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Parasitoid complex of *Phyllonorycter corylifoliella* (Hübner) (Lepidoptera: Gracillariidae) in Fars Province of Iran, with six new species records

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Abstract

The Hawthorn red midget moth, *Phyllonorycter corylifoliella* (HÜBNER) (Lepidoptera: Gracillariidae) is one of the important pests of rosaceous trees in Iran. In this study, parasitoid complex of *P. corylifoliella* were investigated on apple, *Malus domestica* BORKH. (Golden delicious cultivar) during 2006 and 2007 in the Fars province of Iran. As a result of this study, ten parasitoid species of *P. corylifoliella* were reared in the growing season: *Achrysocharoides suprafolius* (ASKEW & RUSE), *Neochrysocharis longiventris* (ASKEW), *Zagrammosoma talitzkii* (BOUCEK), *Sympiesis gordius* (WALKER), *Sympiesis acalle* (WALKER), *Sympiesis sericeicornis* (NEES), *Pnigalio agraules* (WALKER), *Minotetrastichus frontalis* (NEES), *Baryscapus* sp. (Hym., Eulophidae) and *Pholetesor bicolor* (NEES) (Hym., Braconidae). Among them, six species (*A. suprafolius*, *Z. talitzkii*, *S. acalle*, *P. agraules*, *N. longiventris* and *M. frontalis*) are new records for the fauna of Iran. The most common parasitoid was *A. suprafolius* (60.2% in 2006 and 69.0% in 2007) followed by *Z. talitzkii*(14.2% in 2006 and 13.7% in 2007). The mean percentage of seasonal parasitism of *P. corylifoliella* was 11.4 ± 3.9% in 2006 and 13.5 ± 4.7% in 2007. This study revealed the species richness of the parasitoid complex of *P. corylifoliella* in Iran.

Keywords: *Malus domestica*, parasitoids, *Phyllonorychter corylifoliella*, Iran.

Introduction

The Hawthorn red midget moth, (HRMM), *Phyllonorycter corylifoliella* together with other related species such as *Phyllonorycter blancardella* (FABRICIUS), *P. turanica* (GERASIMOV) (Gracillariidae), *Leucoptera scitella* (Lyonetiidae), and *Stigmella malella* (STAINTON) (Nepticulidae) have become important pests of rosaceous trees in many apple-producing areas in Iran (RADJABI, 1986). Among the *Phyllonorycter* species living in fruit orchards, *P. blancardella* is reported to be one of the most widespread in the Holarctic region (CROSS *et al.*, 1999). Leafminers reduce capacity for photosynthesis, and damage is expressed as premature ripening and fruit drop. The HRMM was first reported from Iran in 1970 and gradually spread to across north, northwest and central regions (East Azerbaijan, Khorasan, Tehran, Markazi, Fars and Esfahan provinces) (MODARRES AVAL, 1997) and probably distributed in other parts of Iran (RADJABI, 1986). Development of resistance to insecticides, which are used to control of the other pest such as codling moth, *Cydia pomonella* (L.) (Tortricidae), is considered as the main reason for increase of leafminer moths populations (PREE *et al.*, 1986). Three leafminer species from the genus *Phyllonorycter* (e.g., *P. blancardella*, *P. corylifoliella* and *P. turanica*) are considered to be ecological homologue in terms of general phenology, ecological niche and type of damage (RADJABI, 1986). Of these, the larvae of HRMM and *P. turanica* make mines on the upper surface of leaves of the rosaceous trees. The first three instar larvae feed on the sap from the spongy mesophyll of the leaves and are referred as sap feeders and the last two instar larvae known as tissue feeders (MCGREGOR, 1997).

Several types of natural enemies attack the leafminer species of the genus *Phyllonorycter* and the other closely related species, although numerically the most important are hymenopterous parasitoids. Most species of the parasitoids that attack *Phyllonorycter* species belong to the family Eulophidae (SEFROVA, 2001; NAVONE, 2006; GATES *et al.*, 2002) and superfamily Ichneumonoidea (TOTH & LUKA, 2005). Several species of eulophid wasps are important in natural or applied biological control of lepidopterous, dipterous and hymenopterous leafminers throughout the world (BOUCEK & ASKEW, 1968). Even if many investigations on parasitoid complex of the genus *Phyllonorycter* have previously been carried out in different parts of the world, the majority of them are concentrated, however, on the spotted tentiform leafminer, *P. blancardella* (DIMIC, 1984; CASAS & BAUMYARTNER, 1990). *P. corylifoliella* is frequent in apple orchards in Europe but has not been reported as an important pest in the area (CROSS *et al.*, 1999). Previous studies on the leafminer moths have shown that chalcidoid wasps usually play an important role in regulating their populations, and the level of parasitism in some cases reach over 50% (ASKEW & SHAW, 1979; GIBOGINI *et al.*, 1996). The eulophid wasps sometimes have twice as many generations as leafminers (MAIER, 1994). Survey of the parasitoids of leafmining moths have been done in many countries and showed each leafmining species to be attacked by a complex often over than ten parasitoid species and some of these parasitoids have more

