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کارگاه آنلاین آشنایی با پایگاه های اطلاعات علمی
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کارگاه آنلاین
روش تحقیق و مقاله نویسی علوم انسانی

کارگاه آنلاین
پرپوزال نویسی و پایان نامه نویسی

Appl. Ent. Phytopath.
Vol. 77, No. 1, Sep. 2009

Parasitoid wasp complex of *Phyllonorycter corylifoliella* (Lep.: Gracillariidae) in the Fars Province of Iran, and notes on their morphology and abundances

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(Received: April 2008; Accepted: October 2008)

Abstract

The hawthorn red midget moth, *Phyllonorycter corylifoliella* (Lep.: Gracillariidae) is one of the important pests of apple trees in Fars Province. Parasitoid complex of this leafminer moth were investigated during 2006 and 2007. The leaves containing larvae and pupae were collected from five sites in Sarhad region and maintained in room conditions at temperature of 25±5ºC until the parasitoids were emerged. A total of ten parasitoid species were reared: *Achrysocharoides suprafolius**, *Neochrysocharis longiventris**, *Zagrammosoma talitzkii*, *Sympiesis gordius*, *Sympiesis acalle**, *Sympiesis sericeicornis*, *Pnigalio agraules*, *Minotetrastichus frontalis**, *Baryscapus* sp. (Hym., Eulophidae) and *Pholetesor bicolor* (Hym., Braconidae). Among them, four species (marked with*) are new records for the fauna of Iran. The most common parasitoid was *A. suprafolius* (60.24% in 2006 and 68.98 % in 2007) followed by *Z. talitzkii* (14.20% in 2006 and 14.13% in 2007). Our new findings represent detailed evidence on parasitoid complex of *P. corylifoliella*. Notes on relative frequency, morphology, distribution and host range of the new parasitoid species records are included.

Key words: *Phyllonorycter corylifoliella*, Parasitoid, Apple, Fars Province.

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چکیده

مینوز لکـه تـاولی روی بـرگ *Phyllonorycter corylifoliella* (Lepidoptera: Gracillariidae)، یک آفت مهم در باغات سیب استان فارس است. پارازیتوئیدهای این آفت طی سال‌های ۱۳۸۵ و ۸٦ مورد بررسی قرار گرفت. برگ‌های حاوی لارو و شفیره‌های این گونه مینـوز از ۵ مکـان واقع در منطقه سرخد در شمال استان فارس جمـع آوری و تـا زمـان ظهـور پارازیتوئیـدها در دمای ۵±۲۵ درجه سانتی‌گراد نگهداری شدند. پارازیتوئیـدهای جمـع آوری شـده عبارتنـد از: *Zagramosomma talitzkii*، *Neochrysocharis longiventris*[*]، *Achrysocharoides suprafolus*[*]، *Sympiesis gordius*، *Sympiesis acalle*[*]، *Sympiesis sericeicornis*، *Pnigalio agraules*، *Minotetrastichus frontalis*[*] و *Baryscapus* sp. از خـانواده Eulophidae و *Pholetesor bicolor* از خانواده Braconidae. در میان آن‌ها ٤ گونه (که با ستاره مـشخص شـده‌انـد) بـرای فـون ایـران گزارش جدید هستند. فراوانی نسبی گونه‌ها، ویژگی‌های مورفولوژیک گونه‌هـای جدیـد بـرای فون ایران، دامنه میزبانی و انتشار گونه‌ها مورد بحث و بررسی قرار گرفته است[*].

واژه‌های کلیدی: *Phyllonorycter corylifoliella*، پارازیتوئید، سیب، استان فارس.

