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NATURAL ENEMIES EMERGED FROM *THAUMETOPOEA PITYOCAMPA* (DENIS & SCIFFERMÜLLER) (LEPIDOPTERA NOTODONTIDAE) PUPAE IN SOUTHERN ITALY

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Tarasco E., Triggiani O., Zamoum M., Oreste M. – Natural enemies emerged from *Thaumetopoea pityocampa* (Denis & Sciffermüller) (Lepidoptera Notodontidae) pupae in Southern Italy.

Observation made over a 4-year period in pinewoods of Apulia Region (South Italy) on *Thaumetopoea pityocampa* (Denis Sciffermüller) (Lepidoptera: Notodontidae) prepupae and pupae, indicated the following natural enemies complex: *Hexameris* sp. (Nematoda: Mermithidae), *Phryxe caudata* (Diptera: Tachinidae), *Villa brunnea* and *Hemipenthes velutina* (Diptera: Bombyliidae), *Conomorium pityocampae* (Hymenoptera: Pteromalidae), *Coelichneumon rudis* and *Cryptus* sp. (Hymenoptera: Ichneumonidae), *Beauveria bassiana* and *Isaria farinosa* (formerly *Paecylomyces farinosus*) (Ascomycota: Hypocreales). The moths and the parasitoid species emerged sequentially and, although these natural enemies show irregular distribution, they together controlled up to 60% of *T. pityocampa* pupae. Ecological implications of such parasitism are being discussed.

KEY WORDS: entomopathogenic fungi, virulence, myco-insecticide, microbial pest control

INTRODUCTION

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffemüller) (Lepidoptera: Notodontidae) is a highly destructive pine tree pest, that infects pinewoods, especially *Pinus nigra* and *P. halepensis* (MASUTTI & BATTISTI, 1990; HODAR *et al.*, 2002; STASTNY *et al.*, 2006). This pest is univoltine and widespread throughout the Mediterranean region.

The larvae are ready to pupate in late winter early spring when they leave the tree in a single file, head-to-tail procession, searching for soil sites suitable for pupation. Once the adults emerge they are airborne throughout summer. In pine forests, various natural enemies, including ants (WAY *et al.*, 1999), Tettigonidae (LEDESMA, 1971; GONZALEZ-CANO, 1981), parasitoids (BUXTON, 1990; TARASCO, 1995; SCHMIDT *et al.*, 1999; ZAMOUM *et al.*, 2007) and insectivorous birds, such as the hoopoe (*Upupa epops* L.) (BATTISTI *et al.*, 2000), might have a role in controlling PPM. Currently, its control relies on the use of microbiological agents, aimed primarily at controlling the larvae (TRIGGIANI & TARASCO, 2002; ER *et al.*, 2007). The use of alternative control methods has been confined to identifying efficient biocontrol agents (INCE *et al.*, 2008; HATICE *et al.*, 2009).

The current study analyses the presence of natural enemies of *T. pityocampa* pupae and prepupae in the Apulia region where PPM outbreaks are common (TRIGGIANI *et al.*, 1992). During a search for biological control agents of *T. pityocampa*, surveys of associated entomophagous and entomopathogenic microorganisms were made on prepupae and pupae in infested pinewoods and the most frequent and common ones were investigated in detail.

The first aim of this investigation was to determine the species involved and their importance for the control of the PPM in Apulia region. The second objective of this research was to provide bioethological observations on these natural enemies of *T. pityocampa* pupae with special regard to *Coelichneumon rudis* Fonsc. (Hymenoptera: Ichneumonidae), one of its less known endoparasite.

MATERIALS AND METHODS

More than 7,000 samples of prepupae and pupae of *T. pityocampa* were collected during the period May-June 2008-2011 from the soil in the following pinewoods in Apulia region (Fig. 1): «I Monti», near the municipality of Pietra Montecorvino (Foggia), «Pulicchie» and «Ovile del Castello» near the town of Gravina in Puglia and «Acquatetta» close to Minervino Murge, a town located in the Bari district. In «Monte Scapponi» a pinewood near Mattinata town (Foggia district), «Macchie delle Caselle» near Taranto city and «Montecamplo» near Laterza town (Taranto district). The pickings were carried out only during the period 2008-2010 because the infestation of *T. pityocampa* dropped drastically in 2010-2011. In «San Domino» (belonging to the Tremiti Islands, in the Foggia district) only one sample of 1,180 pupae was performed in June 2010. All the pinewoods examined are characterized by *Pinus halepensis* Milleralone or associated with *P. nigra* Arnold and *P. radiata* D. Don. The populations of *T. pityocampa* were in the declining phase of gradation in all the surveyed sites except the pine forests of S. Domino and Macchia delle Caselle.

