gyumurdzhinsky Snezhnik region in the Eastern Rhodopes, important for the survival of the species as an egg and early-instar larva; (ii) to make a detailed characterization of the complex of egg parasitoids of *T. pityocampa* and their impact on host numbers; (iii) to provide information on the fecundity of *T. pityocampa* and the proportion of unhatched larvae without entomophagous influence.

Material and methods

Study area

The study area is located in the Eastern Rhodopes, surrounded by the following hills: Zhalti dyal in the west, Stramni rid in the east, Arda River in the north and in the south – Gyumyurdzhinski Snezhnik, with the highest peaks Veikata (1463 m a.s.l.) on Bulgarian territory, and Orlitsa (1510 m a.s.l.) in Greece.

Climate analysis

The climate analysis of the temperature regime for the period 2000-2022 is based on climate data from the National Institute of Meteorology and Hydrology. For the same period, the number of days with minimum air temperature ≤-15°C as well as days with maximum air temperature ≥32°C (critical for PPM eggs and larval evolution) was calculated for summer months.

Collection of biological material and laboratory studies

The experimental material includes five generations of *Thaumetopoea pityocampa* collected from 31 villages in the area of four State Forestry Enterprises (SFE) (Fig. 1), as follows:

- SFE Kirkovo – Yanino (2016), Dyulitsa (2016), Medevtsi (2016), Kayaloba (2016), Drangovo (2016), Dzherovo (2016), Domishte (2016), Dobromirtsi (2017, 2022), Gorno Kapinovo (2017), Yakovitsa (2017), Kolarovo (2017), Fotinovo (2018, 2019), Preseka (2019, 2022), Kitna (2022);
- SFE Ardino – Yabalkovets (2018, 2022), Svetulka (2018), Sransko (2018), Ardino (2022), Brezen (2022), Stoyanovo (2022), Yabalkovets (2022);
- SFE Momchilgrad – Mrezhichko (2018, 2019), Ridino (2018), Iliysko (2018), Rogozche (2018), Ustren (2019), Yamino (2019);
- SFE Zlatograd – Startsevo (2018), Kundevo (2018), Nedelino (2018, 2022), Izgrev (2022), Sredets (2022).

The sample for analysis included 693 egg batches with 148420 eggs in them. Part of the egg batches were collected by the Forest Protection Station – Plovdiv. Egg batches were collected after the larvae hatched. After collection, the egg batches were transported to the Forest Research Institute in Sofia. In laboratory conditions, each batch was put in a single test tube, covered with a cotton stopper, and kept at room temperature (20-22 °C). The scales of egg-masses were removed and the samples were analyzed according to the protocol, described by Tsankov et al. (1996). The fi-

Figure 1. Geographical location of the study area

nal laboratory analysis of each sample was performed after the end of the parasitoid flight. Every egg without a hole in its shell was opened carefully and the meconia and remains of the emerged or dead insects were determined using a stereomicroscope (40× magnification). Parasitoids that emerged before collection were determined by their meconia and remains according to Schmidt, Kit (1994), Tanzen, Schmidt (1995), Schmidt et al. (1997) and Tsankov et al. (1996, 1998a), Zaemdzhikova et al. (2021).

Results

Climatic characterization of the studied area

Climate region of East Rhodope river valleys (Kirkovo, Dzhebel, Krumovgrad, Ivailovgrad)

The region covers the East Rhodope river valleys and the relatively low hills separating them from the east of the Maritsa valley to the south of the Harmanliyska River. Compared to the valleys of Struma and Mesta, it is considerably less protected from the influence of cold air masses. The area has an altitude of 50 to 400 m and is relatively close to the Aegean Sea.

With the activation of the Mediterranean cyclones during fall and the beginning of winter, the region of the Eastern Rhodope River valleys gets one of the largest rainfall for the lower part of the country – about 200 mm. Because of the southern position of the area, much of this precipitation is from rain or rain and snow and often leads to a dangerous rise of river levels and local floods.

