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Parasitic wasps related to *Prays oleae* (Bernard, 1788) (Lepidoptera, Praydidae) in olive orchards in Greece

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Abstract

The olive moth, *Prays oleae* (Bernard, 1788) (Lepidoptera: Praydidae) is categorised among the most devastating insect pests of olives, whose anthophagous and carpophagous generations can cause yield loss up to 581 and 846 kg of fruit per ha, respectively. In this study, results of the captured parasitoids in olive tree (*Olea europaea* Linnaeus, 1753) orchards, or infested olive plant material in Crete, Greece, is presented. Five of the six identified species captured in trap devices are related to *P. oleae*, i.e., *Chelonus elaeaphilus* Silvestri, 1908, *Chelonus pellucens* (Nees, 1816), *Apanteles xanthostigma* (Haliday, 1834), *Diadegma armillatum* (Gravenhorst, 1829), and *Exochus lentipes* Gravenhorst, 1829. The species *Eupelmus urozonus* Dalman, 1820 and *Pnigalio mediterraneus* Ferrière & Delucchi, 1957 were reared from infested *P. oleae* leaves. *Chelonus pellucens* is reported for the first time from Greece. According to the international literature, 59 hymenopterous and dipterous parasitoid species are associated with *P. oleae* in Europe.

Keywords

Diptera, Greece, Hymenoptera, parasitoids, *Prays oleae*

Introduction

Olive trees growing has been traditionally localised in the Mediterranean Basin for thousands of years, where almost 97.9% of the cultivated areas are located (Rallo et al. 2018). The list of potentially harmful organisms includes more than 255 species and the losses due to insect pests alone are estimated to be approximately 15% of production (Haniotakis 2003). Among them, the most common species are the olive fruit fly, *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae), the olive moth, *Prays oleae* (Bernard, 1788) (Lepidoptera: Praydidae), and the Mediterranean black scale, *Saissetia oleae* (Olivier, 1791) (Hemiptera: Coccidae) (Haniotakis 2003).

Prays oleae is one of the main pests infesting olives of commercial production, since larvae of the first, second, and third generations attack flowers, fruits, and leaves, respectively (Kavallieratos et al. 2005; Nave et al. 2017). The anthophagous generation can cause yield losses up to 581 kg of fruit per ha and the corresponding carpophagous up to 846 kg per ha, an issue that justifies the imposed control measures (Bento et al. 2001). In recent years, high socioeconomic pressures have forced olive growers to develop alternative control strategies in an effort to mitigate the undesirable side effects of pesticides on trophic chains and biological balances (Nave et al. 2017). In this sense, not only the economic losses due to the pest should be evaluated, but also the possible secondary effects that such control measures can have on beneficial fauna (Ramos et al. 1998).

Previous research has revealed a wide parasitoid spectrum that is related to *P. oleae*, resulting to biological control efforts against this pest. The first parasitoid used in biocontrol program was *Trichogramma embryophagum* (Hartig, 1838) (Hymenoptera: Trichogrammatidae) in former Yugoslavia (Brnetić 1988). In Spain, three species have been released against *P. oleae* with various levels of success; i.e., *Chelonus elaeaphilus* Silvestri, 1908 (Hymenoptera: Braconidae), the specialised *Ageniaspis fuscicollis* (Dalman, 1820) var. *praysincola* Silvestri, 1907 (Hymenoptera: Encyrtidae) and *T. embryophagum* (Civantos and Caballero 1993). *Trichogramma cacaeciae* Marchal, 1927 (Hymenoptera: Trichogrammatidae) has been utilised in Portugal (Bento et al. 1998) and *Trichogramma evanescens* Westwood, 1833 in Egypt (Agamy 2010).

Although there are previous records concerning the occurrence of *P. oleae* parasitoids in Greece, there are no data available from the island of Crete, the most important olive production area with almost 200,000 ha cultivated with olive trees (i.e., nearly 25% of the total island area is covered with olive plantations; Hellenic Statistical Authority 2014). Given that the knowledge of the beneficial entomofauna of the olive crop is clearly linked with the biological control of pests infesting this crop and that indigenous strains of parasitoids occurring in olive groves can be more effective against certain olive pests than the commercially available parasitoids (Herz and Hassan 2006), the objective of this study was to further investigate the parasitoid complex that is associated with *P. oleae* in the overlooked area of Crete by using trap devices and collecting plant material.

