Structure of Egg Batches, Hatching Rate and Egg Parasitoids of the Pine Processionary Moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775) (Lepidoptera: Notodontidae), in Bosnia and Herzegovina

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Abstract:

Thirty eight egg batches containing 8,514 eggs of *Thaumetopoea pityocampa* were collected on 21 September 2013 after hatching caterpillars from *Pinus nigra* trees in the region of Boracko jezero, Bosnia and Herzegovina. Collected batches were singled in test tubes, closed with cotton stoppers and stored under laboratory conditions at 20-22°C. The average number of eggs in one batch was 224 (ranging 115-279). The successfully hatched eggs were 72.1%. The most significant regulators of pine processionary moth number at egg stage were parasitoids and predators. Four primary egg parasitoids of *T. pityocampa* were recorded: *Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus* and *Trichogramma* sp. The hyperparasitoid *Baryscapus transversalis* was also found during this study. Among all parasitoids, *O. pityocampae* was the dominant species, with higher number than all other species. High mortality rate of the host caused by egg parasitoids was registered. In laboratory conditions, the dynamics of emergences of four main parasitoids showed clearly distinguishable periods among the species.

Keywords: Thaumetopoea pityocampa, egg parasitoids, parasitism rate, Bosnia and Herzegovina

Introduction

Pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775), is one of the most dangerous pests in pine forests of the Mediterranean region (Demolin 1969). Caterpillars attack the needles of pine species (Devkota, Schmidt 1990), and their hairs contain urticarial substances causing dermatitis (Lamy 1990).

In the region of former Yugoslavia, *T. pityocampa* occurs in the coastal area and attacks primarily *Pinus nigra* Arn. (Androic 1956). The same author reported *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) as the most commonly found egg parasitoid of this host, followed by *Baryscapus endemus* (Walker) (=*Tetrastichus tibialis* Kurdjumov)

(Hymenoptera: Eulophidae), *Charitolophus* sp. (Hymenoptera: Eupelmidae) and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae).

Dautbašić (2015) considered the pine processionary moth as an economically and ecologically significant pest on *P. nigra* in Bosnia and Herzegovina, which is widely distributed in the vicinity of Adriatic coastline. The author identified three egg parasitoids of *T. pityocampa*: *O. pityocampae*, *Baryscapus servadeii* (Domenichini) (Hymenoptera: Eulophidae) and *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae).

The natural enemies of *T. pityocampa* play an important role in reducing pest number. The most

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common egg parasitoids are *O. pityocampae* and *B. servadeii*, which, together with other less numerous species, can parasitize more than 50% of the pest populations at the egg stage, reaching up 80% in extreme cases (Battisti *et al.* 2015). In Israel, Halperin (1990a) pointed that the egg parasitoids of *Thaumetopoea wilkinsoni* Tams can be used for biological control.

In Bulgaria, a country close to Bosnia and Herzegovina, three species were observed as predators on the eggs of pine processionary moth: the bird *Parus major* Linnaeus (Passeriformes: Paridae) as well as the tettigoniid orthopterans *Ephippiger ephippiger* (Fiebig) and *Pterolepis germanica* (Herrich-Schäffer) (=*Rhacocleis germanica* H.-S.) (Tsankov *et al.* 1996b).

The present study of *T. pityocampa* in egg stage is one of the first for Bosnia and Herzegovina. The aim of this study was to analyse comprehensively the structure, species composition and impact of egg parasitoids on pest numbers.

Material and Methods

Thirty eight egg batches of *T. pityocampa* were collected in the region of Boracko jezero in Bosnia and Herzegovina from *Pinus nigra* trees on 21 September 2013, after hatching of caterpillars (Table 1). They were put in plastic containers and transferred to the entomological laboratory of Forest Research Institute in Sofia.

After arrival, the main indexes of egg batches were described and measured: number of needles on which egg masses were deposited; orientation of scales; length of needles; length and diameter of egg batches; distance of egg batches to the base of needles.

The scale cover of egg batches was removed and each sample was kept singly in a test tube covered with cotton stopper at room temperature (20-22°C). They were observed daily. The emerged parasitoids were taken out and separated in plastic capsules for further determination.