Winter is relatively mild, with average January temperatures above 0 °C (+0.5 °C to +2.5 °C). But after the invasion of a cold front from the north followed by an anticyclonic synoptic situation, the air temperatures in the area fall down rapidly. The orography of the area favors the retention of cold air masses and further fall of temperatures. Under such conditions, the minimum temperatures in the lower parts of the area are sometimes from -12 °C to -15 °C (Dzhebel and Krumovgrad), and in some cases, -20 °C to -22 °C. During the period 2000-2022, the mean annual number of days with minimum air temperature equal to or below -15 °C was about 1.5 days.

The average January soil temperature in the 2-20 cm layer is between 1.5 °C to 2 °C and between 2.5 °C to 3 °C respectively. Nevertheless, at these relatively high temperatures, the soil almost freezes every year to a depth of 10 cm. In extreme cases with prolonged cold weather without snow cover the soil freezes to a greater depth (up to 32 cm).

The summer is sunny, hot and dry. The average July temperatures are 23-25 °C, and during only a few days the average daily temperature is lower than 20 °C. The maximum temperatures in July and August reach values of 41-44 °C. The mean annual number of days with maximum air temperature ≥32°C for this region was above 40 days during the period 2000-2022 and the mean number of consecutive hot days was about 12-13 days/year. The highest number of consecutive days with maximum air temperature ≥32 °C was 25-30 days in 2020, followed by 21 days in 2016.

Eastern Rhodope low-mountain climatic region (south of Kirkovo, Zlatograd, Gyumyurdzhiiski Snezhnik)

The Eastern Rhodope low-mountain climate region is distinguished from the region of the Eastern Rhodope river valleys both by its higher altitude (400-1000 m) and by the quantitative and structural characteristics of the regime of individual meteorological elements.

Due to its higher altitude, the winter in the Eastern Rhodope low mountain region is slightly colder than that in its adjacent valley region. The average January temperature is about 1 °C lower than those in the valley region (-1.5 °C to 1 °C). But in return for its predominance of sloping and salient terrain, this region does not experience such appreciable drops of temperature as the ones possible in the valley region in cases of anticyclonic weather following a more severe cold intrusion. During the period 2000-2022 the mean annual number of days with minimum air temperature ≤-15 °C was below 1 day, and even in the region of Zlatograd, since 2012, there have been no recorded minimum temperatures equal to or lower than -15 °C.

The summer is moderately warm with average July temperatures between 20 °C and 22 °C. The maximum temperatures in July and August reached values of 36-38 °C during the period 2000-2022. The mean annual number of days with maximum air temperature ≥32°C for the same period was 20, while the number of consecutive days with such temperature was about 7 days/year. The highest number of consecutive hot days was 15 in 2007, followed by 14 in 2008 and 2012.

It can be summarized that in lower parts of the region of interest, the climatic conditions in both winter and summer are less favourable for the PPM eggs and larval survival. Unfavourable temperature conditions are almost twice more frequent both in winter and in summer in the East Rhodope river valleys region compared to the Eastern Rhodope low-mountain climatic region.

Spectrum of egg parasitoids

The results obtained by the analysis of the parasitoids that emerged or died in their developing stages in the eggs, are presented in Table 1. Seven primary egg parasitoids were established in this habitat: *Ooencyrtus pityocampae* Mercet, 1921 (Hymenoptera: Encyrtidae); *Baryscapus servadeii* Domenichini, 1965; *Pediobius bruchicida* Rondani, 1872 (Hymenoptera: Eulophidae); *Anastatus bifasciatus* Fonscolombe, 1832; *Eupelmus* (*Macroneura*) *vesicularis* Retzius, 1783; *Eupelmus* (*Macroneura*) *vladimiri* Fusu, 2017 (Hymenoptera: Eupelmidae) and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae), and one hyperparasitoid, *Baryscapus transversalis* Graham, 1991.

