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Comparative studies of egg parasitoids of the pine processionary moth (*Thaumetopoea pityocampa*, Den. & Schiff.) in historic and expansion areas in France and Bulgaria

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To investigate enemy pressure across the range of the pine processionary moth (*Thaumetopoea pityocampa*, Den. & Schiff.) as it spreads northwards in Europe because of climate change, a survey of 206 egg batches from historical and newly colonized areas at both the westernmost and the easternmost ends of the front was carried out in 2016–2018. Out of them, 97 egg batches were collected from nine locations in three regions in France and 109 from four localities in two regions in Bulgaria, both within historical and newly colonized ranges of the pest. The average number of eggs per batch collected in Bulgaria (226.6 ± 43.2) was higher than that in France (194.3 ± 50.1). However, the hatching percentage was higher in French samples, varying from 69.8 to 95.7, *vs* 49.8 to 85.2 per cent in Bulgarian samples. Four primary parasitoids (*Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus*, *Trichogramma* sp.) and a hyperparasitoid (*Baryscapus transversalis*, found only in two regions of the historical range in Bulgaria) were identified. The oligophagous species *B. servadeii* was present at all sites within the historical range of the pest. In newly colonized areas, the impact of primary parasitoids on the host was distinctly low, suggesting that they lag behind the range expansion of pine processionary moth. In France, the most abundant species in the *T. pityocampa* parasitoid complex were *B. servadeii* in Ré Island and Orléans (97.3 and 87.4 per cent, respectively), and *Trichogramma* sp. (99.7 per cent) in a newly colonized locality in Fréhel. *Ooencyrtus pityocampae* prevailed in three of four Bulgarian localities (72.1 per cent in Sandanski, 89.7 per cent in Maglizh and 65.7 per cent in Sladak kladenets), whereas *B. servadeii* was the most abundant in Gega (75.4 per cent). Mortality of *B. servadeii* and *O. pityocampae* caused by the hyperparasitoid *B. transversalis* amounted to 4.8–6.2 per cent. The impact of predators on the pine processionary moth in the egg stage was negligible at most sites, reaching 12.5 per cent in only one site (Maglizh).

Introduction

The pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775) (Lepidoptera: Notodontidae) is one of the most important forest pests in the Mediterranean region. Due to climate change, and particularly winter warming (Battisti *et al.*, 2005, 2006; Robinet *et al.*, 2007), an altitudinal and latitudinal expansion of its range has been observed over the past decades (Hoch *et al.*, 2005; Imber, 2012; Mirchev *et al.*, 2011a; Netherer and Schopf, 2010; Roques *et al.*, 2005; Toffolo *et al.*, 2006). In its current range, *T. pityocampa* lives in different habitats both in its historical range and newly colonized areas.

In France, five egg parasitoids of pine processionary moth have been recorded: *Baryscapus servadeii* (Domenichini, 1965) (Hymenoptera: Eulophidae), *Ooencyrtus pityocampae* (Mercet, 1921) (Hymenoptera: Encyrtidae), *Anastatus bifasciatus* (Geoffroy, 1785), *Eupelmus* sp. (Hymenoptera: Eupelmidae) and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (Auger-Rozenberg *et al.*, 2015). In addition, *Baryscapus transversalis* (Graham, 1991), *Pediobius bruchicida* (Rondani, 1872) (Hymenoptera: Eulophidae) and *Eupelmus* (*Macroneura*) *vesicularis* (Retzius, 1783) (Hymenoptera: Eulophidae) have been established in Bulgaria (Mirchev *et al.*, 2011b; Tsankov *et al.*, 1996a). The performance and distribution of the pine procession-

Table 1 Main characteristics of sample plots in France and Bulgaria.