The final analysis of all egg batches was made in November 2014. Each egg without a hole in its shell was opened carefully and the meconia and remains of emerged or dead insects were determined under a stereomicroscope (40× magnification) by the method presented schematically in Tsankov *et al.* (1996a). Parasitoids that had emerged before collection and transfer to Sofia were determined by their meconia and remains according to Schmidt, Kitt (1994), Tanzen, Schmidt (1995), Schmidt *et al.* (1997b) and Tsankov *et al.* (1996a, 1998a). The egg batches were divided into five equal parts and the parasitization in them was determined.

Results

Characterization of egg batches of T. pityocampa

All analysed 38 egg batches were deposited on two needles of P. nigra. Their length depended on both the number of rows and the number of eggs in them. The number of egg rows per batch varied between eight and ten, 31.6% with 8 rows and 52.6% with 9 rows, respectively, and only 15.8% with 10 rows (Table 1). The number of eggs in the individual batches varied widely, from 115 to 279 (av. 224.05) but it was below 200 only in 4 egg batches (10.5%). Dependence between the distance from egg batch to needle base and scales' orientation was observed. Only in two egg batches the female started laying the eggs and forming the egg batch from the tip of the needle. The results showed that in those cases the scales were orientated from the needle tip to its base, and the distance between the batch and the needles' base was longer than in the cases when the female started laying the eggs from the needles' base, and the batch began from the brachyblast.

Hatching rates and mortality of *T. pityocampa* caterpillars

Out of 8,514 eggs in the sample, the successfully hatched eggs were 72.1% (Table 2). The most significant regulators of *T. pityocampa* number at egg stage were parasitoids and predators. The relative share of unhatched eggs is almost three times lower as a result of observed dead caterpillars, undeveloped eggs with dried-up yolk and totally empty eggs without any remains (Table 3). The groups of undeveloped eggs with dried-up yolk and totally empty eggs are of approximately equal relative shares. The number of caterpillars that died in the eggs before managing to make an emergence opening in egg chorion was over 9 times higher. In 26 died caterpillars, without opening and undeveloped eggs with dried-up yolk, the development of fungal mycelium was observed.

Egg parasitoids

Spectrum of egg parasitoids

Four primary egg parasitoids species of the pine processionary moth were recorded: Ooencyrtus (Hymenoptera: pityocampae (Mercet, 1921) Encyrtidae), Baryscapus servadeii (Domenichini, (Hymenoptera: Eulophidae), 1965) Anastatus bifasciatus (Geoffroy, 1785) (Hymenoptera: Eupelmidae), Trichogramma sp. (Hymenoptera: Trichogrammatidae) (Table 4). One hyperparasitoid, Baryscapus transversalis Graham, 1991 (Hymenoptera: Eulophidae), was also recorded and reported as a new species for the fauna of Bosnia and

Table 1. Main characteristics of site of collection and biometric indices of egg batches of *Thaumetopoea pityocampa*

Parameters	Value		
Region	Boracko jezero		
Locality	Borac		
Altitude	760 m		
Plant host	Pinus nigra Arnold		
Date of collection	September 21, 2013		
Total number of egg batches	38		
Number of egg per batch	$224.05 \pm 33.27 (115 - 279)*$		
Length of needles wrapped by an egg batch, mm	$79.77 \pm 15.38 (49 - 110)*$		
Length of egg batches, mm	$29.45 \pm 4.46 (15 - 39)*$		
Diameter of egg batches, mm	$3.50 \pm 0.33 \ (3.0 - 4.2)^*$		
Distance of egg batches to base of needles, mm	$6.82 \pm 8.37 (0 - 40)$ *		
Number of egg rows per batch	$8.84 \pm 0.68 (8 - 10)$ *		
Orientation of scales:			
- number of egg batches with orientation of scales from base to tip of needles	36		
- number of egg batches with orientation of scales from tip to base of needles	2		

^{*}Average \pm SD (range)

Herzegovina in the course of this study (BOYADZHIEV *et al.* 2015).

Ooencyrtus pityocampae was found as a dominant species in egg parasitoid complex. Its number was higher than the amount of all other primary parasitoids. The number of *B. servadeii* was over four times higher than the polyphagian *A. bifasciatus*, and the participation of *Trichogramma* sp. could be defined as sporadic.