Table 1. Egg parasitoids found in egg batches

	State Forest Enterprise										
	Kirkovo					**Momchilgrad**		**Zlatograd**		**Ardino**	
Parasitoids Year	2016	2017	2018	2019	2022	2018	2019	2018	2022	2019	2022
Total number of parasitoids, of them	4791	6675	382	536	1413	162	1115	615	336	112	2089
Ooencyrtus pityocampae, %	29.1	35.2	35.6	53.5	28.5	0.6	55.2	15.6	37.8	2.7	16.3
Baryscapus servadeii, %	62.4	59.6	44.2	44.8	69.3	93.8	42.6	60.8	48.8	81.2	72.3
Baryscapus transversalis, %	2.5	5.0	17.8	0.6	1.6	1.9	0.1	0.8	4.2	2.7	7.7
Anastatus bifasciatus, %	5.9	-	-	0.9	0.6	3.7	0.5	0.3	1.2	0.9	2.6
Trichogramma sp., %	0.1	0.1	1.6	0.2	-	-	1.6	22.5	8.0	12.5	1.1
Eupelmus vladimiri, %	-	0.1	0.5	-	-	-	-	-	-	-	-
Pediobius bruchicida, %	-	-	0.3	-	-	-	-	-	-	-	-

Dominant parasitoids were *B. servadeii* and *O. pityocampae*. The first species was the most numerous in 9 out of 11 analyzed samples (81.8%) and *O. pityocampa* only in the 2019 samples from Kirkovo and Momchilgrad.

The polyphages *A. bifasciatus* and *Trichogramma* sp. were found in 81.8% of the samples. In the case of the polyembryonic *Trichogramma* sp., the abundance was counted according to the number of eggs of the host, in which it was found. The relative share of *Trichogramma* sp., decreased from 22.5% (Momchilgrad, 2018) to around and below 1% in six of the samples. The participation of *A. bifasciatus* with a maximum value of 5.6% in the 2016 sample from Kirkovo was also in this order.

E. vladimiri and *P. bruchicida* were occasional parasitoids on *T. pityocampa* eggs. Of *P. bruchicida*, only one male individual was imagined from Kirkovo – 2018, and of *E. vladimiri* – 6 and 2 male individuals from the same location in the 2017 and 2018 samples, respectively. The hyperparasitoid *B. transversalis* participated in a complex with relatively low share (0.1-17.8%).

Egg parasitoids and their development rates in egg-batches

Parasitoids have clearly defined differences in imagination time (Table 2). The main part – 72.5 % in *O. pityocampae* were imagined before collecting the sample, i.e. parasitizing happens at an early stage after the appearance of the egg batches. For the other egg parasitoids, these values are: *A. bifasciatus* – 68.1%; *B. servadeii* – 56.4%; *Trichogramma* sp. – 23.8 % and for the hyperparasitoid *B. transversalis* – 1.0 %.

The survival of egg parasitoids in laboratory conditions, in which the samples were kept, was low. In the larval stage, 43.3% died. Following percentage of the parasitoids were found dead as a developed imago in the egg of the host: *Trichogramma* sp., – 70.0 %; the hyperparasitoid *B. transversalis* – 34.0%; *O. pityocampae* – 18.8%, *A. bifasciatus* – 9.8% and the lowest percentage for the specific parasitoid *B. servadeii* – 4.3%.

The total mortality of all parasitoids in larval and imago stages is nearly half of their number – 47.8% (Table 2).

Sexual ratio of the hyperparasitoid *B. transversalis*

After collecting the samples, in laboratory conditions, a total of 442 individuals of the hyperparasitoid *B. transversalis* were imagined, of which 56.3% were females and 43.7% were males.

In the three samples with a significant number of emerged individuals, the following ratios were obtained: Kirkovo (2017): imagined 177 adults, with a female: male ratio of 52.5:47.5; respectively, Kirkovo (2018): 80.3:19.7 out of 61 individuals and Ardino (2022): 145 individuals with 61.4:38.6 ratio (Table 2).

