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Long-term Studies on Egg Parasitoids of Pine Processionary Moth (*Thaumetopoea pityocampa*) in a New Locality in Bulgaria

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ABSTRACT

During the period 1995-2014, the species composition of egg parasitoids of pine processionary moth (*Thaumetopoea pityocampa*) and their impact on host number was studied in Lisets Mt. near the town of Kyustendil, where the pest was first found in 1993. The study site is located 10 km to the west of stands constantly attacked by pine processionary moth and is isolated from them by deciduous forests, farms and urban areas. For all study period a total of 96 egg batches, containing 21 357 eggs were collected in Scots pine (*Pinus sylvestris*) plantations. They were separated singly in test tubes covered with cotton stoppers and were reared in laboratory conditions at room temperature (20-22°C). The emergence of egg parasitoids was observed daily. In the end of the studies, all eggs were analysed in detail. Four primary parasitoids (*Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus*, *Trichogramma embryophagum*) and one hyperparasitoid (*Baryscapus transversalis*) were established in the new locality of the host. In first years, *T. embryophagum* was the most numerous egg parasitoid of *T. pityocampa*. Some aspects of its biology and ecology were examined. The impact of main parasitoids of pine processionary moth (*B. servadeii* and *O. pityocampae*) was unstable. In 2014, twenty-one years after the appearance of *T. pityocampa* in studied habitat, the regulating effect of egg parasitoids is comparable to that in old habitats.

Key words: *Thaumetopoea pityocampa*, egg parasitoids, monitoring, *Trichogramma embryophagum,* Bulgaria.

INTRODUCTION

The population density and distribution area of pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775) (Lepidoptera: Notodontidae, Thaumetopoeinae), in Bulgaria have exhibited a constant growth during the XXth century. It was an extremely rare species at the beginning and has become the most serious pest in pine forests at present. It was detected for the first time in 1906 as a single male specimen caught by a lamp while the first larval colonies were observed only in 1916 (Drenowsky, 1923). The first large scale outbreak was recorded in 1924 (Russkoff, 1929-1930). It gradually increased in number and during the periods 1951-1971, 1972-1976 and 1977-2011 the average annual values of areas attacked in Bulgaria amounted 5,133, 8,648, and 26,009 ha, respectively (Mirchev *et al.*, 2011a).

A prerequisite for the invasion of *T. pityocampa* in Bulgaria was that many new coniferous plantations were formed during the last 50 years of the XXth century at the expense of deciduous forests. Some of the earliest afforestation activities in Bulgaria started in 1890 by planting Scots pine (*Pinus sylvestris* L.) and Austrian black pine (*Pinus nigra* Arn.) in the vicinity of Kyustendil in order to reduce soil erosion (Zahariev *et al.*, 1977). Since 1960, the pine processionary moth has been permanently established and caused repeated outbreaks in these plantations.

Two ecological forms of *T. pityocampa* were established, characterized by substantial differences in their biological cycle (Tsankov, 1966; Tsankov *et al*., 1996a). The natural factors regulating the density of the species were studied (Kaitazova *et al.*, 1978; Tsankov, 1990) and many egg parasitoids were identified: *Ooencyrtus pityocampae* (Mercet, 1921), *Anastasus bifasciatus* (Geoffroy, 1785), *Baryscapus servadeii* (Domenichini, 1965), *Baryscapus transversalis* Graham, 1991, *Eupelmus vesicularis* (Retzius, 1783), *Trichogramma embryophagum* (Hartig, 1838) (Tsankov, 1990; Tsankov *et al*., 1996a) and *Pediobius bruchicida* (Rondani, 1872) (Mirchev *et al.*, 2011b). However, the investigations were carried out mainly in biotopes with high density and permanent colonization of the pest.

The purpose of the present monitoring on limiting factors of *T. pityocampa* is determined by constantly growing areas occupied by the pest in Bulgaria. The aim of this paper is to give information about main biometric, biological, and ecological characteristics of the pest in egg stage and most important egg parasitoids in a new locality, as follows: size and structure of egg-batches, egg fertility, survival rate, unhatched caterpillars share and factors affecting it, species composition, role and sequence of parasitoid settling.