Emergence, viability and sexual ratio of egg parasitoids

The dominant parasitoid *O. pityocampae* was reared from almost 50% of investigated egg batches. Out of 266 adults, 84.6% emerged successfully before the date of sample collection (Table 5). All ones that emerged after this date and the dead adults found in pine processionary moth eggs were female specimens. As to location on egg-shell, the emergence openings of imagoes had appeared before the collection date (i.e. in natural conditions and in the presence of scales) were comparable with those appeared after the sample collection. The emergence openings on top of the eggs were predominated in both cases, whereas the openings appeared on eggs' side were only 4.0% and 7.3%, respectively.

In the case of second most numerous parasitoid, *B. servadeii*, all found specimens were female. Unlike *O. pityocampae*, 87.6% of its adults emerged after the sample collection date. Out of 161 of emergence openings, only one was on the side of egg-shell lateral. This parasitoid was found in 26.3% of the egg batches.

Table 2. Hatching rates and mortality of caterpillars of *Thaumetopoea pityocampa*

Parameters	Number	%
Total number of egg batches	38	100.0
Total number of eggs	8514	100.0
Caterpillars hatched	6135	72.1
Impact of egg parasitoids and predators	1776	20.9
Unhatched caterpillars	603	7.0

Table 3. Structure of unhatched caterpillars without influence of entomophages

Parameters	Number	%
Caterpillars died, of them:	245	40.6
- without opening (of them with fungal mycelium)	221 (3)	
- with opening	24	
Undeveloped eggs with dried-up yolk (of them with fungal mycelium)	177 (23)	29.4
Eggs totally empty, without any remains	181	30.0
Total	603	100.0

Table 4. Egg parasitoids of *T. pityocampa*

Parasitoids	Number	%
Ooencyrtus pityocampae	292	49.2
Baryscapus servadei	163	27.4
Baryscapus transversalis	95	16.0
Anastatus bifasciatus	40	6.7
Trichogramma sp.	4	0.7
Total	594	100.0

Table 5. The egg parasitoids of *Thaumetopoea pityocampa* and their developmental rates in egg batches

Parameters	Value			
Ooencyrtus pityocampae	292			
Emerged before collection of egg batches, from which	225			
♦ emergence opening on top egg-shell	216 (96.0 %)			
♦ emergence opening on the side of egg-shell	9 (4.0 %)			
Emerged after collection of egg batches, from which	4 1♀♀			
♦ emergence opening on top egg-shell	38 (92.7 %)			
♦ emergence opening on the side of egg-shell	3 (7.3 %)			
Adults died in eggs	26 ♀♀			
O. pityocampae found in 18 egg batches	(47.4 %)			
Baryscapus servadeii	163			
Emerged before collection of egg batches, from which	20			
♦ emergence opening on top egg-shell	19 (95.0 %)			
♦ emergence opening on the side of egg-shell	1 (5.0 %)			
Emerged after collection of egg batches, from which	141 ♀♀			
♦ emergence opening on top egg-shell	141 (100 %)			
♦ emergence opening on the side of egg-shell	0			
Adults died in eggs	2 ♀♀			
B. servadeii found in 10 egg batches	(26.3 %)			
Baryscapus transversalis	95			
Emerged before collection of egg batches, from which	1			
♦ emergence opening on top egg-shell	1 (100 %)			
Emerged after collection of egg batches, from which	81 (61 ඉඉ; 20්්්ර්)			
♦ emergence opening on top egg-shell	69 (85.2 %)			
♦ emergence opening on the side of egg-shell	12 (14.8 %)			
Adults died in eggs	13 (11 ♀♀; 2♂♂)			
B. transversalis found in 11 egg batches	(28.9 %)			
Anastatus bifasciatus	40			
Emerged after collection of egg batches, from which	36 ් ්			
♦ emergence opening on top egg-shell	26 (72.2 %)			
♦ emergence opening on the side of egg-shell	10 (27.8 %)			
Adults died in eggs	4 33			
A. bifasciatus found in 4 egg batches	(10.5 %)			
Trichogramma sp.	4			
Emerged before collection of egg batches, from which	1			
♦ emergence opening on top egg-shell	1			
Adults died in eggs	in 3 eggs			
Trichogramma sp. found in 4 egg batches	(10.5 %)			
Undetermined larvae of parasitoids	951			
Eggs destroyed by predators	231			

Table 6. Distribution of the main parasitoids in different parts of the egg batches

Parts of batches		1/5 base	2/5	3/5	4/5	5/5 top	Σ
O. pityocampae	n	55	49	48	54	86	292
	%	18.8	16.8	16.4	18.5	29.5	100.0
B. servadei	n	53	39	28	21	22	163
	%	32.5	23.9	17.2	12.9	13.5	100.0
B. transversalis	n	11	17	22	23	22	95
	%	11.6	17.9	23.2	24.2	23.1	100.0
A. bifasciatus	n	6	4	8	9	13	40
	%	15.0	10.0	20.0	22.5	32.5	100.0

The participation of *A. bifasciatus* in the total number of parasitoids and its occurrence in individual egg batches was modest. No evidence of emerged individuals was found before the sample collection date. All recorded specimens were male. Relatively high percentage (27.8%) of the lateral openings were noticed.