Structure of egg clusters and rate of larval hatching

A total number of 148414 eggs of *T. pityocampa* was analyzed (Table 3). The average number of eggs laid in a batch varied from 176.9 (Kirkovo, 2019) to 226.2 (Momchilgrad, 2019), with an average of 214.2 for the study area.

70.8%. of all the eggs in the samples hatched successfully. In individual samples, these values vary within very wide limits from 57.8% (Kirkovo, 2017) to 94.8% (Ardino, 2019).

The results show that egg parasitoids are a very serious natural factor, regulating the density of the pine processionary moth. However, their impact varied widely from 30.3% 2.1% (Ardino, 2019) to (Kirkovo, 2017), i.e. a difference of 14.4 times. The role of predators was negligibly low, between 0.1 and 1.7%.

Table 2. The egg parasitoids of *T. pityocampa* and their development rates in egg-batches

	State Forest Enterprise										
	Kirkovo					**Momchilgrad**		**Zlatograd**		**Ardino**	
Parasitoids Year	2016	2017	2018	2019	2022	2018	2019	2018	2022	2019	2022
Ooencyrtus pityocampae	1393	2349	136	287	403	1	615	96	127	3	341
Emerged before collection	1098	1620	45	226	328	1	449	33	86	1	285
Emerged after collection	80	201	38	28	10	-	88	39	10	1	5
Adults died in eggs	215	528	53	33	65	-	78	24	31	1	51
Baryscapus servadeii	2992	3980	169	240	979	152	475	374	164	91	1510
Emerged before collection	1483	2326	41	237	541	130	443	171	53	86	760
Emerged after collection	1366	1465	126	-	401	22	7	198	102	1	693
Adults died in eggs	143	189	2	3	37	-	25	5	9	4	57
Baryscapus transversalis	122	331	68	3	23	3	1	5	14	3	160
Emerged before collection	4	3	-	-	-	-	-	-	-	-	-
Emerged after collection, ♀♀	38	93	49	-	3	1	-	1	1	-	89
Emerged after collection, ♂♂	23	84	12	1	9			1	4	3	56
Adults died in eggs	57	151	7	2	11	2	1	3	-	-	15
Anastatus bifasciatus	281	-	-	5	8	6	6	2	4	1	54
Emerged before collection	232	-	-	5	-	3	-	-	-	-	10
Emerged after collection	33	-	-	-	2	3	3	-	1	-	36
Adults died in eggs	16	-	-	-	6			2	3	1	8
***Trichogramma* sp.**	**3**	**9**	**6**	**1**	-	-	18	138	27	14	24
Emerged before collection	3	-	-	1	-	-	5	31	10	3	4
Emerged after collection	-	9	6	-	-	-	-	-		-	-
Adults died in eggs	-	-	-	-	-	-	13	107	17	11	20
Eupelmus vladimiri	**-**	**6**	**2**	-	-	-	-	-	-	-	-
Emerged after collection	-	6♂	2♂	-	-	-	-	-	-	-	-
Pediobius bruchicida	-	-	**1**	-	-	-	-	-	-	-	-
Emerged after collection	-	-	1♂	-	-	-	-	-	-	-	-
Undetermined larvae of parasitoids	2189	6342	736	379	913	64	697	186	172	15	1154
Eggs destroyed by predators	504	667	10	2	17	47	81	139	24	19	144

Table 3. Structure of egg clusters and rate of hatching of larvae of *Thaumetopoea pityocampa*

	State Forest Enterprise										
	Kirkovo					**Momchilgrad**		**Zlatograd**		**Ardino**	
Parameters Year	2016	2017	2018	2019	2022	2018	2019	2018	2022	2019	2022
Total number of egg batches	137	199	30	27	41	17	51	50	30	28	83
Total number of eggs	30426	42952	6367	4775	8116	3639	11536	10225	6939	6058	17381
Number of egg per batch	222.1	215.8	212.3	176.9	198.0	214.1	226.2	204.5	231.3	216.4	209.4
Larvae hatched, %	68.1	57.8	72.6	75.3	63.7	82.9	79.5	84.2	90.3	94.8	76.4
Impact of egg parasitoids, %	22.9	30.3	17.6	19.2	28.7	6.2	15.7	7.8	7.3	2.1	18.7
Impact of predators, %	1.7	1.6	0.2	0.1	0.2	1.3	0.7	1.4	0.3	0.3	0.8