Region	Range	Locality	Coordinates	Altitude, m	Date of collection	Egg batches	Host tree
France							
Ré Island	Historical	Portes-en-Ré	46° 15′ 11.3″ N, 01° 30′ 54.0″ W	14	21.11.2017	15	*Pinus nigra*
		Ars-en-Ré	46° 13′ 15.1″ N, 01° 31′ 49.9″ W	0	21.11.2017	2	*Pinus pinaster*
		St.-Martin-en-Ré	46° 11′ 13.4″ N, 01° 22′ 06.8″ W	14	21.11.2017	4	*P. pinaster*
Fréhel	Newly colonized	Camp de Fréhel	48° 39′ 15.9″ N, 02° 21′ 22.5″ W	14	22.11.2017	40	*Pinus radiata*
		Camp de Fréhel	48° 39′ 15.9″ N, 02° 21′ 22.5″ W	14	22.11.2017	14	*P. pinaster*
		Plurien	48° 38′ 02.5″ N, 02° 24′ 37.0″ W	8	22.11.2017	10	*P. pinaster*
Orléans	Newly colonized	Saran	47° 56′ 58.5″ N, 01° 50′ 16.0″ E	121	23.11.2017	8	*P. nigra*
		Orléans	47° 49′ 22.3″ N, 01° 55′ 29.5″ E	108	23.11.2017	2	*P. nigra*
		Châteaudun	48° 03′ 34.4″ N, 01° 23′ 39.0″ E	127	23.11.2017	2	*P. nigra*
Bulgaria							
Sandanski	Historical	Sandanski	41° 34′ 22.0″ N, 23° 17′ 14.9″ E	325	20.09.2017	22	*P. nigra*
Ograzhden Mt.	Historical	Gega	41° 27′ 09.7″ N, 22° 59′ 54.0″ E	856	31.08.2017	38	*P. nigra*
St. Zagora	Newly colonized	Sladak kladenets	42° 24′ 19.8″ N, 25° 22′ 58.5″ E	403	28.10.2016	36	*P. nigra*
Maglizh	Newly colonized	Maglizh	42° 36′ 08.6″ N, 25° 30′ 31.9″ E	374	03.10.2017	13	*P. nigra*

ary moth and its insect enemies are highly dependent on climatic conditions. Higher temperatures in winter favour larval survival of the pine processionary moth (Battisti *et al.*, 2005), whereas summer temperatures above 32°C are considered lethal for the development of *T. pityocampa* eggs and young larvae (Robinet *et al.*, 2015). The climatic conditions in a particular biotope comprehensively influence the variety of egg parasitoid species as well. Indeed, Masutti (1964) reported that *O. pityocampae* is sensitive to high temperatures and biotopes with prolonged periods above 30°C are not suitable for its survival. In contrast, another hymenopteran species, *B. servadeii*, is more tolerant and develops successfully even at high temperatures. The parasitoid complex in newly colonized areas may also depend on the pace of the range expansion of *T. pityocampa*. The egg parasitoid assemblage of *T. pityocampa* is an indicator of two different types of pest expansion in new habitats: (1) when *T. pityocampa* penetrates into new regions, relatively remote from the primary habitats where the specific *B. servadeii* is absent (Mirchev *et al.*, 2017); (2) when the pine processionary moth gradually enlarges its range in adjacent stands at the rate of 2–3 km y^{-1}, the host is followed by *B. servadeii* (Mirchev *et al.*, 2018).

The aim of this study was to compare the egg parasitoids complex of *T. pityocampa* in historic and expansion areas in France and Bulgaria, the westernmost and easternmost countries where the northwards expansion of the pest is observed.

Materials and methods

During the period 2016–2017, a total of 206 egg batches of *T. pityocampa* were collected from France and Bulgaria between the end of August and November (Table 1). In Bulgaria, all samples were collected in Austrian pine (*Pinus nigra* Arn.) plantations. In France, samples were collected in *P. nigra*, maritime pine (*Pinus pinaster* Aiton) and Monterey pine (*Pinus radiata* D.Don) stands (Table 1).

In France, 97 egg batches were collected in nine localities from three regions corresponding to different colonization dates (Fig. 1):

1. Ré Island, a small island in the Atlantic Ocean situated about 4 km from mainland France, which is within the historical range (there are data since the 1970s);
2. Orléans, in central France, which has been colonized in the 1990s;
3. Fréhel, in Brittany, which has been newly colonized in the 2010s.

In Bulgaria, 109 egg batches were collected in two 'historical' and two 'newly colonized' localities from two distinct regions (Fig. 2):

1. Southwest Bulgaria – Struma valley (town of Sandanski) and Ograzhden Mt. (Gega vill.). In that area, the pine processionary moth has occurred since 1949, considered historical range.
2. Central Bulgaria – in the land of Sladak kladenets vill. In Sredna gora Mt. (the pest was first recorded in 2012) and in the region of Maglizh (newly colonized by *T. pityocampa* since 2015).