MATERIALS AND METHODS

The monitoring was carried out on southern slopes of Lisets Mt., above Garlyano village near town of Kyustendil (42°16’ N, 22°33’ E) (Fig. 1). The egg batches of *T. pityocampa* were collected in plantations of Scots pine at altitude between 940 and 1150 m a.s.l. In the beginning of the study (1995), the pine stands were 30-year-old. The study site is located 10 km to the west of the stands near Kyustendil, constantly attacked by pine processionary moth, and is isolated from them by deciduous forests, farms, and urban areas. In this biotope *T. pityocampa* was detected for first time in 1993. During the study period, the pest density was very low. There were nests only in the stand edges, and on single trees self-originated in an abandoned farm field. Despite its low density, an air treatment with the bacterial insecticide Foray based on *Bacillus thuringiensis kurstaki* was applied in September 1995, with the aim to limit the pest invasion.

Six generations of *T. pityocampa* were studied during the period 1995-2014. A total of 96 egg batches were analysed, containing 21,357 eggs (Table 1). In 1996, only one egg batch was found, that is why no data for that year are included.

After collection, the batches were placed singly in test tubes covered with cotton stoppers and were transported to the laboratory of the Forest Research Institute in Sofia. All laboratory studies were carried out at room temperature (20-22°C). The scales of egg-batches were removed, the parasitoids emerging each day were counted, taken out of the test tubes and put into small plastic capsules for identification. A log was prepared for each egg batch, described in detail by Tsankov *et al*. (1996a).

Every egg without a hole in its shell was opened carefully and the meconia and remains of the emerged or dead insects were determined with a stereomicroscope (40x magnification). Parasitoids emerged before collection were determined after Schmidt and Kitt (1994), Tanzen and Schmidt (1995), Schmidt *et al*. (1997) and Tsankov *et al*. (1996a, 1998).

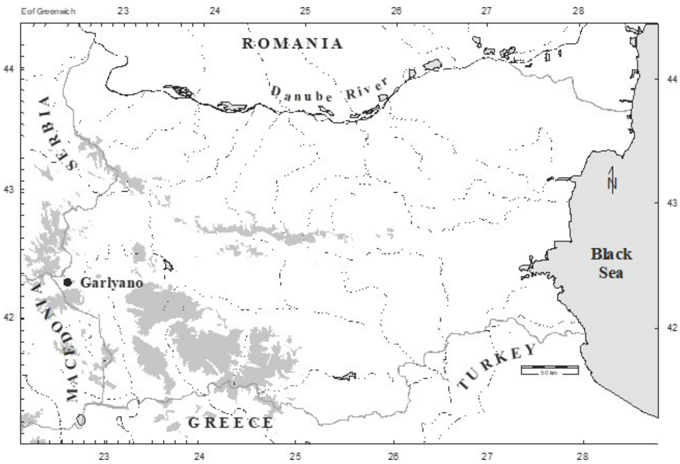

Fig. 1. Studied locality of *T. pityocampa* (the gray color represents the mountain areas above 1000 m a.s.l.)

RESULTS

Biometric characteristics of *T. pityocampa* egg batches

For the entire study period, the number of eggs in egg batches of *T. pityocampa* varied between 37 and 313 (Table 1), with an average number of 222. The highest average egg number in a batch (245) was established in 1994 and the lowest one (180) in 1998. A very wide variation of eggs' number in one batch was observed in different samples. In the first two, the difference was 274 and 269, respectively. The most homogeneous samples were from generations in 1998 and 1999, where this difference was 157 and 146, respectively.

The covering scales in all egg batches were oriented from the top to the base, which means that the female butterfly started depositing the eggs from the needle base. The egg batches were deposited on two needles, and only three batches were found on four needles (Table 1).

The needles of *P. sylvestris* with deposited eggs were of average length between 52.45 and 74.8 mm. The comparatively small needle length probably determines that the egg deposition starts almost from the needle base. Only in three cases, out of 96 batches, the beginning of the egg batch was over 15 mm from the needle base; the cases of deposition at a distance not greater than 10 mm from the base predominated.

Survival of *T. pityocampa* in egg stage

The data in Table 2 show a high percentage of *T. pityocampa* caterpillars' hatching. In half of the studied generations, this share was over 90%. In 1994, the maximum value of 97.2% was established, and the minimum one was in 2014 (72.7%). These differences are due primarily to the very wide variations - from 0.1 to 21.9% - of the share of eggs parasitised and destroyed by predators in individual years. The dead caterpillars, the sterile and empty eggs are around 3% in the first three samples, and about 5% in the last two ones. It should be noted that in 1997 the eggs destroyed by predators (300) were more than the parasitised ones (49).