Unhatched larvae without the influence of entomophages

The natural characteristics of the area, the air temperature during the stages of eggs and young larvae, are favourable for the development of the pine processionary moth. The unsuccessfully hatched larvae were less than a tenth of the eggs – 7.2%, from 2.8% (Ardino, 2019) to 10.4% (Kirkovo, 2017).

Eggs with unhatched larvae were categorized into four groups: dead larvae without opening, the death of the larvae occurred in the egg at various stages of their development; larvae died, with an opening in the egg; undeveloped eggs with dried-up yolk; eggs totally empty, without any remains.

Of the unhatched eggs, on average for the region, the share of the first group is the highest – 35.4% and the difference between individual samples is 41.2 points. On average, undeveloped eggs with dried-up yolk were 29.5%, with a more homogeneous group: max – 42.1%, min – 18.2%. Close to this average value was that of empty eggs, without any remains – 27.4% (max – 42.7%, min – 1.2%). The share of fully developed larvae that died during the hatching process was the lowest – 7.7% (Table 4).

Table 4. Structure of unhatched larvae without the influence of entomophages

	State Forest Enterprise										
	Kirkovo					Momchilgrad		Zlatograd		Ardino	
Parameters Year	2016	2017	2018	2019	2022	2018	2019	2018	2022	2019	2022
Unhatched caterpillars, %	7.3	10.4	9.7	5.5	7.4	6.6	2.0	9.6	4.1	2.8	4.1
Total number of larvae died, of them	2225	4453	615	262	600	675	140	348	472	166	709
Larvae died, without opening, %	27.3	25.2	54.8	66.4	25.8	55.2	47.2	58.1	61.3	64.5	48.5
Larvae died, with opening, %	6.1	5.8	17.1	6.9	5.8	15.3	7.1	13.2	5.9	3.6	11.0
Undeveloped eggs with dried-up yolk, %	23.9	35.6	18.2	24.4	20.2	28.9	42.1	26.7	28.8	30.7	28.2
Eggs totally empty, without any remains, %	42.7	33.4	9.9	2.3	48.2	0.6	3.6	2.0	4.0	1.2	12.3

Discussion

The studied area belongs to the South-Bulgarian subarea of the Continental-Mediterranean climate area, but in it 2 different climatic regions can be distinguished (Sabev, Stanev, 1959) – East Rhodope river valleys and Eastern Rhodope low-mountain regions. Each of them has specific climatic characteristics. The main features of the climate subarea are the mild and very humid winter and sunny, hot and dry summer. During the second half of the autumn (in November) or at the beginning of winter (in December) maximum precipitation sums are observed – about 2.5-3 times more than in August or September. The summer is characterized by dry, hot weather – most pronounced in the lower parts of the considered area (up to 400 m a.s.l.), along the river valleys, with average July temperatures 22-25 °C. The driest month of the year for this area is August or September.

The comparison of the ecological conditions with the habitats in the lower reaches of the Struma River, based on the research and assessments for the period 1991-2017, according to data from the meteorological database of NIMH, it can be concluded that the summer in Sandanski and Kirkovo is dry and hot, but in the first region, it is significantly warmer and drier, especially in June.