Materials and methods

All parasitoids were collected in olive orchards from the island of Crete, Greece from June to October 2017. A part of the material was captured in five glass McPhail trap devices, installed from June to October in an olive orchard at Messara (Crete) that covers an area of approx. 0.5 ha baited with 200 ml aqueous solution of 2% hydrolysed protein (Entomela 75 SL, 25% w/w urea; BASF Hellas, Amaroussion, Greece). Each trap device was placed with its lower part at a height of 2 m from the ground. The distances among trap devices were approx. 100 m. The solution was replaced every week. Additional specimens were reared from *P. oleae* infested plant material (*O. europaea* var. *koroneiki*). Infested leaves by *P. oleae* larvae were collected from olive trees, separately transferred into plastic vials covered with mesh, and transferred to the laboratory. Vials were maintained at 25 °C and 60% relative humidity and inspected daily for emergence of parasitoids. All parasitoid individuals, either from trap devices or plant material, were preserved in 96% alcohol. Specimens were dissected and slide mounted in Berlese medium. The identification of the captured and reared specimens was conducted under a Nikon SM2 745T binocular stereomicroscope (Nikon CEE GmbH, Wien, Austria) or an Olympus SZX9 (Olympus Corporation, Tokyo, Japan) using appropriate keys (Tobias et al. 1986; Askew and Nieves Aldrey 2000; Tolkanitz 2007; Broad 2011). Part of the specimens was deposited in the insect collection of the Laboratory of Agricultural Zoology of Entomology, Agricultural University of Athens, Greece, and a part was deposited in the insect collection of the Faculty of Sciences and Mathematics, Department of Biology and Ecology, University of Niš, Serbia.

Additional to field research, we critically reviewed all recorded parasitoids of *P. oleae* in Greece and Europe indicating the pest's stage they attack. The synonymy among taxa was checked and adopted according to online databases (van Achterberg 2013; Fernandez Triana and Ward 2015; Noyes 2017; Tschorsnig 2017), and the database provided by Yu et al. (2012).

Results

In total, five out of six species captured in McPhail trap devices are related to *P. oleae*, i.e., *C. elaeaphilus*, *Chelonus pellucens* (Nees, 1816) (Hymenoptera: Braconidae), *Apanteles xanthostigma* (Haliday, 1834) (Hymenoptera: Braconidae), *Diadegma armillatum* (Gravenhorst, 1829) (Hymenoptera: Ichneumonidae), and *Exochus lentipes* Gravenhorst, 1829 (Hymenoptera: Ichneumonidae), while two species were reared from *P. oleae* infested olive leaves.

The exhaustive investigation of the international literature revealed 59 hymenopterous and dipterous parasitoid species that attack *P. oleae* in Europe; 14 Braconidae, 2 Chalcididae, 1 Encyrtidae, 20 Eulophidae, 1 Eupelmidae, 7 Icheumonidae, 1 Platygastridae, 3 Pteromalidae, 2 Tachinidae, and 8 Trichogrammatidae (Table 1). Thirty-one out of

Table 1. Parasitoids of *Prays oleae* recorded in Europe and their presence in Greece: (+) recorded, (-) not recorded.

