

## *New records and impact of tachinid parasitoids of *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) in Bulgaria*

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**Abstract.** The impact of larval and pupal parasitoids of *Thaumetopoea pityocampa* was studied in laboratory conditions, during the period February-October 2021. The material, 138 hibernating caterpillars of summer form and 415 larvae of winter form intercepted during processions, was collected from two localities – Dobrostan Vill. and Gotse Delchev, respectively. In the land of Kovil Vill., which is occupied by the winter form, the winter nests of *T. pityocampa* were checked for infection by parasitoids. In this study, all isolated parasitoids belong to the family Tachinidae. As a result, 3 species – *Phryxe vulgaris* (Fallén, 1810), *Compsilura concinnata* (Meigen, 1824) and *Bothria frontosa* (Meigen, 1824) were found to parasitized *T. pityocampa*. A relatively high parasitism rate of *T. pityocampa* winter form by tachinids was observed – 21%, of which *P. vulgaris* parasitized 15.2% of the sample. Conversely, the parasitism rate of *T. pityocampa* summer form is low – 5%, in which *C. concinnata* parasitized 1.4% from the sample. Two puparia of *B. frontosa* were collected from larval winter nests of *T. pityocampa* in the region of Kovil Vill. This is the first record of the Notodontidae family as a host of *Bothria frontosa*. A complete up-to-date list of trophic connections of *T. pityocampa* with tachinid parasitoids, reported in Bulgarian and international publications is provided, as well as their impact on pest populations.

**Key words:** pine processionary moth, Tachinidae, parasitism rate, host record.

### Introduction

*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is a forest insect, which caterpillars cause severe defoliation of pine and cedar plantations throughout the Mediterranean region, including Southwestern Europe and Balkan Peninsula (Hódar et al., 2003; Kanat et al., 2005; Jacquet et al., 2012; Roques et al., 2015; Mirchev et al., 2017). A part of the northern border of its range passes Bulgaria (Roques et al., 2015). Pine processionary moth (hereafter

abbreviated as PPM) is present in over 20 countries with Atlantic and Mediterranean climate (Battisti et al., 2015), with the exception of Bulgaria, where Continental climate predominates (Sabev & Stanev, 1959). Globally, a worldwide vertical and horizontal expansion of PPM range to the north and to higher altitudes is being reported (Battisti et al., 2006), also in Bulgaria (Mirchev et al., 2017; Zaemdzhikova et al., 2018). This gradual increase of PPM attacks in recent decades has a negative impact on the economic,

social and environmental functions of the pine forests (Arnaldo et al., 2010; Aimi et al., 2006; Carus, 2010). In this regard, natural enemies play an important role in controlling the population density of pests, and they also have a great impact suppressing incipient pests' outbreaks in various ecosystems (Žikić et al., 2017). The natural enemies, such as parasitoids, predators, insectivorous birds and pathogens might have a role in controlling processionary moth species (Battisti et al., 2015).

In the Mediterranean area, 18 parasitoids and approximately 14 predators have been reported as natural regulators of *T. pityocampa* (Battisti et al., 2015). A list of all known entomophagous of *T. pityocampa* in Bulgaria is given in Table 1. In the country, 13 primary parasitoids (six chalcidoid wasps,

one ichneumonid, *Trichogramma* sp., five tachinid species) and 5 predators have been identified. Among them, the regulatory role of egg parasites has been well studied (Tsankov et al., 1996 a, b; Mirchev et al., 2021). Given the recent increase in the frequency of PPM outbreaks in pine forests of Central Bulgaria reported by Zaemdzhikova et al. (2018), as well as the real potential for increasing their frequency in the future, it is important to know the role of other enemies on pest populations in the country. In this context, the aim of the present work is to establish the species composition and impact of tachinid parasitoids of *T. pityocampa* in Bulgaria. To achieve this proposal, our own observations were supplemented with all known country data, as well as these from international publications.