For both regions, the warmest month is August, while the absolute summer maximum air temperature is recorded in July. The average number of days with maximum air temperature ≥ 32 °C (critical for the development of PPM larvae) for the Kirkovo region is 4 days in June, 12 in July, and 14 in August. For the Sandanski region, they are as follows: 11 days in June, 21 days in July, and 22 days in August. In addition to the significantly higher average number of days with air temperature ≥32 °C, significantly longer periods of retention of these temperatures were also recorded in Sandanski: 43 days in 2012 (from June 29 to August 10); 41 days in 2015, and 38 days in 1998 and 2013. For the Petrichko-Sandanski climate region, the typical retention period of maximum temperatures above the specified limit is 15-20 days during the summer months. At the same time, this number for the Kirkovo region was only 10-12 days. The longest periods of retention of maximum air temperatures ≥32 °C for the region of the Eastern Rhodope river valleys were observed in 2016 – 21 days (from July 22 to August 11) and in 1998 – 20 days (Mirchev et al., 2018).

In the easternmost part of the Rhodopes, in the region of Ivaylovgrad, five parasitoids were found: *B. servadeii*, *O. pityocampae*, *A. bifasciatus*, *B. transversalis* and *P. bruchicida.* The most numerous is *Baryscapus servadei,* with relative participation of 45.9–85.6%, followed by *O. pityocampae* (7.2–38.4%). The relative participation of *A. bifasciatus* and the hyperparasitoid *B. transversalis* is about 10%. *P. bruchicida* has sporadic participation (Mirchev et al., 2012; 2014; Zaemdzhikova et al., 2019).

In the westernmost part of southern Bulgaria, in habitats along the Struma River, *Trichogramma embryophagum* and *E. vesicularis* (in Marikostinovo) were also found in addition to the parasitoids mentioned above. In all localities (Ploski, Sandanski, and Marikostinovo), *O. pityocampae* is the most numerous. The abundance of *A. bifasciatus* and *B. servadeii* is much lower. The hyperparasitoid *B. transversalis* was found at a low density (Tsankov et al., 1996; 1998b; 1998c; Mirchev, Tsankov, 2003).

Halfway between the above two geographical points in the Western Rhodope region (Satovcha, Valkosel, and Slashten) 6 parasitoids have been reported, without the one mentioned in Marikostinovo – *E. vesicularis*. The most frequent parasitoids were *O. pityocampae* and *B. servadeii* (85% of total parasitoids counted). A clear trend in these two parasitoids over the years is an increase in the proportion of *O. pityocampae*, while *B. servadeii* is in sharp decline (Tsankov et al., 1998c; Mirchev et al., 2010).

There are several hypotheses in the literature that seek to explain why a given parasitoid is more numerous in a certain area.

Massuti (1964) pointed out that temperature is the limiting factor, the representatives of Eulophidae are more plastic and develop successfully in areas with temperatures above 30 °C, conditions that hinder the development of *O. pityocampae*. Therefore, in Italy, *B. servadeii* has the highest abundance in the warmer areas of the central and southern part of the country but was not found in Sicily and in the pine forests of Abruzzo at low altitude (Tiberi, 1990).

B. servadeii can successfully parasitize the pine processionary moth eggs from their laying until the caterpillar's hatch, while for *O. pityocampae* this period is shorter. When encyrtid parasitizes eggs after 32 days of the host incubation period, the parasitoid cannot develop (Halperin, 1990a).

The successful development of *O. pityocampae* requires a richer species diversity in the floristic aspect of the given habitat, which would create a favourable environment for the development of these hosts. Eighteen alternative hosts have been reported for this parasitoid (Thompson, 1954; Battisti et al., 1988; Tiberi et al., 1993; Masuti et al., 1993; Halperin, 1963, 1990b; El-Yousfi, 1989; Schmidt and Kitt, 1993, 1994, 1995; Kitt, Schmidt, 1993; Trjapitzin, 1978, 1989).

In summary, the essential factors for the development of *O. pityocampae* are (i) certain temperature conditions – below 30 °C (ii) a particular stage of embryonic development of the host; (iii) availability of alternative hosts. Knowledge of the biology and ecology of *B. servadeii* and *O. pityocampae* does not explain the obtained data on the number and ratio between them in the southern limit of the distribution of the pine processionary moth in Bulgaria. For example, the low number of *B. servadeii* in Marikostinovo is striking, and radically different from the results and conclusions indicated by Tiberi (1990).