Family	Species	Source of host record	Host stage attacked	Recorded or not in Greece
Braconidae	*Aleiodes circumscriptus* (Nees, 1834)	Beyarslan (2015)	larva	+
	Aleiodes gastritor (Thunberg, 1822)	Halperin (1986)	larva	+
	Apanteles xanthostigma (Haliday, 1834)	Nave et al. (2016)	larva	+
	Bracon hebetor Say, 1836	Falcó et al. (1993)	larva	+
	Bracon laetus (Wesmael, 1838)	Aubert (1966)	larva	+
	Bracon crassicornis Thomson, 1892	Silvestri (1908)	larva	+
	Chelonus (*Microchelonus*) *elaeaphilus* Silvestri, 1908	Nave et al. (2016)	larva	+
	Chelonus (Microchelonus) silvestrii (Papp, 1999)	Papp (1999)	larva	-
	Chelonus (*Parachelonus) pellucens* (Nees, 1816)	Texeira et al. (2000)	larva	-
	Clinocentrus testaceus (Kriechbaumer, 1894)	Texeira et al. (2000)	larva	-
	Dolichogenidea dilecta (Haliday, 1834)	Telenga (1955)	larva	-
	Dolichogenidea ultor (Reinhard, 1880)	Arambourg (1969)	larva	-
	Meteorus rubens (Nees, 1811)	Texeira et al. (2000)	larva	+
	Phanerotoma dentata (Panzer, 1805)	Texeira et al. (2000)	larva	+
Chalcididae	*Hockeria bifasciata* Walker, 1834	Madl (2008)	larva	-
	Hockeria unicolor Walker, 1834	Stavraki (1977)	larva	+
Encyrtidae	*Ageniaspis fuscicollis* (Dalman, 1820) var. *praysincola* Silvestri, 1907	Nave et al. (2016)	larva	+
Eulophidae	*Asecodes erxias* (Walker, 1848)	Silvestri (1908)	larva	+
	Baryscapus nigroviolaceus (Nees, 1834)	Noyes (2017)	larva	-
	Chrysocharis gemma (Walker, 1839)	Noyes (2017)	larva	+
	Chrysocharis nephereus (Walker, 1839)	Noyes (2017)	larva	+
	Cirrospilus elongatus Boucek, 1959	Noyes (2017)	larva	-
	Dicladocerus westwoodii Westwood, 1832	Ramos and Panis (1975)	larva	+
	Elasmus arcuatus Ferrière, 1947	Ferrière (1947)	larva	-
	Elasmus flabellatus (Fonscolombe, 1832)	Nave et al. (2016)	larva	+
	Elasmus masii Ferrière, 1929	Anonymous (2006)	larva	-
	Elasmus nudus (Nees, 1834)	Ramos and Panis (1975)	larva	-
	Elasmus steffani Viggiani, 1967	Redolfi and Campos (2010)	larva	+
	Elasmus westwoodi Giraud, 1856	Noyes (2017)	larva	+
	Euderus albitarsis (Zetterstedt, 1838)	Nave et al. (2016)	larva	-
	Hemiptarsenus unguicellus (Zetterstedt, 1838)	Noyes (2017)	larva	-
	Pediobius bruchicida (Rondani, 1872)	Bouček (1974)	larva/ pupa	+
	Pnigalio agraules (Walker, 1839)	Nave et al. (2016)	larva/ pupa	+
	Pnigalio epilobii Bouchek, 1966	Stavraki (1970)	larva/ pupa	+
	Pnigalio longulus (Zetterstedt, 1838)	Stavraki (1970)	larva/ pupa	+
	Pnigalio mediterraneus Ferrière & Delucchi, 1957	Stavraki (1970)	larva/ pupa	+
	Pnigalio pectinicornis (Linnaeus, 1758)	Ramos and Panis (1975)	larva/ pupa	+
Eupelmidae	*Eupelmus urozonus* Dalman, 1820	Noyes (2017)	larva	+
Ichneumonidae	*Diadegma armillatum* (Gravenhorst, 1829)	Bento et al. (1998)	larva/ pupa	+

Family	Species	Source of host record	Host stage attacked	Recorded or not in Greece
Ichneumonidae	*Diadegma semiclausum* (Hellén, 1949)	Torres (2010)	larva/ pupa	+
	Exochus lentipes Gravenhorst, 1829	Texeira et al. (2000)	larva	-
	Himertosoma superbum Schmiedeknecht, 1900	Vidal (1997)	larva/ pupa	-
	Itoplectis alternans (Gravenhorst, 1829)	Silvestri (1908)	larva/ pupa	-
	Lissonota superbator Aubert, 1967	Aubert (1969)	larva	+
	Scambus elegans (Woldstedt, 1877)	Nave et al. (2017)	larva	-
Platygastridae	*Platygaster apicalis* Thomson, 1859	Stavraki (1970)	larva	+
Pteromalidae	*Mesopolobus mediterraneus* (Mayr, 1903)	Bozbuğa and Elekçioğlu (2008)	pupa	-
	Pteromalus chrysos Walker, 1836	Noyes (2017)	pupa	-
	Pteromalus semotus Walker, 1834	Noyes (2017)	pupa	-
Tachinidae	*Phytomyptera nigrina* (Meigen, 1824)	Kara and Tschorsnig (2003)	larva	-
	Phytomyptera vaccinii Sintenis, 1897	Tschorsnig (2017)	larva	-
Trichogrammatidae	*Trichogramma bourarachae* Pintureau & Babault, 1988	Polaszek (2009)	egg	-
	Trichogramma brassicae Bezdenko, 1968	Polaszek (2009)	egg	-
	Trichogramma cordubensis Vargas & Cabello, 1985	Jardak (1980)	egg	-
	Trichogramma dendrolimi Matsumura, 1926	Polaszek (2009)	egg	-
	Trichogramma euproctidis (Girault, 1911)	Pereira et al. (2004)	egg	+
	Trichogramma minutum Riley, 1871	Stavraki (1985)	egg	-
	Trichogramma oleae Voegele & Pointel, 1979	Polaszek (2009)	egg	+
	Trichogramma pretiosum Riley, 1879	Polaszek (2009)	egg	-