Egg parasitoids of *T. pityocampa*

Four primary parasitoids (*Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus* and *Trichogramma embryophagum*) and one hyperparasitoid (*Baryscapus transversalis*) were reared from egg batches of *T. pityocampa* (Table 2). This parasitoid species diversity is due, to a certain extent, to the sample from 14 November 1995, when four of all of five species were found. No parasitized eggs were established in 1999 sample. In the first examined generation only the polyembryonic parasitoid *T. embryophagum* was reared, and in the 1997 sample in addition to it, *O. pityocampae* was also found. *T. embryophagum* was not found in 1998 samples. In the first three studied generations of *T. pityocampa* (1994, 1995 and 1997), the highest number of parasitised host eggs were destroyed by *T. embryophagum* (69.4-100.0%) (Table 2).

The data of parasitoid number show that the main parasitoids of pine processionary moth - *O. pityocampae*, *B. servadeii* and *A. bifasciatus* - in most of the material examined are either absent, or have parasitised few eggs. As it has been mentioned above, the first sample was taken in the second year after the appearance of pine processionary moth in this site. The relatively great distance from the areas with continuous presence of the pest could be taken as the primary reason for the absence of specific parasitoids. Only one specimen of *B. transversalis* was reared in 1998 (Table 2).

In the sample from 1995, it was established that the maximum parasitization of *Trichogramma* in one batch was 39.8%. At the same time, out of 37 batches subjected to examination, 20 batches (54.1%) were parasitized at varying degrees.

After collection of the batches, 72 *B. servadeii* individuals emerged in laboratory conditions, two more were found dead as developed adults in the host eggs. Out of all, only 3 were male (4.1%).

The emerged *A. bifasciatus* specimens were male, and the only one *B. transversalis* was female. For *O. pityocampae, A. bifasciatus, B. servadeii* and *B. transversalis,* due to their small number, no conclusions could be drawn about their survival rate in the host egg.

In the sample from 26 August 1998, out of the 51 *B. servadeii*, which emerged after the winter diapause, 41 (80.4%) were with remains from a caterpillar, which shows that the parasitisation happened when the development of the embryo was at a very advanced stage.

Table 1. Structure of *T. pityocampa* egg batches collected in a new locality near Kyustendil

Date of collection	28.04.1995	14.11.1995	24.09.1997	26.08.1998	04.11.1999	25. 02. 2014
Generation	1994	1995	1997	1998	1999	2013
Number of egg batches	24	37	16	5	5	9
Length of needles of *Pinus sylvestris*, mm	34-80	37-85	40-115	61-100	55-85	46-108
Mean of length of egg batches, mm (range)	32.1 (10-40)	29.1 (8-44)	27.6 (10-39)	28.7 (21-35)	24.0 (15-32)	29.1 (12-40)
Number of needles used:						
- 2 needles	23	36	16	5	5	8
- 4 needles	1	1	0	0	0	1
Mean of diameter of egg batches, mm						
- on 2 needles (range)	3.3 (3.1-3.6)	3.4 (3.0-4.0)	3.2 (3.0-3.6)	3.4 (3.0-3.6)	3.7 (3.4-3.9)	3.4 (3.1-3.8)
- on 4 needles (range)	4.7	4.8	-	-	-	3.5-5.0
Number of egg rows per batch	6-9	7-11	8-10	8-9	9-10	7-11
Distance of egg batches to base of needles, mm (range)	3.5 (0-27)	2.1 (0-10)	5.3 (0-18)	5.3 (1-8)	6.6 (3-15)	4.3 (0-15)
Oviposition direction	Oviposition in all cases from base to top					
Number of eggs per batch	37-311	44-313	85-293	93-250	119-265	76-291
Total number of eggs studied	5874	8113	3427	904	1021	2018
Mean of eggs per batch	245	219	214	180	204	224

Table 2. Hatching rate, mortality of caterpillars, and parasitoid species found in *T. pityocampa* egg batches.