Introduction

The genus *Phyllonorycter* includes 400 described species worldwide, with the greatest species richness in the palaearctic Region (285 species) (De Prins & De Prins, 2005). The *Phyllonorycter* species have been studied extensively and some of them are well known as pests of fruit orchards (Baggiolini, 1960; Potinger & Leroux, 1971). Outbreaks of leaf mining moths have occurred repeatedly in last decades and caused economic damage in apple orchards (Cross *et al*., 1999). Heavy infestation can cause early leaf drop, reduction in terminal growth, small fruit size and premature ripening and fruit drop (Pottinger & Leroux, 1971). Until now, six species of the genus *Phyllonorycter* have been reported to feed in apple orchards in Europe: *Phyllonorycter blancardella* (F.), *P. mespilella* (Hübner), *P. pomonella* (Zeller), *P. gerasimovi* (Hering), *P. cerasicolella* (Herrich-Schäffer), and the howthorn red

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midget moth (HRMM), *P. corylifoliella* (Hubner). Of these, only the larvae of HRMM make mines on the upper surface of leaves of rosaceous trees (Olivella, 1997). Acording to Radjabi (1986) three leafmining moth species of the genus *Phyllonorycter* including *P. blancardella*, *P. turanica* (Gerasimov) and HRMM with other related apple leafminer species such as *Leucoptera scitella* Zeller and *Stigmella malella* (Stainton) have been increasing important in majority regions of apple producing in Iran. The HRMM was firstly reported from Iran in 1970 (Radjabi, 1986) and gradually spread to across north, northwest and central regions (East Azarbaijan, Khorasan, Tehran, Markazi, Fars and Esfahan provinces) and probably distributed in other parts of Iran (Radjabi, 1986). Application of broad spectrum insecticides against apple key pests such as codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae) and side effects of these compounds on beneficial arthropods and resistance to insecticides are the main reasons to increase the population density of leaf miner moths (Georghiou & Saito, 1983).

The HRMM has become an important pest in commercial apple orchards in Fars province (Amiri, 2008). It is frequent in apple orchards in Europe but has never been reported as an important pest in the area. The HRMM has four generations per year in the north of Fars Province conditions and hibernates as 5th instar larvae (Amiri, 2008). Overwintering generation emerged in early spring and adult females deposited their eggs singly on the upper surface of the leaves (Radjabi, 1986; Amiri, 2008).

Natural enemies of leaf miner moths were investigated in the world and among them, hymenopterous parasitic wasps are valuable components in maintaining ecological balance of these insects (Lasalle & Gauld, 1993). Acording to Gates *et al.* (2002) eulophid wasps are consisted over 80% of leaf miner parasitoids. In northeastern Spain, the dominant parasitoids of HRMM were *Sympiesis gordius* (Walker), *S. acalle* (Walker) and *P. bicolor* (Nees) (Bellostas *et al*., 1998) while, in Hungary, the more abundant parasitoid species was *S. sericeicornis* (Balazs, 1989).

Here, we report the results of a 2-year survey of the hymenopterous parasitoids of HRMM in the north of Fars Province of Iran. This research is a part of a larger project for biological control program against HRMM. As the first important step in this process, we try to identify, re-describe and illustrate newly recorded parasitoid species of HRMM from Fars province. Descriptions of the newly recorded species are provided to help Iranian researchers in identifying those species.

Materials and Methods

Parasitoid complex of HRMM were investigated during May to October 2006 and 2007 in the Sarhad region, (52˚, 12' E and 30˚, 43' N), located in the north of Fars province of Iran. Parasitoid complex were collected by weekly sampling of infested leaves in five apple orchards in the Sarhad region at five sites: Sedeh, Tange Boragh, Bande Bahram, Baseri, Bakan, within 80 Km radiuses. On each sampling date, 100 infested leaves were selected from each orchard. Samples were placed into clean plastic bags and transferred to the laboratory. Each sample (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 in room temperature (25±5 ºC) for at least two months. The rearing boxes were checked daily, and parasitoid adults were collected. The specimens were preserved in 75% ethanol. The parasitoid species were confirmed by the fourth and fifth authors of this paper (Z. Yefremova and P. Navone). Some of the ethanol-preserved specimens were mounted on slides for microscopic study in Canada balsam using the techniques outlined in Noyes (1982).