than one host (CROSS *et al.*, 1999). For example, the studies of TOMOV (2002) in Bulgaria showed that the dominant parasitoids of HRMM were *Achrysocharoides zwoelferi* (DELUCCHI), *A. altilis* (DELUCCHI) and *A. nivepies* (THOMSON). This author lists 22 parasitoid species, all of which reared from mines of HRMM.

Here, we report the results of a two-year survey of the hymenopterous parasitoids of HRMM in the Fars Province of Iran. This research is part of a larger study to identify parasitoid faunas throughout Iran for future use in biological control programs against HRMM.

Material and Methods

This survey was conducted in the Sarhad region (52°12'E and 30°43'N) located in the north of Fars province (Iran) during the period May to October 2006 and 2007. Samples of infested leaves were collected weekly in five apple orchards (Golden delicious variety) at five sites: Sedeh, Tange Boragh, Bande Bahram, Baseri, and Bakan, within 80 Km radius. On each sampling date, a number of 10 infested leaves from each of the 10 trees (i.e., 100 leaves in total) were randomly collected at each of five sample sites. Each of the collected leaves was indeed infested with one late-instar larva and pupa. The samples were placed into plastic bags and taken to the laboratory. Each sample of 100 infested leaves was placed in a plastic culture container (25 × 10 × 8 cm) and covered with fine-mesh nylon. The rearing boxes were maintained at room temperature (approximately 25 ± 5ºC) for at least two months. The rearing boxes were checked daily and the number of emerged moth and parasitoid adults were counted. Parasitoid adult specimens were preserved in 75% ethanol for future work. The parasitoid species were identified by authors of this paper (YEFREMOVA and NAVONE).

The percentage of parasitism of leafminer larvae and pupae was calculated based on the number of parasitoids divided by the total number of either moths or parasitoids emerged. The relative frequency (in %) was calculated based on the number of a particular parasitoid species divided by the number of total parasitoids.

The identified specimens were deposited in the collection of the Department of Entomology, Tarbiat Modares University, and some specimens in the Department of Zoology, Ul'yanovsk State University, and in the University of Torino.

Results

A total of ten hymenopterous parasitoid species were reared from HEMM in the studied area: *Achrysocharoides suprafolius* (ASKEW & RUSE), *Neochrysocharis longiventris* (ASKEW), *Zagrammosoma talitzkii* (BOUCEK), *Sympiesis gordius* (WALKER), *Sympiesis acalle* (WALKER), *Sympiesis sericeicornis* (NEES), *Pnigalio agraules* (WALKER), *Minotetrastichus frontalis* (NEES), *Baryscapus* sp. (Eulophidae) and *Pholetesor bicolor* (NEES) (Braconidae).

Table 1. Relative frequency of parasitoid complex of *Phyllonoeycter corylifoliella* in Fars province of Iran in 2006 and 2007.

Parasitoids	Number of parasitoid specimens		Relative frequency (%)	
	2006	2007	2006	2007
Achrysocharoides suprafolius (ASKEW & RUSE)	509	576	60.2	69.0
Zagrammosoma talitzkii (BOUCEK)	120	114	14.2	13.7
Sympiesis gordius (WALKER)	56	17	6.6	2.0
S. sericeicornis (NEES)	2	-	0.2	-
S. acalle (WALKER)	47	31	5.6	3.7
Neochrysocharis longiventris (ASKEW)	47	67	5.6	8.0
Pnigalio agraules (WALKER)	41	10	4.9	1.2
Baryscapus sp.	8	4	1.0	0.5
Minotetrastichus frontalis (NEES)	7	3	0.8	0.4
Pholetesor bicolor (NEES)	8	13	1.0	1.6

Among them six parasitoid species: *A. suprafolius, Z. talitzkii, S. acalle, P. agraules, N. longiventris*, and *M. frontalis* are new records for Iran.