Generation	1994	1995	1997	1998	1999	2013
Date of collection	28.04.1995	14.11.1995	24.09.1997	26.08.1998	04.11.1999	25. 02. 2014
Caterpillars dead in eggs without opening	56	202	29	11	16	25
Undeveloped caterpillars dead	0	0	12	0	0	0
Caterpillars dead with opening	12	38	30	3	3	6
Undeveloped eggs with dried-up yolk	69	45	36	14	33	40
Eggs totally empty, without any remains	0	11	21	0	1	38
Total number of caterpillars dead, undeveloped eggs with dried-up yolk and eggs totally empty, number (%)	137 (2.3)	296 (3.7)	128 (3.7)	28 (3.1)	53 (5.2)	109 (5.4)
Caterpillars hatched, number (%)	5708 (97.2)	7370 (90.8)	2 950 (86.1)	735 (81.3)	967 (94.7)	1 467 (72.7)
Total number of parasitised eggs	24	257	49	102	0	406
Total number of eggs destroyed by predators	5	190	300	39	1	36
Impact of parasitoids and predators (%)	0.5	5.5	10.2	15.6	0.1	21.9
Parasitoid species						
Ooencyrtus pityocampae, number (%)	-	**5 (1.9)**	**-**	**9 (8.8)**	-	**337 (85.8)**
Emerged before collection of egg batches	-	4	-	8	-	134
Emerged after collection of egg batches	-	1♀	-	-	-	189♀♀
Adults dead in eggs without opening	-	-	-	-	-	14♀♀
Pupae dead in the eggs	-	-	-	1	-	-
Baryscapus servadeii, number (%)	-	**11 (4.3)**	**15 (30.6)**	**92 (90.2)**	-	-
Emerged before collection of egg batches	-	2	1	37	-	-
Emerged after collection of egg batches	-	9 (7♀♀; 2♂♂)	12♀♀	51 (50♀♀; 1♂)	-	-
Adults dead in eggs without opening	-	-	-	2 ♀♀	-	-
Pupae dead in the eggs	-	-	-	1	-	-
Larvae of different stages dead in the eggs	-	-	2	1	-	-
Baryscapus transversalis, number (%)	-	-	**-**	**1 (1.0 %)**	-	-
Emerged before collection of egg batches	-	-	-	-	-	-
Emerged after collection of egg batches	-	-	-	1♀	-	-
Anastatus bifasciatus, number (%)	-	**1 (0.4)**	-	-	-	**55 (14.0)**
Emerged before collection of egg batches	-	-	-	-	-	3
Emerged after collection of egg batches	-	1♂	-	-	-	48♂♂
Adults dead in eggs without opening	-	-	-	-	-	4♂♂
Trichogramma embryophagum, number (%)	**24 (100.0)**	**240 (93.4)**	**34 (69.4)**	-	-	**1 (0.2)**
Emerged before collection of egg batches	11	43	11	-	-	-
Emerged after collection of egg batches	13	164	23	-	-	1
Adults dead in eggs without opening	-	2	-	-	-	-
Pupae dead in the eggs	-	14	-	-	-	-
Undetermined larvae of parasitoids	-	-	-	-	-	13

Biology of *T. embryophagum*

In the sample from 14 November 1995, the highest number of pine processionary moth eggs parasitized by T. embryophagum was found. By dissection, dead adults or pupae of this parasitoid were found in 16 eggs. In addition, in two more cases emerged adults were reared from individual eggs. Its number in one host egg varied from 5 to 14 (Fig. 2). In 10 of the eggs, which is the predominant part, the individuals were over 10.

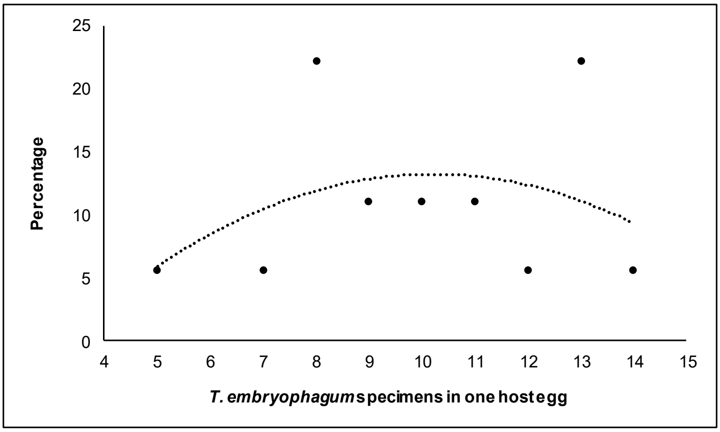

Fig. 2. Number of T. embryophagum specimens developing in one egg of T. pityocampa (N=18)

From the same sample, a total of 1425 individuals from 164 eggs emerged in the laboratory - the average number of *T. embryophagum* individuals in one *T. pityocampa* egg was 8.7.