Upon collection, all egg batches were transported to the laboratory of entomology of Forest Research Institute in Sofia. The scales of egg masses were removed, and the samples were analysed according to the protocol described in Tsankov *et al.* (1996a). The egg-batches were put singly in test tubes covered with cotton stoppers and kept at room temperature (20–22°C).

During the laboratory studies, the samples were checked five times and the emerged parasitoids were separated in plastic capsules for identification. In November–December 2018, the eggs without exit holes were dissected, and the meconia and remains of emerged or dead insects were determined under a stereomicroscope (40×). Parasitoids that emerged before collection were determined by their meconia and remains according to Schmidt and Kitt (1994), Tanzen and Schmidt (1995), Schmidt *et al.* (1997a) and Tsankov *et al.* (1996a, 1998).

The data were analysed using descriptive statistic of MS Excel 2013. The Clopper–Pearson method (Hollander and Wolfe, 1973) was used to compare relative share of the specific *B. servadeii* in parasitoid assemblages of *T. pityocampa* in old and newly colonized areas.

Figure 1 Studied localities in France.

Results

Viability of the pine processionary moth in the egg stage

The number of eggs in *T. pityocampa* batches varied from 185.7 ± 47.7 to 232.2 ± 43.5 in France and from 216.4 ± 41.0 to 251.3 ± 31.3 in Bulgaria (Table 2). In Bulgaria, the average number of eggs in a batch (226.6 ± 43.2) was higher than that obtained from France (194.3 ± 50.1). The highest average egg production per female (251.3 ± 31.3) and the smallest dispersion between samples (204–299 eggs/batch) were recorded in Maglizh. In this locality, the proportion of hatched larvae was almost half (49.8 per cent) of all analysed eggs. A significant share (41.5 per cent) of parasitized and destroyed by predators eggs was registered. In the rest of the localities, the rate of parasitized and destroyed eggs varied between 10.3 per cent (Sladak kladenets) and 19.2 per cent (Sandanski).

The lowest average number of eggs per batch (185.7 ± 47.7) and a large dispersion between samples (55–278) were recorded in the recently infested by the pine processionary moth region of Fréhel. The highest proportion of hatched larvae was established in this region (95.7 per cent), and the rate of eggs parasitized and destroyed by predators was only 2.9 per cent. In the rest French localities, the impact of parasitoids and predators reached up to 13.6 per cent (Ré Island) and 24.5 per cent (Orléans) (Table 2).

Another factor that also affected the rate of successfully hatching was the abortion of eggs, which varied between 1.4 per cent (Fréhel) and 10.8 per cent (Sandanski) (Table 2). The number of dead larvae in the eggs (without exit holes) varied between 0.7 and 2.5 per cent in France, and between 2.1 and 4.7 per cent in Bulgaria (Table 2). The proportion of sterile, non-fertilized eggs recorded in all samples varied between 0.6 per cent (Fréhel) and 5.0 per cent (Sandanski).

The predatory effect in most localities was negligible (0.02–2.5 per cent) with exception of Maglizh in Bulgaria (12.5 per cent) (Table 2).

Complex of egg parasitoids in different localities

In this study, the egg parasitoids were the main factor causing pine processionary moth mortality. The mortality rate in France varied between 2.9 per cent (Fréhel) and 24.4 per cent (Orléans) (Table 2). In Bulgaria, the parasitoids affected between 7.8 per cent (Sladak kladenets) and 29.0 per cent (Maglizh) of host eggs.

Four primary egg parasitoids were identified in both France and Bulgaria: *O. pityocampae*, *B. servadeii*, *A. bifasciatus*, *Trichogramma* sp. (Table 3).

In a recently colonized area in France (Fréhel), only *Trichogramma* sp. and a single specimen of *O. pityocampae* represented the complex of parasitoids (Table 3). In areas of historical range (Orléans and Ré Island), *B. servadeii* was

Table 2 Viability of *Thaumetopoea pityocampa* in egg stage in different localities.