The hyperparasitoid *B. transversalis* was found in 28.9% of egg batches. It primarily emerged after September. Representatives of both sexes were found but the female individuals' participation was considerably higher (76.6%). Compared to *O. pityocampae* and *B. servadeii*, the share of its lateral emergence openings was higher.

Trichogramma sp. adults were found only in four eggs. Dead adults were found in three of them, and in one egg - an emergence opening of individuals emerged before the sample collection date was noticed.

The relative share of pine processionary moth eggs destroyed by predators was 2.7%.

A significant part (1,541) of *T. pityocampa* eggs were impacted by the monoembryonic parasitoids *O. pityocampae*, *B. servadeii*, *B. transversalis* and *A. bifasciatus*. Specimens of those four parasitoids successfully completed their development and emerged in only 35.4% of the cases. The bulk of the found dead specimens were in larval stage, and an insignificant part (4.5%) were adults.

Due to the high share of dead unidentified larvae, it is not possible to draw a conclusion as to the viability of different parasitoids. The dead imagoes found were within the range of about 10%, with exception of *B. servadeii* which the rate of mortality was around 10 times lower (1.2%).

Distribution of egg parasitoids in different parts of egg batches

Normally, eggs without scales are attacked more than eggs with scales. First ones are primarily located in the base and apex parts of the batches. Consequently, the parasitization of eggs at batches' ends should be expected to be higher than in other their parts (Table 6). With an even distribution of parasitization in 5 separate virtual parts of the batches, the parasitization in the individual sectors should amount to 20%. However, the sum of parasitization caused by four main parasitoids in both end parts was higher than this percentage.

Emergence dynamics of egg parasitoids

The emergence dynamics of four main parasitoids, recorded under laboratory conditions, showed clearly distinguishable periods among the species (Fig. 1). Firstly, the adults of hyperparasitoid *B. transversalis* and *A. bifasciatus* emerged, and

their emergence period ended within a month.

The results are interesting in the case of the two most numerous parasitoids - *O. pityocampae* and *B. servadeii*. It is known that *B. servadeii* is specific for pine processionary moth eggs and no other host has been reported. In this context, a later emergence should be expected for this parasitoid, i.e. it is synchronized with the appearance of next generation pine processionary moth eggs. In the present study, noting once again that it was conducted under laboratory conditions and the eggs had been taken out of their natural environment, characterized primarily by low winter temperatures, the behaviour of *O. pityocampae* was observed as more typical than *B. servadeii*.

Discussion

It is well known that female moths of T. pityocampa usually lay just one egg batch (Douma-Petridou 1990). For that reason the average number of eggs in egg batches corresponds to real average fertility rate of the species in the region. Many studies (Carvalho et al. 1999, Tiberi et al. 1999, Moura et al. 1999) show that the biochemical composition of the host plant is one of the significant ecological factors influencing the pine processionary moth number and egg productivity of female moths, respectively. MIRCHEV et al. (2004) reported that the batches formed on *P. nigra* are considerably smaller in size and the number of eggs in them is lower compared to *P. brutia*. Long-term observations in *P. nigra* stand in Marikostinovo, Bulgaria, showed that the average number of eggs in one batch varied between 203 and 253 (TSANKOV et al. 1998b). The average egg productivity in the present study was higher than the one reported for neighbouring countries such as Albania (Mirchev et al. 1999) and comparable to the one in Macedonia (Tsankov et al. 2006), where the plant nutrient was also *P. nigra*.

The plant nutrient with its needles' morphological characteristics has a distinct influence on the female moth's orientation when they start laying eggs. In case of *P. nigra*, both present study results and ones reported by other investigations (TSANKOV *et al.* 1997, MIRCHEV *et al.* 1999), reveal that egg deposition started from needle apex was extremely rare or limited. Unlike *P. nigra*, the egg-laying on *Pinus halepensis* MILL. in 45% of the cases has started from the apex of needles (TSANKOV *et al.* 1997).