these 59 parasitoid species have been recorded in Greece: 9 Braconidae, 1 Chalcididae, 1 Encyrtidae, 13 Eulophidae, 1 Eupelmidae, 3 Icheumonidae, 1 Platygastridae, and 2 Trichogrammatidae. All Braconidae, Chalcididae, Encyrtidae, Eupelmidae, Platygastridae, and Tachinidae which are parasitoids of *P. oleae* attack only larvae. All eulophids parasitise larvae of *P. oleae* while some of them attack both larvae and pupae. Three icheumonids parasitise larvae exclusively and four both larvae and pupae. All pteromalids are pupal parasitoids whilst all trichogrammatids are egg parasitoids.

Family Braconidae

***Apanteles xanthostigma* (Haliday, 1834)**
Material examined: 11 ♀, Messara (Crete) (35°2'20"N, 24°50'54"E), 16–23.06.2017, captured in McPhail trap device.

***Chelonus* (*Microchelonus*) *elaeaphilus* (Silvestri, 1907)**
Material examined: 4 ♀, 4 ♂, Messara (Crete) (35°2'20"N, 24°50'54"E), 09–16.06.2017, captured in McPhail trap device.

***Chelonus* (*Parachelonus*) *pellucens* (Nees, 1816)**
Material examined: 6 ♀, Messara (Crete) (35°2'20"N, 24°50'54"E), 09–16.06.2017, captured in McPhail trap device.

***Glyptapanteles vitripennis* (Curtis, 1830)**
Material examined: 2 ♀, 7 ♂, Messara (Crete) (35°2'20"N, 24°50'54"E), 23–30.06.2017, captured in McPhail trap device.

Family Eulophidae

***Pnigalio mediterraneus* Ferrière & Delucchi, 1957**
Material examined: 12 ♂, Heraklion, Voutes, (Crete) (35°15'54"N, 25°03'26"E), 15.03.2017 (date of host collection). Host: *Prays oleae* on *Olea europaea* var. *koroneiki*.

Family Eupelmidae

***Eupelmus urozonus* Dalman, 1820**
Material examined: 8 ♀, 12 ♂, Heraklion, Voutes, (Crete) (35°15'54"N, 25°03'26"E), 15.03.2017 (date of host collection). Host: *Prays oleae* on *Olea europaea* var. *koroneiki*.

Family Ichneumonidae

***Diadegma armillatum* (Gravenhorst, 1829)**
Material examined: 3 ♀, 5 ♂, Messara (Crete) (35°2'20"N, 24°50'54"E), 16–23.08.2017, captured in McPhail trap device.

***Exochus lentipes* Gravenhorst, 1829**
Material examined: 6 ♀, 8 ♂, Messara (Crete) (35°2'20"N, 24°50'54"E), 16–23.09.2017, captured in McPhail trap device.

Discussion

Microgastrinae is one of the largest subfamilies of Braconidae with about 2,000 described species worldwide (Pérez Rodríguez et al. 2013). Very recently, the hymenopteran parasitoid complex of *P. oleae* was studied in Portugal where, among the 22 recorded parasitoid taxa, *A. xanthostigma* was the major natural enemy (Nave et al. 2017). Furthermore, in Egypt *A. xanthostigma* was found to parasitise the larval stage of *P. oleae* at a rate of more than 50% (Herz et al. 2005). Apart from *P. oleae*, this parasitoid species parasitises a high number of microlepidopterous species, mainly Tortricidae, Gracillariidae, and Yponomeutidae, particularly the genera *Paraswammerdamia* Friese, 1960 and *Swammerdamia* Hübner, 1825 (Yu et al. 2012). *Glyptapanteles* Ash-