Parameters	France			Bulgaria			
	Ré Island	Fréhel	Orléans	Sandanski	Gega	Sladak kladenets	Maglizh
Generation of pine processionary moth (PPM)	2017	2017	2017	2017	2017	2016	2017
Total number (*n*) of egg batches	21	64	12	22	38	36	13
Larvae died without opening, *n* (%)	30 (0.7)	84 (0.7)	70 (2.5)	232 (4.7)	242 (2.9)	173 (2.1)	112 (3.4)
Larvae died with opening, *n* (%)	9 (0.2)	13 (0.1)	3 (0.1)	49 (1.0)	115 (1.4)	26 (0.3)	35 (1.1)
Undeveloped eggs with dried-up yolk, *n* (%)	76 (1.7)	74 (0.6)	72 (2.6)	245 (5.0)	226 (2.7)	149 (1.8)	97 (3.0)
Eggs totally empty, without any remains, *n* (%)	7 (0.2)	–	11 (0.4)	4 (0.1)	99 (1.2)	26 (0.3)	41 (1.3)
Total number of aborted eggs, *n* (%)	122 (2.8)	171 (1.4)	156 (5.6)	530 (10.8)	682 (8.3)	374 (4.5)	285 (8.7)
Number of larvae hatched, *n* (%)	3642 (83.6)	11 198 (95.7)	1947 (69.8)	3452 (70.0)	6120 (74.4)	7052 (85.2)	1626 (49.8)
Impact of parasitoids, *n* (%)	593 (13.6)	332 (2.9)	681 (24.4)	947 (19.2)	1392 (16.9)	650 (7.8)	947 (29.0)
Impact of predators, *n* (%)	1 (0.02)	2 (0.02)	3 (0.1)	–	32 (0.4)	203 (2.5)	409 (12.5)
Total number of eggs, *n* (%)	4358 (100)	11 703 (100)	2787 (100)	4929 (100)	8226 (100)	8279 (100)	3267 (100)
Range of eggs per egg batches	106–287	55–278	172–305	105–284	94–277	92–306	204–299
Mean eggs per egg batches ± Standart deviation (Std)	207.5 ± 50.5	185.7 ± 47.7	232.2 ± 43.5	224.0 ± 41.8	216.4 ± 41.0	230.0 ± 48.8	251.3 ± 31.3