The results of present study bear out the inferences of a number of investigations that, at the egg stage, parasitoids were the most significant natural regulator of pine processionary moth number (Tsankov *et al.* 1995, Schmidt *et al.* 1997, Mirchev *et al.* 2004). The

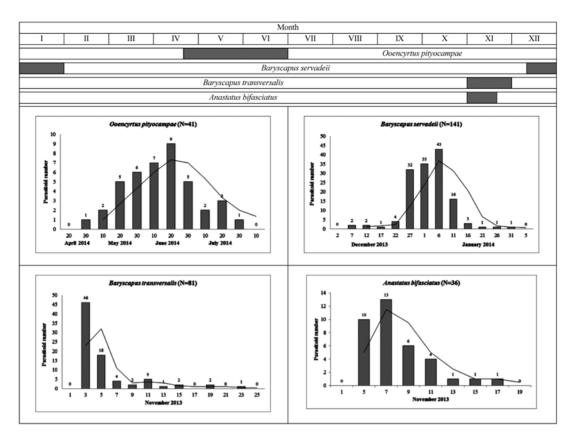


Fig. 1. Terms and emergence dynamics of main parasitoid species in laboratory conditions

regulating effect of egg parasitoids in countries close to Bosnia and Herzegovina is in order of 15.2-23.2% for Albania (Mirchev *et al.* 1999) and 10.4-16.9% for FYRMacedonia (Tsankov *et al.* 2006). However, higher levels of parasitism of *T. pityocampa* eggs have been registered in some samples in other neighbouring countries: 44.7% and 45.8% in Bulgaria and Greece, respectively (Mirchev *et al.* 1998, 2010).

With exception of the species recorded in the present study, Pediobius bruchicida (RONDANI) (Hymenoptera: Eulophidae) and Eupelmus vesicularis (Retzius) (Hymenoptera: Eupelmidae) have also been reported as egg parasitoids of T. pityocampa. The former parasitoid has been found in Bulgaria (MIRCHEV 2005), Macedonia (TSANKOV et al. 2006), Greece (MIRCHEV et al. 2010) and Turkey (MIRCHEV et al. 2004), and the latter – in two regions of Bulgaria as individual specimens (TSANKOV et al. 1996a; 1998b). Eupelmus vesicularis was found as parasitoid of *T. wilkinsoni* in Israel as well (HALPERIN 1990b). The relative share of *P. bruchicida* compared to the total number of parasitoids in the places where it has been found was reported as very small. With respect to both parasitoids as polyphagians with a wide range of hosts, it could be mentioned that the parasitization of pine processionary moth is of rather an accidental nature, and finding them in a specific biotope can also depend on the size of experimental material and on how long the study has lasted.

Bellin (1995) reported B. transversalis as an obligatory hyperparasitoid of the primary egg parasitoids of T. pityocampa - B. servadeii and O. pityocampae.

Two more species, reported as egg parasitoids of pine processionary moth, were not recorded during the present study. In Southern Italy (Apulia), in addition to *O. pityocampae*, another species of the genus *Ooencyrtus*, *O. telenomicida* (Vassiliev), was found (Tiberi 1990). This species has not been reported by other authors on pine processionary moth but has been observed as a primary and sometimes secondary parasitoid in the eggs of species from Pentatomidae and Scutelleridae families (Hemiptera) (Trjapitzin 1989). In France, Biliotti (1958) identified *B. endemus* (syn. *Baryscapus tibialis* Kurdjumov) as a secondary parasitoid of *T. pityocampa*. This species has also been reported as a parasitoid of pine processionary moth in Spain (Cadahia & Cuevas 1964).

In present study, out of primary parasitoids, a high participation rate belongs to *O. pityocampae* and *B. servadeii*, whereas the participation of *A. bifasciatus* and *Trichogramma* sp. is quite modest. Zovi *et al.* (2006) recorded relatively high number of the latter two parasitoids in a newly-arising pine processionary moth attack in the expansion of its

area in Italy, up to the appearance of *B. servadeii* and subsequent steady increase in its number and its transformation into a dominant species.