Of the two parasitoids that have sporadic involvement, *E. vladimiri* was recorded for the first time in trophic association with *T. pityocampa* in the region of Kirkovo (Boyadzhiev et al., 2020), and *P. bruchicida* has been reported for Marikostinovo, Central Bulgaria (Mirchev et al., 2021) Western Rhodopes (Mirchev et al., 2010) and Ivaylovgrad (Mirchev et al., 2012).

In the study area for the imago of *B. transversalis* the female: male individuals ratio is 56.3:43.7, i.e. the proportion of females is lower than the established average values for the hyperparasitoid population in Bulgaria, which are 63.1:36.9 (Mirchev et al., 2022).

There is no data in the literature on the influence of environmental factors on the formation of the sex ratio in this parasitoid. In other parasitoids, such an influence has been demonstrated. The sex of *O. pityocampae* depends on the temperature at which they develop. At a constant temperature of 32 °C, only females imagine (theliotoky), at 32.5–33.0 °C males are also found (deuterotoky, amphitoky), while at 34 °C or more, all offspring are male (arenotoky) (Halperin, 1990b). The influence of temperature on the formation of the sex in another member of this genus, *O. submetallicus* (Howard, 1897), was reported by Wilson, Woolcock (1960). Schmidt, Tanzen (1998) reported that the offspring of fertilized females were of both sexes.

Stahl et al. (2018) found that in *A. bifasciatus*, sex depends on host egg size.

In the present study, only male individuals of *E. vladimiri* emerged. For another representative of this genus – *Eupelmus vuilleti* (Crawford, 1913) it was established that, in larger hosts, fertilized eggs are laid, from which female adults appear, and in smaller hosts – unfertilized eggs and male generation (Terrasse et al., 1996).

In this study, *T. pityocampa* fecundity averaged 214.2, ranging from 176.9 to 226.2. In the neighbouring areas of the southern border of the pine processionary moth in Bulgaria, these values are: Ivaylovgrad 250-279 (Mirchev et al., 2012); Western Rhodopes 207-225 (Mirchev et al., 2010); Marikostinovo 203-253 (Tsankov et al., 1998b); Ploski 198-241 (Tsankov et al., 1998a) and Sandanski 193-253 (Mirchev, Tsankov, 2003). The data show that fecundity is neither a constant value for the species nor for a given region. In the present study, the deviations around the mean value were 17.5%, similar to the reported data for the neighboring districts. It is determined both by several environmental factors and by the phase of the population size in the given year. Masutti, Battisti (1990) considered that the fecundity of the pine processionary moth depends on the climatic conditions, the host plant, and the population cycle. Özkazans (1987) noted that the egg productivity of the pine processionary moth increases with increasing altitude of the species' habitats.

With all the conventions, it can be concluded that the fertility of *T. pityocampa* in the studied area is comparable to that of the neighbouring areas.

70.8% (57.8–94.8%) of all the eggs in the samples hatched successfully. Parasitoids are the most serious regulator – 20.9% parasitized eggs, with a large variation in samples from 2.1 to 30.3%. The role of predators is in the range of 1.1% (0.1–1.7%). Unhatched larvae were 7.2% (2.8–10.4%). The values of hatched larvae, the impact of parasitoids, predators and the relatively low relative proportion of unhatched larvae are indicators of favourable ecological conditions of the environment for the development of *T. pityocampa* and entomophages.

In conclusion, it could be noted, that the region of Gyumurdzhinsky Snezhnik in Eastern Rhodopes is a very specific habitat for the pine processionary moth in the southernmost part of Bulgaria in which summer air temperatures are quite favourable for its survival in egg and early-instar larval stage. On the other hand, the rich complex of egg parasitoids in the study area is a very serious natural factor, regulating the density of the pest by up to 30%.

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