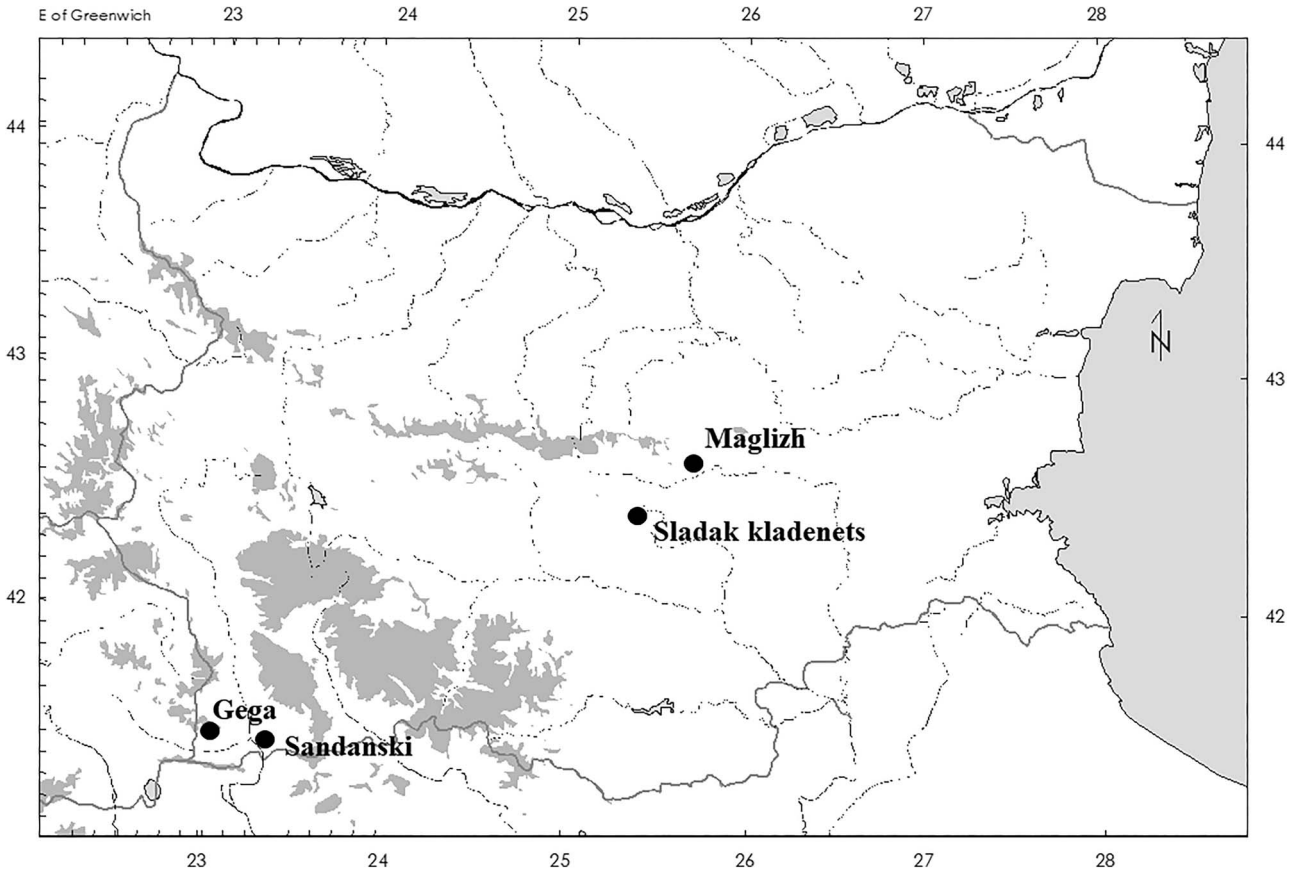

Figure 2 Studied localities in Bulgaria.

dominant with 84.7 and 97.3 per cent, respectively. A minor presence of *O. pityocampae* and *Trichogramma* sp. was also recorded in them, whereas in Orléans, four specimens of *A. bifasciatus* were detected.

In the historical range of *T. pityocampa* in Bulgaria, the most numerous were the polyphagous *O. pityocampae* in Sandanski (72.1 per cent) and the oligophagous *B. servadeii* in Gega (75.4 per cent). In the newly colonized sites, the polyphagous parasitoids prevailed. In Sladak kladenets, only *O. pityocampae* and *A. bifasciatus* were identified, with a significant prevalence of the former species. In Maglizh, four primary parasitoids were reared from host eggs but the specific species *B. servadeii* occupied only 2.1 per cent of the parasitoid complex.

In general, *B. servadeii* is common at all sites where the pine processionary moth has occurred for a long time (Ré Island, Orléans, Sandanski, Gega) and it is insignificant in all sites where the pest has penetrated recently (Fréhel, Sladak kladenets,

Table 3 Percentage of egg parasitoids of *Thaumetopoea pityocampa* in different localities.

Parasitoids	France						Bulgaria							
	Ré Island		Fréhel		Orléans		Sandanski		Gega		Sladak kladenets		Maglizh	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Ooencyrtus pityocampae	9	1.6	1	0.3	91	14.7	457	72.1	130	13.3	236	65.7	549	89.7
Baryscapus servadeii	550	97.3	–	–	524	84.7	130	20.5	737	75.4	–	–	13	2.1
Baryscapus transversalis	–	–	–	–	–	–	28	4.4	54	5.5	–	–	–	–
Anastatus bifasciatus	–	–	–	–	4	0.6	19	3.0	36	3.7	123	34.3	30	4.9
Trichogramma sp.	6	1.1	331	99.7	–	–	–	–	21	2.1	–	–	20	3.3
Total	565	100.0	332	100.0	619	100.0	634	100.0	978	100.0	359	100.0	612	100.0

Maglizh) (Table 3). This regularity of *B. servadeii* distribution has been confirmed in all studied areas. The Clopper–Pearson method determined the confidence interval (59, 100) of the percentage of cases in which the rule is confirmed with at a confidence level of 0.95. This suggests that the spread of *B. servadeii* is related to the old habitats of pine processionary moth and exceptions to this rule are rare (maximum 31 per cent).

The hyperparasitoid *B. transversalis* was found only in Bulgaria at Sandanski and Gega, both within the historical range of the pest.