Every year 50% of the pupae and all the prepupae of each sample were dissected to check the percentage of

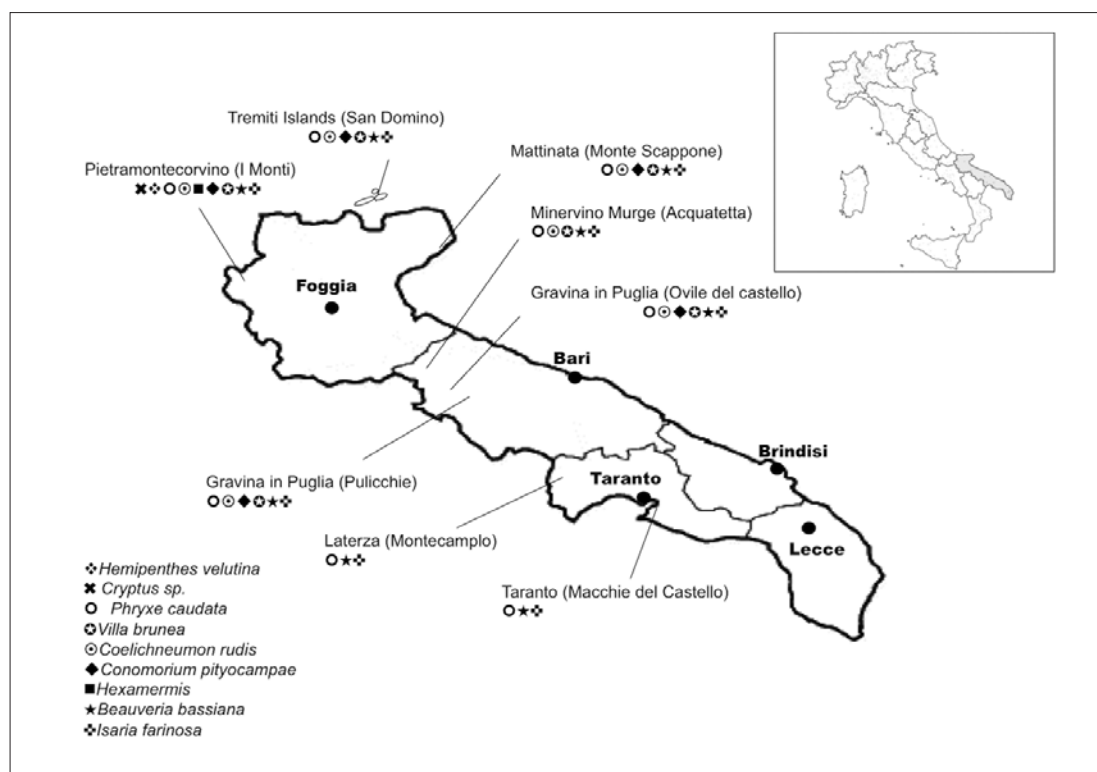


Fig. I – Pine woods of Apulia Region in which the observation were pointed out and natural enemies found.

parasitism while the remaining pupae were kept separately in test tubes at outdoor temperature. The members and the species of parasitoids were determined and adults of *Coelichneumon rudis* were reared and let reproduce in laboratory. In order to observe the behaviour of the *C. rudis*, separate groups formed by 3-5 fertilized females and 3-5 virgins were kept in the presence of 30 pupae of *T. pityocampa* set in 1-cm thick circular transparent chambers of 24 cm in diameter and covered with a 8-10 cm layer of soil. The adults were fed utilizing a solution of honey (10%) in water.

RESULTS

During the research period prepupae and pupae of *T. pityocampa* proved to be affected by 8 parasitoid and hyperparasite organisms, as summarized in Table 1 and in Fig. I.

DISCUSSIONS AND CONCLUSIONS

Though single natural enemies did not considerably affect pupae, their combined action on the Apulian pinewoods was undoubtedly effective with parasitization rates in excess of 60%.