**Table 1.** List of primary parasitoids and predators of *Thaumetopoea pityocampa* in Bulgaria.

Stage	Parasitoids	Source
Eggs	<i>Ooencyrtus pityocampae</i> (Mercet, 1921) (Hymenoptera: Encyrtidae)	Mirchev et al. (1998)
	<i>Anastatus bifasciatus</i> (Geoffroy, 1785) (Hymenoptera: Eupelmidae)	
	<i>Eupelmus vesicularis</i> (Retzius, 1783) (Hymenoptera: Eupelmidae)	
	<i>Baryscapus servadeii</i> (Domenichini, 1965) (Hymenoptera: Eulophidae)	Boyadzhiev et al. (2020)
	<i>Eupelmus vladimiri</i> Fusu, 2017 (Hymenoptera: Eupelmidae)	
	<i>Pediobius bruchicida</i> (Rondani, 1872) (Hymenoptera: Eulophidae)	
Larvae/ Pupae	<i>Trichogramma embryophagum</i> (Hartig, 1838) (Hymenoptera: Trichogrammatidae)	Mirchev et al. (1998)
	<i>Heteropelma megarthrum</i> (Ratzeburg, 1848) (Hymenoptera: Ichneumonidae)	Zankov (1960)
Larvae Pupae	<i>Compsilura concinnata</i> (Meigen, 1824) (Diptera: Tachinidae)	Russkoff (1929-1930)
	<i>Exorista fasciata</i> (Fallén, 1820) (Diptera: Tachinidae)	Georgiev et al. (2022)
Larvae	<i>Exorista segregata</i> (Rondani, 1859) (Diptera: Tachinidae)	Russkoff (1929-1930)
Pupae	<i>Phryxe vulgaris</i> (Fallén, 1810) (Diptera: Tachinidae)	Hubenov (1983)
Larvae	<i>Phorocera grandis</i> (Rondani, 1859) (Diptera: Tachinidae)	
<b>Predators</b>		
Eggs	<i>Ephippiger ephippiger</i> (Fiebig, 1784) (Orthoptera: Tettigoniidae)	Mirchev et al. (2019a)
	<i>Pterolepis germanica</i> (Herrich-Schäffer, 1840) (Orthoptera: Tettigoniidae)	
	<i>Dermestes lardarius</i> Linnaeus, 1758 (Coleoptera: Dermestidae)	Zankov (1960)
Larvae	<i>Formica rufa</i> Linnaeus, 1761 (Hymenoptera: Formicidae)	Zaemdzhikova & Doychev (2020)
Larvae	<i>Oecanthus pellucens</i> (Scopoli, 1763) (Orthoptera: Gryllidae)	

### Material and Methods

The biological material, a total of 553 *T. pityocampa* larvae, was collected during the period February-April 2021 in two localities

of *Pinus nigra* J.F. Arnold - Dobrostan Vill. (Rhodope Mts.) and Gotse Delchev (Southern Pirin Mt.). In Dobrostan the PPM summer form occurs (Mirchev et al., 2019b)

and the winter one is widespread in the region of Gotse Delchev (Zaemdzhikova 2020). From the first locality, 138 overwintering larvae were dug out from the soil, and from the second one – 415 larvae were intercepted at the beginning of processions. In the laboratory of Entomology at Forest Research Institute (Bulgarian Academy of Sciences), the collected larvae were placed in plastic containers with ventilated holes (40x30x20 cm). A layer of 5 cm sterilized sand was added to each box, simulating the soil environment required for burying of the caterpillars. After larvae built cocoons, they were removed from the boxes and stored in dark at room temperature until their pupation. The cocoons were checked

once a week for pupae present. All cocoons and pupae were isolated in separate containers. All samples (larvae, prepupae, pupae) were checked 3 times a week for the emergence of adults or puparia of parasitoids. In addition to the collected samples, in the vicinity of Kovil Vill. (Rhodope Mts.), where the PPM winter form occurs, the nests of caterpillars have been directly observed for infestation by parasitoids (Fig. 1). Twenty PPM winter nests were checked.

The parasitism rate was calculated as the number of insects in a sample from which parasitoids had emerged divided by the total number of insects, including these parasitoids, which were not yet identified (conserved as puparia).