Bioecological characteristics of egg parasitoids

A difference in the phenology of *O. pityocampae* was observed in France and Bulgaria. In samples from Ré Island and Orléans, most of the individuals emerged after collection of egg batches – 77.8 and 67.0 per cent, respectively (Table 4). In contrast, in three Bulgarian localities (Gega, Sladak kladenets and Maglizh), the predominant part of *O. pityocampae* adults (60.6–73.6 per cent) had emerged before collection of egg batches, and only in Sandanski, most of them (55.8 per cent) emerged after collection. The proportion of established dead *O. pityocampae* adults in host's eggs was the lowest in Orléans (3.3 per cent), whereas it ranged from 10.2 to 32.2 per cent in Bulgarian localities. The average survival rate of the parasitoid in French and Bulgarian localities was 96.0 and 85.4 per cent, respectively.

High average survival rate of *B. servadeii* 97.3 per cent was recorded for both countries, and the mortality was low in both French and Bulgarian sites (1.5–3.4 per cent). There was no difference in phenology for this parasitoid in both countries. The polyphagous *A. bifasciatus* was established in one locality in France (Orléans) and all studied localities in Bulgaria (Table 3). In Bulgaria, the majority of adults emerged after hibernation.

The polyembryonic *Trichogramma* sp. was found in 9.5–13.2 per cent of *T. pityocampa* egg batches in the localities of historical range in France and Bulgaria, and in 38.5–39.1 per cent in the newly colonized areas (Table 4). A high mortality (up to 70.0 per cent) of adults was observed in the eggs. The hyperparasitoid *B. transversalis* parasitized 4.8–6.2 per cent of *B. servadeii* and *O. pityocampae*. A high mortality (16.7–21.4 per cent) of the hyperparasitoid was recorded in Sandanski and Gega (Table 4).

In this study, all emerged adults of *B. servadeii* and *O. pityocampae* were female, unlike *A. bifasciatus* that only male specimens were recorded.

Discussion

Female moths of *T. pityocampa* usually lay only one egg batch (Douma-Petridou, 1990), and therefore the average number of eggs in batches corresponds to fertility of the species. The average number of eggs per batch collected in Bulgaria (226.6) was higher than that collected in France (194.3). The fertility of *T. pityocampa* depends on climatic conditions, host plant quality and population dynamics (Masutti and Battisti, 1990). It is higher in the northern parts of its range (Mirchev, 2005) and increases with altitude (Özkazans, 1987; Tiberi and Roversi, 1987). In contrast, Masutti and Battisti (1990) reported lower fertility in colder areas.

The biochemical composition of the host plant is one of the significant environmental factors affecting *T. pityocampa* (Carvalho *et al.*, 1999; Tiberi *et al.*, 1999, Moura *et al.*, 1999; Roussis *et al.*, 1994). In mixed stands of *P. radiata* and *P. pinaster*, the pine processionary moth prefers the former species on which caterpillars grow faster, reach a larger size and pupate earlier (Buxton, 1990). The parasitoid assemblage of *T. pityocampa* and its impact on the pest may also be affected by the identity of the host plant. According to Mirchev (2005), the rate of parasitism in different pine species varies greatly between years and localities: in Turkish pine (*Pinus brutia* Tenore) stands in Turkey, the average parasitism in Köycegiz was 9.8 per cent, but in Iskenderun – 34.9 per cent; in Bulgaria, in *P. nigra* plantations, the parasitism varied from 4.4 per cent (Kurtovo) to 43.7 per cent (Ploski) and in *P. sylvestris* – from 0 per cent (Kyustendil) to 23.5 per cent (Satovcha); in Greece, the lowest and highest rates of parasitism in Aleppo pine (*Pinus halepensis* Mill.) stands were 13.3 per cent (Fteri-Egio) and 39.6 per cent (Hydra Island), respectively.