The collected numbers and relative frequencies of the parasitoids are given in Table 1. In both years, *A. suprafolius* was the most common parasitoid species, the second common species being *Z. talitzkii*. The four parasitoids, *S. gordius*, *S. acalle*, *N. longiventris*, and *P. agraules*, were moderately abundant. *Baryscapus* sp., *M. frontalis*, S. *sericeicornis*, and *P. bicolor* occurred in small numbers and they apparently had little significance as regulating agents of HRMM in both years (Table 1). Most parasitoids emerged and were collected from mid July to early September. The species and abundance of the parasitoids vary between the different generations of the HRMM. Among parasitoid species, *S. sericeicornis* was not found in 2007. *S. gordius* and *S. acalle* were found in lower numbers in 2007 than in 2006, whereas *A. suprafolius* was more abundant in 2007 (Table 1).

The most important parasitoids in the first generation of HRMM were *A. suprafolius*, *Z. talitzkii*, *S. gordius*, *S. acalle*, and *P. agraules*. In the second generation that occurs in the summer the most important parasitoids were *A. suprafolius* and *Z. talitzkii*. Parasitoids of third generation were *N. longiventris*, *A. suprafolius, S. gordius*, *S. acalle* and *P. agraules*. Among parasitoids, *S. acalle* and *P. agraules* only emerged in the early and late growing season, whereas *S. gordius* was recorded throughout the growing season. *N. longiventris* was only collected in samples from the third generation. Its abundance represented more than 45% of collected wasps, however its parasitism rate was significantly lower than *A. suprafolius* and *Z. talitzkii*. Seasonal percentage of parasitism by *S. acalle* was obtained 1.4% in 2006 and 0.8% in 2007. We found the highest parasitism levels during August in both years, with peaks clearly reaching over 50% (Table 2). Mean total seasonal percentage of parasitism was obtained 11.4 ± 3.9% in 2006 and 13.5 ± 4.7% in 2007.

Table 2- Seasonal parasitism of *Phyllonoeycter corylifoliella* in Fars province of Iran in 2006 and 2007.

Sampling dates	Percentage of parasitism (Mean ± SE)	
	2006	2007
14 June	0.0	0.0
21 June	3.9 ± 0.9	4.1 ± 0.6
28 June	11.1 ± 1.1	12.2 ± 1.7
05 July	16.5 ± 3.1	21.1 ± 2.5
12 July	0.0	0.0
19 July	0.0	0.0
26 July	0.0	0.0
02 August	5.0 ± 1.2	16.7 ± 0.3
09 August	23.4 ± 4.1	38.3 ± 3.5
16 August	34.4 ± 9.9	61.5 ± 5.2
23 August	54.9 ± 6.2	43.3 ± 8.2
30 August	21.6 ± 1.2	13.8 ± 3.3
06 September	6.7 ± 1.1	5.5 ± 0.7
13 September	4.4 ± 0.0	0.1 ± 0.0
20 September	0.3 ± 0.0	0.0
27 September	0.0	0.0

Discussion

A majority of chalcidoid species that we collected are polyphagous and develop not only on leafmining moths but also on other leafminer insects such as Agromyzidae flies (ASKEW & SHAW, 1986; ASADI *et al.*, 2006; NOYES, 2007). Our findings showed that *A. suprafolius,* which is a new record for Iran, was the most abundant parasitoid of *P. corylifoliella* in the Fars province of Iran (Table 1). Most species of the genus *Achrysocharoides* are known as endoparasitoid wasps in the larvae of leafminer moths of the family Gracilariidae, mainly the genus *Phyllonorycter* (HANSSON, 1983). The eulophid wasps of this genus prefer the mine produced on upper surface of leaves (SLAVOV, 1980). According to NOYES (2007) *A. suprafolius* is a very common species in European countries (Germany, The Netherlands, England, and Scotland) but strictly associated with HRMM. The studies of KLADUBOWSKI & WLKANIEC (1982) in Poland showed that *P. blancardella* was parasitized by ten species of Hymenoptera. The dominant species were *Holcothorax testaceipes* (RATZEBURG) (Encyrtidae) and *A. circumscriptus* (Braconidae). In northeastern Spain, the dominant parasitoids of HRMM are *S. gordius*, *S. acalle* and *P. bicolor* (BELLOSTAS *et al.*, 1998). In eastern North America, the braconid wasp *Polestesor ornigis* (WEED) and the eulophid wasp *Sympiesis marylandensis* (GIRAULT) are the main parasitoids of *P. blancardella* (POTTINGER & LEROUX, 1971; RIDGWAY & MAHR, 1985). In western North America the eulophid wasps *Pnigalio flavipes* (ASHEMEAD) and *S. marylandensis* were reported as main parasitoids of *Phyllonorycter mespiella* (HUBNER) (VAREIA & WELTER, 1992; COSSENTINE & JENSEN,

1994). In Bulgaria, parasitoid complex of *P. blancardella* and *P. corylifoliella* was studied by IVANOV (1976). He found that the most important parasitoids were *S. sericeicornis* and *Apanteles circumscriptus* (NEES).