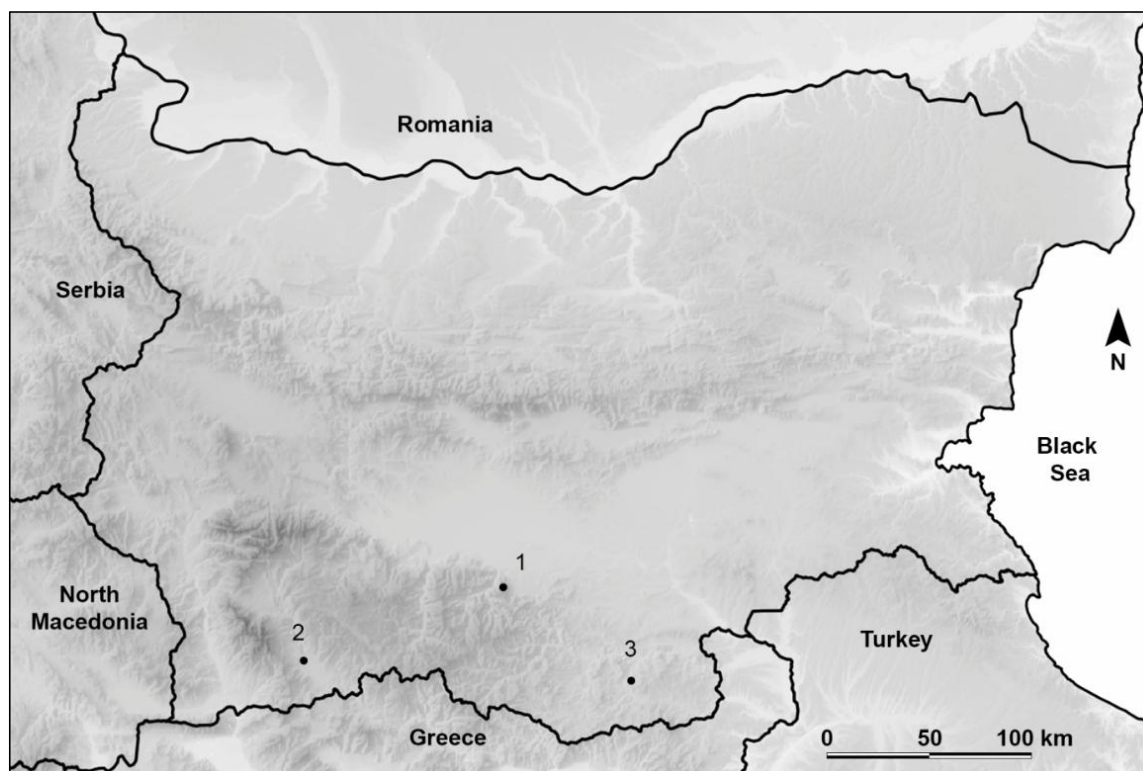


Fig. 1. New localities of *Compsilura concinnata* (1), *Phryxe vulgaris* (2) and *Bothria frontosa* (3) in Bulgaria.

## Results

*General.* In the present study, all isolated parasitoids from *T. pityocampa* belong to the subfamily Exoristinae (Diptera:

Tachinidae). In the result, three species were established by the host – *Phryxe vulgaris* (Fallén, 1810), *Compsilura concinnata* (Meigen, 1824) and *Bothria frontosa* (Meigen,

1824). For one species, *B. frontosa* – a host of Notodontidae family has been reported for the first time.

*Compsilura concinnata*

*Material examined:* Western Rhodope Mts., land of Dobrostan Vill. (41°54'10"N/24°55'07"E), 1100 m a. s. l., date of coll. 12.III.2021, 2 ♀♀, emerged 4.IV.2021 (det. Z. Hubenov). The adults were reared from prepupae of PPM summer form.

*Distribution:* Holarctic-Paleotropical-Australian. *In Bulgaria* – common: Western Danubian Plain, Popovo-Provadiya district, Loudogorie-Dobroudzha district, Western and Middle Predbalkan, Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Podbalkan Basins; Thracian Lowland, Belasitsa Mts., Boboshevo-Simitli Valley, Kroupnik-Sandanski-Petrich Valley, Rila Mts., Pirin Mts., Slavyanka Mt., Western Rhodope Mts., Eastern Rhodope Mts., Northern Black Sea Coast (Hubenov, 2021).