Hexameris sp. (Nematoda: Mermithidae) (Fig. II, 1) was one of the most uncommon parasites which emerged from the pupae and prepupae of the *T. pityocampa*. During the samplings, dead or dying prepupae in completely formed cocoons were found in the area of Pietra Montecorvino. The 7% percent of them were parasitized by *Hexameris* sp. and contained 1-4 juveniles stages of the nematode. Although *Hexameris* sp. has been found

infesting several insects (RUIZ-NÁJERA *et al.*, 2013), including forest species as *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae) and *Diloboderus abderus* Sturm (Coleoptera: Scarabaeidae) (NIKDEL *et al.*, 2008; ACHINELLY & CAMINO 2008), this represents the first finding of this genus in *Thaumetopoea pityocampa*.

Phryxe caudata Rond. (Diptera: Tachinidae) (Fig. II, 2) is a well known endoparasite of *T. pityocampa* larvae (BILIOTTI, 1956) which completes its second generation at the expense of *T. pityocampa* pupae; it emerged from the pupae during August and September. It was always present in all examined biotopes but it never caused severe mortality events. The rate of parasitization was normally below 20%. Only one *P. caudata* adult usually emerged from each pupae, infrequently two or three.

Conomorium pityocampae Graham (Hymenoptera: Pteromalidae) (Fig. II, 3) was previously described as *Conomorium patulum* (Walker) and rediscrised by Graham as *C. pityocampae* (1992). A gregarious pupal endoparasite of *T. pityocampa* and *T. wilkinsoni* Tams, it was sporadically present and never exceeded 17.2% of parasitization. In some cases it also developed as a hyperparasite of *Coelichneumon rudis* (Fonsc.).

So far only one male and probably one larva of *Cryptus* sp. (Hymenoptera : Ichneumonidae) was found in May 2011 from pupae collected in Pietra Montecorvino (Fig. II, 4). It can be considered the first known case in which it presented as a parasite of *T. pityocampa*.

Coelichneumon rudis (Boyer de Fonscolombe) (Hymenoptera: Ichneumonidae) was detected by BOYER DE FONSCOLOMBE (1847) in France, in almost all central and Southern Europe and in some African and Middle Eastern Countries of the Mediterranean basin (BERTHOUMIEU, 1894 and 1925; SMITS VAN BURGST, 1918; SCMIEDEKNECHT, 1928; ANDROIC, 1956; COSTANTINIANU,

Table 1 – Percentage of natural enemies emerged from *T. pityocampae* eopupae and pupae.

Location	Collection date	<i>Hexameris</i>	<i>P. caudata</i>	<i>C. pityocampae</i>	<i>Cryptus</i> sp.	<i>C. rudis</i>	<i>V. brunnea</i>	<i>H. velutinus</i>	<i>B. bassiana</i> <i>I. farinosa</i>	Total
Gravina in Puglia (BA)	May 2008	-	4.5	-	-	-	-	-	-	4.5
Pulicchie	May 2009	-	3.2	-	-	-	-	-	-	3.2
	May 2010	-	4.8	0.8	-	9.2	-	-	3.6	18.4
	June 2011	-	5.3	17.2	-	23.3	-	-	-	45.8
Gravina in Puglia (BA)	May 2008	-	0.5	-	-	-	-	-	-	0.5
Ovile del Castello	May 2009	-	4.9	-	-	15.1	-	-	-	20.0
	May 2010	-	0.5	2.4	-	8.7	-	-	-	11.9
	June 2011	-	2.4	8.9	-	9.3	-	-	9.3	29.9
Minervino Murge (BA)	May 2008	-	1.3	-	-	1.3	-	-	-	2.6
Acquatetta	May 2009	-	3.7	-	-	-	-	-	-	3.7
	May 2010	-	4.5	1.4	-	-	-	-	3.1	9.0
	June 2011	-	-	-	-	2.8	-	-	-	2.8
Mattinata (FG)										
Monte Scappone	May 2008	-	5.5	1.1	-	5.1	0.7	-	-	15.4
Pietra Montecorvino (FG)	May 2008	-	18.9	-	-	10.7	-	-	-	29.6
I Monti	May 2009	-	5.0	0.3	-	-	-	-	-	5.3
	May 2010	7	4.2	1.3	-	16.0	6.6	-	15.8	50.9
	June 2011	-	0.9	11.0	0.1	38.6	-	1.1	11.0	61.7
Tremi Islands (FG)										
San Domino	June 2011	-	6.2	2.5	-	8.0	16.0	-	11.0	43.7
Taranto (TA)										
Macchie delle Caselle	May 2008	-	0.7	-	-	-	-	-	-	0.7
Laterza (TA)										
Montecamplo	May 2008	-	0.5	-	-	-	-	-	-	0.5