Z. talitskii, which is newly recorded from Iran, was the second dominant species in the parasitoid complex. The parasitoid density was lower in the first generation of HRMM, but its abundance increased in the second generation and no parasitism occurred in the third generation of the host. *Z. talitskii* has also been reported as parasitoid of HRMM in Moldavia and Ukraine (BOUCEK & ASKEW, 1968). It is a solitary ectoparasitoid of leafminer moths (STOYANOVIC & MARKOVIC, 2005), and may also attack other lepidopterous and dipterous leafminers (YEFREMOVA, 1995).

S. gordius, *S. sericeicornis*, and *S. acalle* are primary and solitary ectoparasitoid of leafminers (CROSS *et al.*, 1999). *S. gordius* was previously recorded as parasitoid of HRMM, *P. blancardella*, *P. turanica*, *L. malifoliella*, and *Lithocolletis platani* (STAUDINGER) in Iran (MODARRES AVAL, 1997). *S. acalle*, which is newly recorded from Iran, was reared from collected leaf samples in the first and third generation but not observed in the second generation of the host. This species is recorded as dominant parasitoid of HRMM (BELLOSTAS *et al.*, 1998) and *P. robiniella* (STOYANOVIC & MARKOVIC, 2005). We collected only two specimens of *S. sericeicornis* as parasitoid of HRMM in the studied area in 2006, and no one in 2007. In contrast, *S. sericeicornis* is the predominant parasitoid of leafminers in the fruit orchards of Croatia (Cross *et al.*, 1999) and in the Sarajevo area (DIMIC, 1984).

P. agraules is a solitary ectoparasitoid of leafminers. It is one of the larger parasitoids attacking *C. ohridella* (GRABENWEGER, 2003; FREISE *et al.*, 2002). This species has previously been reported as parasitoid of *P. coryliofoliella* (NOYES, 2007) and *P. robiniella* (GRABENWEGER, 2003). ASKEW & SHAW (1974) found that *P. pectinicornis* was one of the commonest species reared from *Phyllonorycter* mines in the leaves of *Malus* trees in northern England.

M. frontalis is one of the most common and polyphagous parasitoids of various groups of leafminers. It is distributed in Europe, Canada and the USA (NOYES, 2007). It was previously reared from *P. corylifoliella*, *P. blancardella* (TOMOV, 2002), *P. robiniella*, and *C. ohridella* (GRABENWEGER, 2003). We reared a small number of *M. frontalis* specimens during this study (Table 1). According to STOJANOVIC & MARKOVIC (2004), *M. frontalis* was a dominant species in the parasitoid complex of *P. robiniella*.

Baryscapus spp. have a wide host range including many species that are hyperparasitoids (ELEKCIOGLU & UJGUN, 2006). This species were reared in small numbers from mines of HRMM, in both years.

N. longiventris is a polyphagous species and reared as parasitod of Coleoptera (Curculionidae) and Lepidoptera such as *Phyllonorycter maestingella* ZELLER, *P. quinnata* (STAINTON), *P. tenella* (JOANNIS) (NOYES, 2007). *N. longiventris* is recorded here for the first time as parasitoid of HRMM. It was dominant parasitoid in samples taken from third generation of HRMM. Species of the genus *Neochrysocharis* developed as solitary or gregarious endoparasites in immature stage of mainly phytophagous insects

(HANSSON, 1990). This parasitoid is morphologically similar to *Neochrysocharis formosa* WESTWOOD, that was previously reported as parasitoid of HRMM in Iran (MODARRES AVAL, 1997).

P. bicolor is an endoparasitic wasp and prefers first instar larvae of leafminers. This species was reported as parasitoid of HRMM in the central part of Iran (RADJABI, 1986) and east of Romania (COJOCARU, 1997). In northeastern Spain, *P. bicolor* is also recorded as one of the dominant parasitoids of HRMM (CROSS *et al.*, 1999).

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