Egg parasitoids are considered the most important regulator of *T. pityocampa* at the egg stage in both Bulgaria and France that was found in many previous studies (Battisti, 1989; Biliotti, 1958; Biliotti *et al.*, 1962; Cadahia and Cuevas, 1964; Ceballos, 1969; Demolin and Delmos, 1967; Harapin, 1986; Jamaâ *et al.*, 1996; El-Yousfi, 1989; Kitt and Schmidt, 1993; Tiberi and Roversi, 1987; Tsankov, 1990; Szeczepański and Tzankov, 1967). Percent parasitism is an indirect indicator of parasitoid effects on host populations, although life table studies that consider stage-specific effects of parasitoids and other mortality factors provide a more accurate picture of the role of different parasitoids (Van Driesche *et al.*, 1991). In the present study, a higher rate of parasitized eggs was detected at the localities of historical range of the species in France – Ré Island and Orléans, compared with newly colonized one, Fréhel. In Bulgaria, the host mortality caused by parasitoids in historical localities (Sandanski and Gega) was

Table 4 Characteristics of egg parasitoids of *T. pityocampa* in different localities.

Species and parameters	France			Bulgaria			
	Ré Island	Fréhel	Orléans	Sandanski	Gega	Sladak kladenets	Maglizh
Ooencyrtus pityocampae, *n*	9	1	91	457	130	236	549
Emerged before collection of egg batches, *n* (%)	2 (22.2)	–	27 (29.7)	149 (32.6)	79 (60.8)	143 (60.6)	404 (73.6)
Emerged after collection of egg batches, *n* (%)	7 (77.8)	–	61 (67.0)	255 (55.8)	36 (27.7)	17 (7.2)	89 (16.2)
Adults died in eggs, *n* (%)	–	1 (100.0)	3 (3.3)	53 (11.6)	15 (11.5)	76 (32.2)	56 (10.2)
Egg batches with *O. pityocampae*, *n* (%)	3 (14.3)	1 (1.6)	7 (58.3)	21 (95.5)	16 (42.1)	22 (61.1)	12 (92.3)
Baryscapus servadeii, *n*	550	–	524	130	737	–	13
Emerged before collection of egg batches, *n* (%)	113 (20.5)	–	100 (19.1)	2 (1.5)	374 (50.7)	–	3 (23.1)
Emerged after collection of egg batches, *n* (%)	429 (78.0)	–	406 (77.5)	126 (97.0)	339 (46.0)	–	10 (76.9)
Adults died in eggs, *n* (%)	8 (1.5)	–	18 (3.4)	2 (1.5)	24 (3.3)	–	–
Egg batches with *B. servadeii*, *n* (%)	12 (57.1)	–	9 (75.0)	8 (36.4)	29 (76.3)	–	4 (30.8)
Baryscapus transversalis, *n*	–	–	–	28	54	–	–
Emerged after collection of egg batches, ♀, *n* (%)	–	–	–	12 (42.9)	24 (44.4)	–	–
Emerged after collection of egg batches, ♂, *n* (%)	–	–	–	10 (35.7)	21 (38.9)	–	–
Adults died in eggs, *n* (%)	–	–	–	6 (21.4)	9 (16.7)	–	–
Egg batches with *B. transversalis*, *n* (%)	–	–	–	5 (22.7)	13 (34.2)	–	–
Anastatus bifasciatus, *n*	–	–	4	19	36	123	30
Emerged before collection of egg batches, *n* (%)	–	–	2 (50.0)	1 (5.3)	19 (52.8)	97 (78.9)	25 (83.3)
Emerged after collection of egg batches, *n* (%)	–	–	2 (50.0)	18 (94.7)	17 (47.2)	17 (13.8)	2 (6.7)
Adults died in eggs, *n* (%)	–	–	–	–	–	9 (7.3)	3 (10.0)
Egg batches with *A. bifasciatus*, *n* (%)	–	–	1 (8.3)	1 (4.5)	5 (13.2)	14 (38.9)	5 (38.5)
Trichogramma sp., *n*	6	331	–	–	21	–	20
Emerged before collection of egg batches, *n* (%)	5 (83.3)	147 (44.4)	–	–	10 (47.6)	–	6 (30.0)
Emerged after collection of egg batches, *n* (%)	–	44 (13.3)	–	–	6 (28.6)	–	–
Adults died in eggs, *n* (%)	1 (16.7)	140 (42.3)	–	–	5 (23.8)	–	14 (70.0)
Egg batches with *Trichogramma* sp., *n* (%)	2 (9.5)	25 (39.1)	–	–	5 (13.2)	–	5 (38.5)
Undetermined larvae of parasitoids, *n*	28	–	68	313	411	291	335

19.2–17.3 per cent. In one of the new localities, Sladak kladenets, a typical low parasitism rate was recorded. In contrast, an unusually high parasitism rate (29.0 per cent) was established in the second newly colonized locality, Maglizh. The high value of parasitism in Maglizh is due to the polyphagous *O. pityocampae* which is associated with a wide range of hemipteran and lepidopteran hosts (Noyes, 2018). As this parasitoid is less specialized and adapted to *T. pityocampa* (the parasitoid's emergence occurs in late autumn or spring), survival on other hosts is probably responsible for its high impact to the pest.