The external morphology of the parasitoid was illustrated using a phase-contrast OLYMPUS BH2 microscope with a drawing tube. The morphological terminology used in the parasitoid species morphology is based on Gibson *et al*. (1997) and Noyes (2007). The synonym names of the newly recorded species were standardized according to Noyes (2007). The material is deposited mainly in the collection of the Department of Entomology, College of Agriculture, Tarbiat Modares University, Tehran Iran, with duplicate in the Department of Zoology, Ul'yanovsk State University, Russia, and in the University of Torino, Italy.

Result and discussion

A total of 10 hymenopterous parasitoid species were reared from HEMM in the studied area during May to October 2006 and 2007: *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* (Hym., Braconidae). Among them four parasitoid species: *A. suprafolius, S. acalle, N. longiventris* and *M. frontalis* are new records for Iran.

A. suprafolius is found to be the most common parasitoid (60.24 % and 68.98% in 2006 and 2007, respectively) followed by *Z. talitzkii* (14.20% and 14.13% in 2006 and 2007, respectively). The other parasitoids, e.g. *S. gordius*, *S. acalle*, *N. longiventris*, *P. agraules* had

moderately abundant and the remaining species, *Baryscapus* sp., *M. frontalis*, S. *sericeicornis*, and *A. bicolor* occurred in small numbers (Table 1). Previous studies on the leafminer moths have shown that chalcidoid wasps usually play an important role in regulating of these pests population and level of parasitism in some cases reach over 50% (Askew and Shaw, 1979; Gibogini *et al*., 1996). Parasitoids of HRMM were identified in Northern Spain and their dominant parasitoids were *Sympiesis gordius*, *S. acalle* and *Pholetesor bicolor* (Bellostas *et al*., 1998).

Table 1- 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	509	576	60.24	68.98
Zagrammosoma talitzkii	120	114	14.20	13.65
Sympiesis gordius	56	17	6.63	2.04
S. sericeicornis	2	-	0.24	_
S. acalle	47	31	5.56	3.71
Neochrysocharis longiventris	47	67	5.56	8.02
Pnigalio agraules	41	10	4.85	1.19
***Baryscapus* sp.**	8	4	0.95	0.47
Minotetrastichus frontalis	7	3	0.83	0.36
Pholetesor bicolor	8	13	0.95	1.56

Notes on the Newly Recorded Parasitoid Species

***Achrysocharoides suprafolius* (Askew, 1974)**

Syn.: *Enaysma suprafolia* Askew, 1974

Diagnosis: Female: Body length 1.2-1.5 mm**;** vertex golden green, face brown, compound eyes red, frontal suture T-shape; mandibles tridentate; antenna 7-segmented, scrobe reticulated, antennal segments yellowish to white, scape length 3x as long as pedicel, funicle 3-segmented, clava 2-segmented (Fig. 5), antenna with three discoid anelli in both sex; thorax and abdomen metallic green, pronotal collar without carina, antero-median part of

propodeum smooth without carina (Fig. 12), thorax included propodeum 0.86x as long as abdomen; legs white and pretarsus pale brown; post marginal vein as long as stigmal vein, fore wing 2.04 as long as wide, ratio of submarginal/marginal/postmarginal/stigmal veins is 0.9: 2.3: 0.3: 0.27 (Fig. 1); **Male**: Body length 1.1-1.3 mm; resembles female but its antennal segment paler than female, width of antennal scape about 2x as female (Fig. 6).

Distribution and host range: *A. suprafolius* was the most abundant parasitoid of HRMM in the studied area (Table 1). This species is distributed in Germany, the Netherlands and United Kingdom (Noyes, 2007). *Achrysocharoides* species attack *Phyllonorycter* larvae that mine lower surfaces of leaves, except for *A. suprafolius*, which feeds only on the HRMM larvae (Askew & Ruse, 1974).