The number of O. pityocampae and B. servadeii in a specific biotope was determined by several ecological factors and biological characteristics of them. From ecological factors, the decisive role belongs to the fauna diversity and temperature conditions. Masutti (1964) points out that temperature is the limiting factor, the representatives of Eulophidae are more plastic and develop successfully on regions with temperatures of over 30°C – conditions which impede the development of O. pityocampae. The latter parasitoid is a polyphagian and its participation is determined by the conditions of the environment, ensuring the presence and number of its wide range of hosts. From biological characteristics of these two parasitoids Mirchev (2005) pointed out that B. servadeii can successfully parasitize the pine processionary moth eggs from their deposition to the moment of caterpillar hatching, whereas for O. pityocampae this period is shorter. When eggs are parasitised by the encyrtid after 32-nd day of host incubation period, the parasitoid cannot develop (HALPERIN 1990b).

A relatively high mortality rate of parasitoids was registered in this study. Similar results have also been reported in other studies. Analyzing literature data, MIRCHEV *et al.* (1999) reported that the mortality rate can reach up to 73.5%. As it has already been pointed out, *O. pityocampae* is sensitive to temperatures over 30°C. It is difficult to say at what extent the temperature is a reason for the high mortality rate. It could be supposed that very likely after collecting of the experimental material, the egg batches were not kept under controlled laboratory conditions at temperature of 20-22°C. There are no available data of site climatic conditions for the period before the sample collection date.

In present study, all imagoes of *O. pityocampae* and *B. servadeii* emerged in laboratory conditions or have been found dead in the host's eggs, were female. About *O. pityocampae*, this finds its logical explanation in the investigations of Halperin (1990b). The author has d that the sex of the encyrtid depends on the temperature in which the individuals developed. In temperature close to the optimum for the development and survival of the parasitoid (32°C), only females imago were found. Above these temperature males were also found, and in extreme for the species over 34°C the entire offspring were male. Like *O. pityocampae*, *B. servadeii* propagate parthenogenetically, through thelytoky (Halperin 1990b).

Different plasticity in the behaviour of *O. pityo-campae* and *B. servadeii* has also been observed with

regard to their abilities in the parasitization of pine processionary moth eggs. The presence of scales is a barrier for the encyrtid, whereas they are not an obstacle for the eulophid (BILIOTTI 1958). MASUTTI (1964) describes that B. servadeii slides between the scales, reaching the eggs of pine processionary moth and attacking them. It can be said that the results of present study bear out such findings, having in mind that the number of O. pityocampae is almost 1.8 higher than those of B. servadeii and, evidently, the scales of egg batches have not been an insurmountable obstacle. It must be noted that reports of different authors on this subject are contradictory. KITT & SCHMIDT (1993) found that O. pityocampae parasitised primarily the apex part of the batch, whereas B. servadeii shows preference for the base part. These results contrast with those obtained by TIBERI (1983).

The distribution of the emergence openings of various parasitoid species could be regarded as an indicator of their adaptation to a particular host. MIRCHEV (2005) reported that when the parasitoids made side openings in the egg walls, the final results were not always favourable for them. In this respect, higher percentage of emergence openings in the top part of the eggs shows that like the specific parasitoid *B. servadeii*, the polyphagous *O. pityocampae* also has high adaptability to the pine processionary moth, in contrast to another polyphagous species - *A. bifasciatus*.

The emergence dynamics of parasitoids was determined by their biological characteristics. It is normal for the hyperparasitoid B. transversalis to appear in a period when its hosts, O. pityocampae and B. servadeii have not emerged yet but are still developing or to be at stage of diapausing larvae. O. pityocampae and A. bifasciatus are polyphagians and their survival as species does not necessarily require synchronization between the time for their emergence and the availability of new, unhatched pine processionary moth eggs. Such synchronization is necessary for *B. servadeii* because this parasitoid does have no other host than pine processionary moth (Halperin, 1990b). There are reports of adult emergence of the species in late autumn of the same year in Albania (MIRCHEV et al. 1999), Morocco (SCHMIDT et al. 1997) and Turkey (Mirchev et al. 2004) when it was unlikely to find unhatched eggs of this host for parasitizing the eulophid. Kitt & Schmidt (1993) reported that in nature the temperature has to be low to prevent the premature emergence in the biotopes but to be forced to wait for the host's next generation. The hypotheses put forward about presumable temperature anomalies preventing *B. servadeii* from being in a state of diapause are just assumptions and there is a need of focused studies.

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