*Parasitoid-host list:* many lepidopteran species, most of the reported hosts are species of the Notodontidae family (Tschorsinig, 2017). *Trophic connections in Bulgaria:* *Calyptra thalictri* (Borkhausen, 1790), *Lymantria dispar* (Linnaeus, 1758), *Euproctis chryssorrhoea* (Linnaeus, 1758), *Hyphantria cunea* (Drury, 1773), *Leucoma salicis* (Linnaeus, 1758) (Lepidoptera: Erebidae); *Eriogaster lanestris* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae); *Archips xylosteana* (Linnaeus, 1758) (Lepidoptera: Tortricidae); *Orthosia cruda* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae); *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) (Hubenov, 1985); *Subacronicta megacephala* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) (Hubenov, 1985; 2001); *Thaumetopoea solitaria* (Freyer, 1838), *T. processionea* (Linnaeus, 1758) (Hubenov, 1985) and *T. pityocampa* (Lepidoptera: Notodontidae) (Russkoff, 1929-1930; Georgiev et al., 2022).

*Phryxe vulgaris*

*Material examined:* Pirin Mts., region of Gotse Delchev (41°35'17"N/23°44'24"E), 554

m a. s. l., date of coll. 30.IV.2021, 32 ♀♀, 31 ♂♂, emerged 15-19.IX.2021 (det. Z. Hubenov). All specimens were reared from pupae of PPM winter form.

*Distribution:* Holarctic. *In Bulgaria:* Loudogorie-Dobroudzha district, Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Lozenska Planina Mt., Thracian Lowland, Belasitsa Mts., Kroupnik-Sandanski-Petrich Valley, Rila Mts., Pirin Mts., Slavyanka Mt., Rhodope Mts., Northern Black Sea Coast (Hubenov, 2021).

*Parasitoid-host list:* Many hosts from the Coleoptera, Hymenoptera and Lepidoptera orders. Only two hosts are known of the family Notodontidae – *Thaumetopoea processionea* and *T. pityocampa* (Tschorsinig, 2017). *Trophic connections in Bulgaria:* *Sphinx pinastri* Linnaeus, 1758 (Lepidoptera: Sphingidae); *T. pityocampa* (Lepidoptera: Notodontidae) (Hubenov, 1983; Georgiev et al., 2022); *Lycia hirtaria* (Clerck, 1759), *Erannis defoliaria* (Clerck, 1759) (Lepidoptera: Geometridae) (Hubenov, 1985).

*Bothria frontosa*

*Material examined:* Eastern Rhodope Mts., in the vicinity of the Kovil Vill. (41°30'17"N/25°39'41"E), 500 m a. s. l., date of coll. 27.II.2021, two puparia were observed in the winter nest of PPM winter form (they were reared in laboratory), 1 ♀, emerged 04.IV.2021 (det. Z. Hubenov).

*Distribution:* Transpalaeartic, Holoeurosiberian. *In Bulgaria* – Western Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Thracian Lowland; Pirin Mts. (Hubenov 2021).

*Parasitoid-host list:* Until now, only two hosts are known – *Mesogona acetosellae* (Denis & Schiffermüller, 1775) and *Noctua comes* Hübner, 1813 (Lepidoptera: Noctuidae) (Tschorsinig, 2017). *Trophic connections in Bulgaria:* No records.

*Parasitism rate.* Significant differences in the parasitism rate of *T. pityocampa* were observed in the localities (Table 2). In the region of Gotse Delchev, where the winter form is widespread, the rate of parasitism is

high – 21%, while in Dobrostan, where summer form occurs, it is only – 5%. The parasitism rate in both localities is 12% of an average.

Relatively high parasitism rate of *T. pityocampa* by *Phryxe vulgaris* has been found, which is the main result of this study. It parasitized 15.2% of the larvae of PPM

winter form, while the other tachinid species – *Compsilura concinnata* parasitized 1.4% of the larvae of PPM summer form. All adults of *P. vulgaris* emerged (in laboratory condition) in the middle of September, while *C. concinnata* and *B. frontosa* – at the beginning of April.