mead, 1904 is a genus with about 200 species in Palaearctic and Nearctic regions and, like all Microgastrinae, are koinobiont endoparasitoids of lepidopteran larvae (Penteado Dias et al. 2011). *Glyptapanteles vitripennis* was first reported in southern Greece in 1978 (Papp 2007) without further records since then. This parasitoid species was the second most abundant recovered from Malaise traps placed in the Artikutza forest of Pyrenees (Spain) (Pérez Rodríguez et al. 2013) while it is also known that it attacks *Yponomeuta malinellus* (Zeller, 1838) (Lepidoptera: Yponomeutidae) (Velcheva et al. 2012). Given that this species parasitises numerous other lepidopterous species belonging to Geometridae, Noctuidae, Plutellidae, and Tortricidae (Nixon 1973), it could be a good candidate for biological control purposes. Whether *G. vitripennis* parasitises *P. oleae*, it remains to be confirmed with additional field efforts.

The subfamily Cheloninae is formed by more than 1,300 species belonging to 15 genera, thus constituting a quite large part of Braconidae (Kittel and Austin 2014). They oviposit into eggs and larvae of various lepidopterous species, a fact that makes them valuable potential biocontrol agents (Inayatullah and Naeem 2004; Walker and Huddleston 1987; Edmardash et al. 2011). The subgenus *Microchelonus* Szepligeti, 1908 is even considered as a valid genus, following the standpoints of Papp (2014a, b). The genus *Chelonus* Panzer, 1806 counts 601 species in the Holarctic region (Papp 2014c) with *M. elaeaphilus* being known in the Mediterranean region, either as *M. elaeaphilus* or *C. elaeaphilus* (Papp 2012; Nave et al. 2017). This species has been introduced and established in Greece from France (Yamvrias 1998). On the other hand, *C. pellucens* has a wider European distribution than *M. elaeaphilus* (van Achterberg 2013). *Chelonus pellucens* is reported for the first time from Greece and although *C. elaeaphilus* parasitises *P. oleae* (Bento et al. 1998), there are no relevant records for *C. pellucens*, an issue that merits further investigation.

Although Eupelmidae is a relatively small family with approximately 1000 species, the genus *Eupelmus* Dalman, 1820 is a large taxon containing more than 300 species (Gibson and Fusu 2016) whilst Eulophidae is one of the largest families within chalcidoid wasps, with almost 5,000 species (Aguiar et al. 2013). The genus *Pnigalio* Schrank, 1802 is comprised by 61 valid species (Li et al. 2017). Several hosts of *Pnigalio mediterraneus* Ferrière & Delucchi, 1957 (Hymenoptera: Eulophidae) are major pests of plants of ornamental and agricultural importance belonging to different orders, such as *B. oleae*, *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae), and *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera: Gracillariidae) (Gebiola et al. 2009). Both *Eupelmus urozonus* Dalman, 1820 (Hymenoptera: Eupelmidae) and *P. mediterraneus* were found in the Greek island of Corfu as primary parasitoids of *B. oleae* (Kapatos and Fletcher 1986). Based on our results, these species are also parasitoids of *P. oleae* that occur in Greece since they were recorded from infested olive leaves.

The genus *Diadegma* Förster, 1869 constitutes a large group of Ichneumonid wasps with more than 200 known species worldwide (Wagener et al. 2006). *Diadegma armillatum* is a known parasitoid of various lepidopterous species (Velcheva et al. 2012; Fernandez Triana et al. 2014) that has been recently recorded attacking *P. oleae* larvae (Nave et al. 2017). The genus *Exochus* Gravenhorst, 1829 is the largest group of

Metopiinae including the widely distributed in Europe, *E. lentipes* that attacks various Tortricidae and Gelechiidae larvae (Yu et al. 2012).

Our original findings on associated parasitoids of *P. oleae* and the compiled information revealed could trigger further studies that deal with the management of this noxious insect species in the target area from a biological control point of view. The identified parasitoid spectrum was broad, despite the short interval of obtaining the data, indicating a potential positive impact of natural enemies to *P. oleae*, an issue however that merits further field efforts. Last but not least, given that *C. pellucens* is identified as a new member of the entomofauna of Greece during the present first attempt to record the beneficial parasitoids in olive orchards in Crete, we may expect that additional parasitoid species may occur in this agroecosystem.

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