The data in Table 3 show that after hibernation in laboratory conditions, the emergence of trichogramma lasts about 3 months. However, the main part (87.8%) of the individuals emerged in the course of 20 days - from the end of March to mid-April. It should be noted that in all four years when *T. embryophagum* was established, the bulk emerged during the following year after hibernation. With the first sample, 45.8% emerged in the year when the eggs were deposed, and in the next sample this share was only 21.8%, and in 1997 - 32.3%.

The data in Table 4, based on the sample with the highest number of *T. embryophagum,* show a relatively higher distribution of eggs parasitized at the ends of the batches, although initial analysis did not establish any visibly lacking scales.

Table 3. Emergence dynamics of *T. embryophagum* under laboratory conditions (collected 14.11.1995).

Date		1996, March	1996, April			1996, May			1996, June
		21-31	1-10	11-20	21-30	1-10	11-20	21-31	1-10
T. pityocampa eggs from which emerged *T. embryophagum*	N	100	44	0	15	0	0	0	5
	%	61.0	26.8	0.0	9.1	0.0	0.0	0.0	3.1

Table 4. Distribution of *T. embryophagum* in the egg batches of *T. pityocampa* (collected 14.11.1995.

Frequency of *T. pityocampa* eggs with *T. embryophagum* (n=240)	Part of batch (%) base → top				
	1/5 (22.5)	2/5(9.6)	3/5(19.6)	4/5(23.3)	5/5(25.0)

DISCUSSION

In all cases, the laying of the eggs started from needle base. The same pattern was observed during the analysis of 147 egg-batches from the villages of Marikostinovo and Ploski, located in Southwest Bulgaria, and the village of Kurtovo in Central Bulgaria (Tsankov *et al.*, 1996a). The host plant in these three cases was *P. nigra* and the longer needles are a prerequisite for the considerably wider range of variation (0 - 58 mm) of the distance from needle base to the beginning of egg-batch in comparison with our results from Kyustendil region, where the host plant is *P. sylvestris*, its needles being much shorter.

Schmidt (1990) reported that on *Pinus halepensis* Miller in Greece, 62.5% of the cover scales are directed to the top and 37.5% - to the base of the needle. In all analysed 20 egg-batches, on the same host plant in Algeria, the eggs' laying started from needles base (Tsankov *et al.*, 1995). A similar condition was recorded also for *Thaumetopoea wilkinsoni* Tams in Israel on *P. halepensis*, *Pinus brutia* Ten. and *Pinus canariensis* (Kitt and Schmidt, 1993), while for *Thaumetopoea pinivora* (Tr.) from the Baltic Sea coast, developed on *P. sylvestris,* the egg laying started from needles top for 80 from all the 81 egg batches investigated (Tsankov *et al.*, 1993).

No substantial differences have been observed in one of the basic biometric parameters, characterizing *T. pityocampa* - namely its fecundity. Our data for the mean values of egg number in an egg batch are close to the above mentioned 3 sites in Bulgaria (Marikostinovo, Ploski and Kurtovo), where they vary from 220 to 253 (Tsankov *et al*., 1996a). Masutti and Battisti (1990) considered that *T. pityocampa* fertility depends on climatic conditions, the host plant and the population cycle, and it varies for Italy from 210 to 280 eggs. They have established that in *P. nigra* stands in colder regions pine processionary moth has a lower fertility. Low fertility of the species was established in Algeria - a mean value of 154 with a minimum of 125 and maximum 245 eggs (Tsankov *et al*., 1995).

There might be different reasons for the existence of many small egg batches during the present investigation - 16.2% of them being with an egg number less than 150 during the second year. The ecological conditions in the region provide possibilities for adequate nourishment of caterpillars and for normal egg productivity. The small number of eggs in separate batches might be due to diseases, but most likely it is a result from interruption of egg laying due to predators.