**Table 2.** Parasitism rate of *Thaumetopoea pityocampa* by tachinid species.

Locality	Phenological form	Collected sample, number	Parasitism		Emerged adults of identified parasitoids				
			N	%	Species	Reared from (host stage)	Emerged	Parasitism	
				N	%			N	%
Dobrostan Vill.	summer	138 (overwintering larvae)	7	5	<i>Compsilura concinnata</i>	prepupae	4.IV.2021	2	1.4
Gotse Delchev	winter	415 (larvae in procession)	87	21	<i>Phryxe vulgaris</i>	pupae	15-19.IX.2021	63	15.2

### Discussion

*Trophic connections recorded by us.* Three trophic connections of *Thaumetopoea pityocampa* with tachinid parasitoids were found, as follows – *Compsilura concinnata*, *Phryxe vulgaris* and *Bothria frontosa*. Of these, *B. frontosa* is a new parasitoid record for *T. pityocampa*. The other two tachinids are known to parasitize on *T. pityocampa* in the country. The first report for *C. concinnata* is by Russkoff (1929-1930) and *P. vulgaris* by Hubenov (1983). From our findings and literature sources, a complete up-to-date list of trophic connections of tachinid parasitoids with *T. pityocampa* is given in Table 3.

*Trophic connections evidenced in Bulgaria.* Six tachinid species have been associated with *T. pityocampa* in the country so far. For three parasitoids (*Bothria frontosa*, *Exorista fasciata* and *Phryxe vulgaris*) this host is known only for the country, and for two of them – *B. frontosa* and *E. fasciata* (Georgiev et al., 2022) the host record is new of the family Notodontidae (Tschorsinig, 2017).

*World-wide trophic connections.* Until now, 14 parasitoid-host relationships are known. Five of them are considered questionable – *Townsendiellomyia nidicola* (Townsend, 1908), *Pelatachina tibialis* (Fallén,

1810), *Siphona cristata* (Fabricius, 1805), *Cyrtophloebe ruricola* (Meigen, 1824) and *Zenillia* sp. The remaining nine parasitoid-host associations were confirmed in different studies (Tschorsinig, 2017).

*Unobserved trophic connections in Bulgaria.* Among the known 14 trophic connections, 8 remain unknown for the country, of which 5 are questionable (Tschorsinig, 2017). The remaining three are unconfirmed at this time. Among them, *Phryxe caudata* (Rondani, 1859) is absent for the list of Bulgarian dipterans (Hubenov, 2021). The other two tachinids – *Carcelia iliaca* (Ratzeburg, 1840) and *Pales pavida* (Meigen, 1824) are present in the country (Hubenov, 2021), but they have not been associated with *T. pityocampa*.

*Parasitism rate.* In this study, a relatively high mortality of PPM winter form larvae caused by tachinid parasitoids in the region of Gotse Delchev was found, where *Phryxe vulgaris* parasitized 15.2% from the larvae in the sample. According to Georgiev et al. (2022) the mortality of PPM larvae, caused by *P. vulgaris* in the region in Eastern Rhodopes (Kandilka) and Struma Valley (town of Sandanski) is 2.5 and 2.4%, respectively. This is 6 times lower than the observed parasitism rate in this study.

Surprisingly but so far, this cosmopolitan species has been reported as a parasitoid of the pine processionary moth only in the country (Tschorsnig, 2017). *P. vulgaris* is extremely polyphagous on different families of Lepidoptera with relatively large caterpillars (Tschorsnig & Herting 1994). Probably it parasitized the larvae of the PPM in the winter nest or during their moving to pupation in the soil, similarly to *Phryxe caudata* (Buxton, 1990; Zamoum et al., 2017).