Baryscapus servadeii and *O. pityocampae* are widespread across the distribution range of *T. pityocampa* (Mirchev, 2005). The oligophagous *B. servadeii* parasitizes not only pine processionary moth, but also two other species of *Thaumetopoea* genus – *T. bonjeani* (Demolin, 1988) and *T. wilkinsoni* (Halperin, 1990). The number of *B. servadeii* and *O. pityocampae* may be limited by different adaptability to environmental conditions. *Baryscapus servadeii* is more adaptive to thermal stressors (Masutti, 1964). It successfully infests *T. pityocampa* eggs from their deposition to larval hatching, whereas *O. pityocampae* cannot develop after 32 days of host incubation period (Halperin, 1990). The number of egg parasitoids of *T. pityocampa* is also influenced by the presence of scales on egg batches (Biliotti, 1958). This is obviously much more relevant for small-sized parasitoids such as *Trichogramma* sp., as females cannot overcome the scales and can only parasitize uncovered eggs in the ends of batches (Tsankov, 1990).

The hyperparasitoid *B. transversalis* was found in Bulgarian samples. It is distributed in Southeast Europe: Albania, Bulgaria, Bosnia and Herzegovina, Greece and Turkey (Noyes, 2018). Another representative of the genus, *Baryscapus endemus* (Walker, 1839) (syn. *Tetrastichus tibialis*, Kurdjumov, 1913) was reported as a secondary parasitoid in France (Biliotti, 1958). It was also found on *T. pityocampa* in Spain (Cadahia and Cuevas, 1964) and North Macedonia (Roques *et al.*, 2015). *Baryscapus endemus* is a polyphage of many insect hosts from Coleoptera, Hemiptera, Hymenoptera and Lepidoptera orders (Noyes, 2018).

Abiotic factors (high temperature, low humidity, etc.) are the main causes for embryonal mortality of *T. pityocampa* (Mirchev, 2005). When the egg batches are exposed to direct sunlight, their temperature might rise by 14°C (Milani, 1990). Sterile, unfertilized or empty eggs in *T. pityocampa* batches were reported in Greece (Bellin *et al.*, 1990), Morocco (Schmidt *et al.*, 1997b), Portugal (Mirchev and Tsankov, 2000) and other countries.

The proportion of eggs destroyed by predators is usually negligible, but in Maglizh, the regulating effect reached high values (>10 per cent). In Bulgaria, *Ephippiger ephippiger* (Fiebig, 1784) (Orthoptera: Bradyporidae) and *Pterolepis germanica* (Herrich-Schäffer, 1840) (Orthoptera: Tettigoniidae) were observed to destroy *T. pityocampa* egg batches (Tsankov *et al.*, 1996b). The first species was reported as a predator of pine processionary moth in France as well (Demolin and Delmos, 1967). Halperin (1990) reported *Monomorium dentigerum* (Roger, 1862) (Hymenoptera: Formicidae) and tetigonid species as predators of *T. wilkinsoni* in Israel, and *Monomorium destructor* (Jerdon, 1851) (syn. *Myrmica gracillima*, Smith, 1861) in Cyprus.

In conclusion, parasitoids were the most important regulating factor at the egg stage of *T. pityocampa* in both France and

Bulgaria. In newly colonized areas, the impact of the parasitoids on the host is distinctly low. This suggests that parasitoids lag behind the expansion of the pine processionary moth. In one of the most important species, *O. pityocampae*, the temperature conditions and presence of alternative hosts are essential factors for its effectiveness. The hyperparasitoid *B. transversalis* caused relatively low mortality (4.8–6.2 per cent) of primary parasitoids at sites within the historical range of *T. pityocampa* in Bulgaria.

Conflict of interest statement

None declared.

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