One of the most significant parameters characterizing the new species habitats is the high percentage of hatched caterpillars. They exceed substantially the values in the permanent habitats, as for instance 52-54% in Marikostinovo, 62% in Ploski and 66% in Kurtovo (Bulgaria) (Tsankov *et al*., 1996a). The values in Kalogria and Delphi (Greece) - 68.5% (Schmidt, 1990) are close to the above-mentioned ones.

No predators destroying *T. pityocampa* eggs were observed in the field during collection of biological material. Halperin (1990) pointed *Monomorium dentiger* (Roger) (Hymenoptera: Formicidae) and representatives of Tettigoniidae family (Orthoptera) as predators for *T. wilkinsoni* eggs in Israel, and *Monomorium gracillimum* (F. Smith) in Cyprus, and mentioned that on average 2.5% of the eggs were destroyed by ant species. The egg batches can also be destroyed in winter by birds from the *Parus* genus (Schmidt *et al*., 1990). Demolin and Delmos (1967) found *Ephippiger ephippiger* (Fieb.) (Orthoptera: Bradyporidae) as a predator of *T. pityocampa*. In Bulgaria this species and another tettigoniid, *Pterolepis germanica* (H.-S.) (Hymenoptera: Tettigoniidae) were found to destroy *T. pityocampa* eggs (Tsankov *et al*., 1996b).

The species composition and the quantity of parasitoids was also a significant feature for the new habitat of certain species. The practically absent parasitism was recorded for *T. pinivora* in the Baltic without information for the time of development of the species in this biotope (Tsankov *et al*., 1993).

The most numerous parasitoid in the investigated biotope during first years of observation (1994-1997) was *T. embryophagum*. This parasitoid is less numerous in the core areas of *T. pityocampa*. No parasitism was established in the eggs of the pine processionary moth in Marikostinovo and it amounted to only 0.1% in Ploski and 0.5% - in Kurtovo (Tsankov *et al*., 1996a). Tiberi (1990) pointed out that *Trichogramma* attacks *T. pityocampa* from time to time mainly in new habitats, and the maximum number of parasitized eggs in Italy reaching up to 2.65%. Tsankov (1990) considered the cover scales of egg batches, which the female *Trichogramma* cannot overcome, as a limiting factor restricting the possibility of parasitism in a greater number of eggs. It is much more advantageous to parasitize end parts of the batches without scales, so that the female does not need to spend much energy to overcome the scales when laying eggs. Low percentage of parasitism caused by *Trichogramma* was recorded by other authors. The share of *T. embryophagum* from the total of eggs with parasitism was only 0.5% for Yugoslavia (Harapin, 1986). Bellin *et al.* (1990) determined *Trichogramma* only in Kassandra, North Greece to 0.1-0.2% from all parasitized eggs. The same authors did not observe *Trichogramma* attack of eggs in Peloponnesus. In Algeria, only 0.3% of the eggs were attacked by this parasitoid (Tsankov *et al*., 1995).

The almost uniform parasitism along the whole length of egg batch observed during the present investigation differs substantially from *Trichogramma* effect in Central Bulgaria, where 45.3% of the attacked eggs are concentrated in its base (Tsankov *et al*., 1996b).

The high number of *B. servadeii* in 1998 was surprising. In new localities of pine processionary moth where no alternative hosts of *B. servadeii* have been found, it is normal to expect the appearance of polyphagous *O. pityocampae* and *A. bifasciatus*.

CONCLUSION

In the new locality, no difference in *T. pityocampa* fertility was established, expressed by the average number of eggs in an egg batch, compared to its old habitats.

In the first years, a very high percentage of successfully hatched caterpillars was observed because of weaker influence of biotic regulating factors. At the same time, it is a pre-condition for the rapid increase in *T. pityocampa* number in this biotope.

In the site studied, there is a substantial difference in species composition of egg parasitoid complex of *T. pityocampa* and regulating effect of different species on the pest number. Parasitism was almost absent during the first years. It initially started by the polyphagous *T. embryophagum*. Gradually the specialized parasitoids were also settled, their role as regulators at this stage being quite modest. For the study period, the presence and impact of both main parasitoids of pine processionary moth *B. servadeii* and *O. pityocampae* - was evaluated as unstable. However, after 21 years, since the appearance of the pest in this habitat, the regulating effect of egg parasitoids is comparable to that in old habitats.

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