Relatively low parasitism rate of the larvae sample of PPM summer form has been found - 5%. In this case - *Compsilura concinnata* parasitized 1.4% of the collected overwintering PPM larvae. For Bulgaria, the known parasitization level of PPM by this tachinid fly ranged between 0.5% (Fotinovo Vill.) and 5.3% and (Dobrostan Vill.) (Rhodope Mts.) (Georgiev et al., 2022). Overall the observed country's maximum

mortality is lower than known maximum parasitism rate reported by Battisti et al. (2015) - 7%. *C. concinnata* is polyphagous generalist species, attacking over 100 species in the Palearctic region (Herting, 1960). Most of the hosts are lepidopterous larvae (Tschorsnig, 2017). Three to four generations of *C. concinnata* occur per year, with the larvae overwintering in host larvae or pupae (Culver, 1919). In this case, the fly probably attacked the host larvae on trees or on the soil during their autumn migration, then it overwintered in larval stage inside the host prepupae. Only two host records for *Bothria frontosa* dating back to the middle of the last century have been found in the literature (Tschorsnig, 2017). For the first time in this study data on a host of *B. frontosa* of the Notodontidae family are provided, which is the third host record for the tachinid parasitoid.

**Table 3.** List of known tachinid parasitoids of developmental stages of *Thaumetopoea pityocampa*. The data recorded by: <sup>R</sup> Russkoff, 1929-1930; <sup>H</sup> - Hubenov, 1983; <sup>G</sup> - Georgiev et al., 2022; <sup>ZD</sup> - current study (Zaemdzhikova & Doychev, 2020); <sup>T</sup> - Tschorsnig, 2017; <sup>B</sup> - Battisti et al., 2015. Legend: \* - first record for Bulgaria; (Q) - the parasitoids-host connection is questionable.

Stage	Species	New connection found by us	Confirmed known connections in this study	Total of connections evidenced in Bulgaria	Total worldwide trophic connections evidenced - number, author, country	Known connections not observed in Bulgaria	Connection known only for Bulgaria	First record of the Notodontidae family	Period of activity (month)	Max. mortality (%)
Larva	<i>Phorocera grandis</i>			1 <sup>H*</sup>	numerous <sup>T</sup>				IV <sup>H</sup>	unknown
Pupa	<i>Phryxe vulgaris</i>		1 <sup>H</sup>	1 <sup>H*,G,ZD</sup>	3 <sup>H,G,ZD</sup> Bulgaria		1		XIII - XI <sup>G</sup> , IX <sup>ZD</sup>	15.2 <sup>ZD</sup>
Larva	<i>Phryxe caudata</i>				numerous <sup>T</sup>	1			IX-X and II-III-IV <sup>B</sup>	10 <sup>B</sup>
Larva	<i>Compsilura concinnata</i>		1 <sup>R</sup>	1 <sup>R*,G</sup>	numerous <sup>T</sup>				IV-V <sup>B</sup> , III <sup>G</sup>	7 <sup>B</sup>
Larva	<i>Bothria frontosa</i>	1		1 <sup>ZD*</sup>	1 <sup>ZD</sup> Bulgaria		1	1 <sup>ZD</sup>	IV <sup>ZD</sup>	unknown
Larva / Pupae	<i>Exorista fasciata</i>			1 <sup>G*</sup>	1 <sup>G</sup> Bulgaria		1	1 <sup>G</sup>	III-IV <sup>G</sup>	2.2 <sup>G</sup>
Larva	<i>Exorista segregata</i>			1 <sup>R*</sup>	numerous <sup>T</sup>				IV <sup>B</sup>	5 <sup>B</sup>
Not found	<i>Carcelia iliaca</i>				1 <sup>T</sup> Southern France	1				
	<i>Townsendiella myia nidicola</i>				1 <sup>T</sup> only for Italy (Q)	1 (Q)				
	<i>Zenillia sp.</i>				1 <sup>T</sup> only for Portugal (Q)	1 (Q)				
	<i>Pales pavida</i>				1 <sup>T</sup> only for	1				



					Corsica					
	<i>Pelatachina tibialis</i>				1 <sup>T</sup> only for Italy (Q)	1(Q)				
	<i>Siphona cristata</i>				1 <sup>T</sup> only for Austria (Q)	1(Q)				
Larva	<i>Cyrtophloeba ruricola</i>				1 <sup>T</sup> only for Morocco (Q)	1(Q)				
Total result		1	2	6	14	8(5)	3	2		

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