1959; RASNITSYN & SIJMAN, 1981; AUBERT *et al.*, 1984). *Coelichneumon rudis* was also found in the Northern Italy, in the Eastern Alps, by Battisti (1985), and in Southern Italy, in Apulia Region (TRIGGIANI *et al.*, 1992; TARASCO, 1995) and in Calabria Region (BONSIGNORE *et al.*, 2011). In Apulia the *Coelichneumon rudis* presence varied from year to year and, in some pinewoods, it was completely absent during the whole observation period. The samples collected in «Macchie delle Caselle» and «Montecamplo» exhibited a very low presence of natural enemies and no *C. rudis* was found in pupae or caught in the traps-tests. The *Coelichneumon rudis* presence was confirmed at «Acquatetta», «Ovile del Castello», «Pulicchie», «Mattinata» and «Pietra Montecorvino», where the level of parasitization reached 38.6% in 2010 (table 1). In the

field the first adults of *C. rudis* were collected in the trap-tests from the beginning of May to the first days of June at the «Pulicchie» and «Ovile del Castello» pinewoods at 500-575 m a.s.l., and from about mid-May to the last ten days of June at «Pietra Montecorvino» (700-750 m a.s.l.). Observations carried out in laboratory (TARASCO, 1995) showed that both virgin and fertilized females start to lay eggs into pupae (Fig. II, 5) one or two days after emerging from the pupa in which they developed. At room temperature the eggs hatch after 24-36 hours and the larva reaches the fifth stage after 7-10 days. Summer and winter are spent as last-instar larvae that pupate at the beginning of spring. Females start to mate few hours after birth preferring 2-3 day older males and rejecting coeval males. They match only once and then strongly refuse other

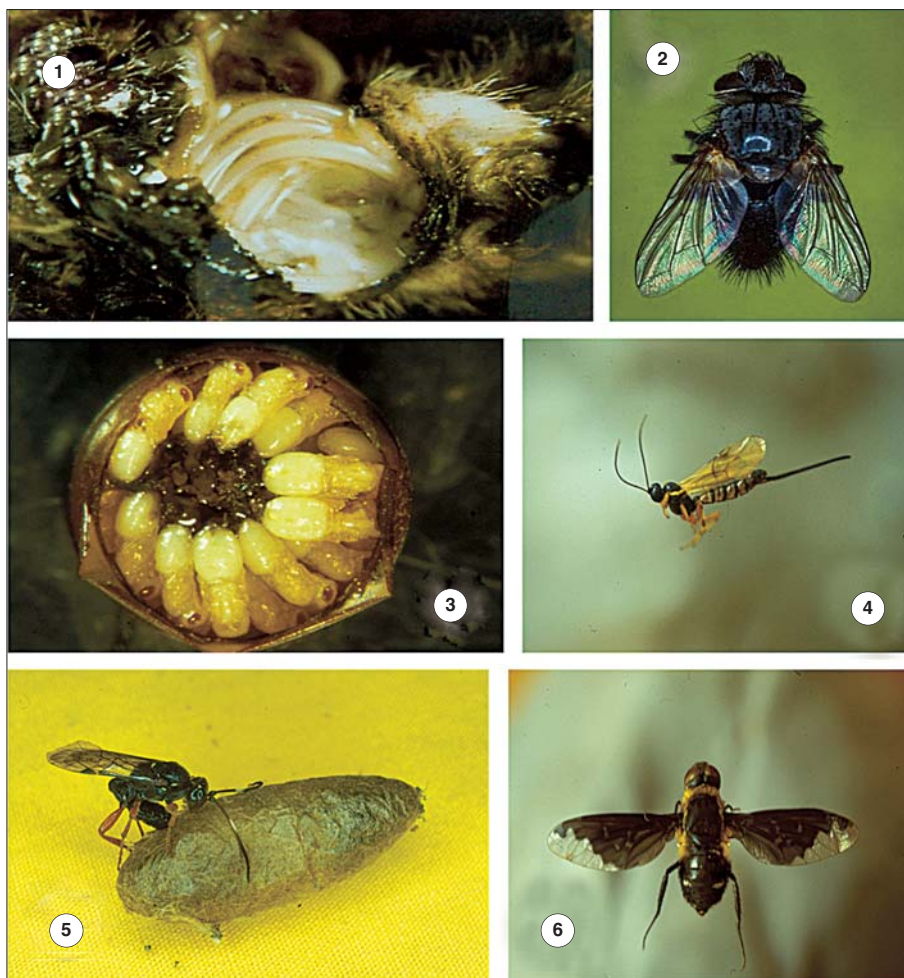


Fig. II – Natural enemies emerged from *T. pityocampa* pupae and eopupae 1) *Hexameris* sp.; 2) *P. caudata*; 3) *C. pityocampa*; 4) *Cryptus* sp., female; 5) *C. rudis*, female; 6) *H. velutina*.

males. One or two days later, the females approach the *T. pityocampa* pupae, tear the cocoon with their mandibles making a 2-4 mm hole and, once they ensure that the pupae are viable by means of their antennae, they start to lay their eggs in the pupa. Only one *C. rudis* larva is able to reach maturity even if more eggs are laid: the strongest larva rapidly kills the others. Under laboratory conditions, in a thermostat chamber at 24 °C and with a humidity at 75%, *C. rudis* females lived 18 days and males 22 days on average. By computing the eggs laid and those present in the oocytes of a single female it is possible to assume that each female can lay from 32 to 80 eggs during her life. This species is characterized by protandry and arrhenotokous parthenogenesis: only males emerge from the eggs laid by virgin females while males and females emerge from the eggs of fertilized females.

Villa brunnea Beck. (Diptera: Bombyliidae) is an uncommon endoparasite that parasitized the pupae only at «Mattinata» and «Pietra Montecorvino» in 2010 and 2011 respectively, but it never exceeded 6.6%. *Villa brunnea* Beck was more numerous at S. Domino than in other pinewoods reaching a 16% of parasitization and it was previously found in association with *T. pityocampa* by BATTISTI *et al.*, (2000) and BONSIGNORE *et al.* (2011).

Only one sample of the hyperparasite *Hemipenthes velutina* (Meigen) (Diptera: Bombyliidae) (Fig. II, 6) was obtained in July 2011 from a pupa of *T. pityocampa*

parasitized by *C. rudis* and collected at the end of January 2011 at «Pietra Montecorvino». It is widespread in Europe, North Africa and Asia (BIRTELE, 2007) and in Italy it was found on the mainland, in Sicily and Sardinia (CONTINI *et al.*, 1995; EVENHUIS & GREATHEAD, 1999; GREATHEAD, 2004). This is its first record in association with *T. pityocampa*.

Considering the entomopathogenic fungi, *Metarhizium anisopliae* (Metch.) Sorokin, *Lecanicillium lecanii* (Zimm.) Zare & W.Gams (2001) and *Beauveria bassiana* (Balsamo) Vuillemin were reported as fungal pathogens on *T. pityocampa* by several authors (BILIOTTI, 1959; VEEN, 1968; PAPARATTI & FABOZZI, 1988; VARGAS-OSUNA *et al.*, 1994) and studies about their effectiveness in controlling *T. pityocampa* were performed in laboratory conditions (ER *et al.*, 2007). During our survey, the most common species isolated from *T. pityocampa* pupae were *Beauveria bassiana* (Vuill) Balsam. and *Isaria farinosa* (Ascomycota: Hypocreales). Their action in reducing *T. pityocampa* populations was ineffective. The percentage of parasitization was about 10% for *B. bassiana* and 4% for *I. farinosa*. However, their action, though at a low level, also affected larvae and pupae of *C. rudis*.

Among the biotopes examined, the «I Monti» pinewood in «Pietra Montecorvino» exhibited the greatest rate of parasitization (61.7% in 2011) and the broadest range of natural enemy species of *T. pityocampa*. Also, this is the

first time that the hyperparasite *H. velutina* as well as *Cryptus* sp. and *Hexameris* sp., were observed to be pupal parasitoids of *T. pityocampa* and the action of *C. rudis* was also of interest. In the biotopes where *C. rudis* was detected, it exhibited higher parasitization than the other natural enemies, thus showing its leading role in the natural control of *T. pityocampa* pupae. Although the single low parasitization level, the complex of these natural enemies may be considered as a consistent contributor to natural mortality of *T. pityocampa*.

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