***Sympiesis acalle* (Walker, 1848)**

Syn.: *Astichus bimaculatipennis* Girault, 1912; *Entedon nubeculatus* Ratzeburg, 1848; *Eulophus acalle* Walker, 1848; *Eulophus bifasciatus* Thomson 1878; *Sympiesis bimaculata* Crawford, 1913; *Sympiesis bimaculatipennis* (Girault, 1912); *Sympiesis meteori* Girault, 1916.

Diagnosis: Female: Body length 2.5-2.9 mm; body dark green with bluish tint; antennae 8 segmented in both sexes, scape yellowish to white, with black dorsal surface, funicle 4 segmented, clava 2-segmented (Fig. 7); thorax 2x as long as wide, propodeum with complete median carina (Fig. 13); coxa and femur in all legs brownish to black; fore wings with two dark strips, post marginal vein about 2x as long as stigmal vein (Fig. 2); **Male**: Body length 1.8–2 mm; three first segments of funicle with branch–like projections (Fig. 8), forewings without dark stripes.

Distribution and host range: This species was reported from Azerbaijan, Bosnia, Bulgaria, Canada, United Kingdom, Czech Republic, Germany, Hungary, Italy, Japan, Korea, Rumania, Russia and the Netherlands (Noyes, 2007). This species is a parasitoid of *Phyllonorycter* (Lepidoptera: Gracillariidae) and *Agromyza* (Diptera: Agromyzidae) (Boucek & Askew, 1968; Yefremova, 2007).

***Neochrysocharis longiventris* (Askew, 1979)**

Syn.: *Chrysonotomyia longiventris* Askew, 1979

Diagnosis: Female: Body length 0.9–1.2 mm; Body dark green, anterior margin of clypeus almost straight, mandibles bidentate and brown; eyes with pubescence, hairs minute and visible only under high magnification; scape somewhat paler than flagellum and palest at base, pedicel in profile 1.6x as long as broad, clava 3-segmented with an apical spine, longer

than funicle segments plus anelli (Fig. 11); thorax somewhat flattened, scutellum very slightly longer than broad with one pair of long setae, thorax in combination with propodeum 1.4x as long as broad in dorsal view (Fig. 14); gaster with dark green reflection on the first tergite; ovipositor dark brown; wings hyaline with a small fuscous spot extending from stigma, venation brownish, fore wings 1.9x as long as broad (Fig. 3). coxa green metallic, *N. longiventris* is very similar to *Closterocerus formosus* in morphological chrachteristics but *N. longiventris* have relatively longer gaster; **Male**: Body length 0.7- 1.1 mm**;** resembles female, but gaster trapezoidal and its length about as long as rest of body, antennae rather longer and more slender.

Distribution and host range: *N. longiventris* was reported from Denmark, United Kingdom (Askew, 1979), Sweden (Hansson, 1990). It is a polyphagous species and reared as parasitoid 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. Species of the genus *Neochrysocharis* developed as solitary or gregarious endoparasites in immature stage of mainly phytophagous insects (Hansson, 1990).

***Minotetrastichus frontalis* (Nees, 1834)**

Syn.: *Aprostocetus budensis* (Erdös, 1954); *Aprostocetus ecus* (Walker, 1839); *Cirrospilus ecus* Walker, 1839; *Entedon cyclogaster* (Ratzeburg, 1844); *Entedon rivillellae* Rondani, 1877; *Entedon xanthops* (Ratzeburg, 1844); *Eulophus cyclogaster* Ratzeburg, 1844; *Eulophus frontalis* Nees, 1834; *Eulophus xanthops* Ratzeburg, 1844; *Geniocerus budensis* Erdös, 1954; *Geniocerus cyclogaster* (Ratzeburg, 1844); *Geniocerus ecus* (Walker, 1839); *Geniocerus xanthops* (Ratzeburg, 1844); *Minotetrastichus ecus* (Walker, 1839); *Tetrastichus cimbicis* Kostjukov, 1976; *Tetrastichus cyclogaster* (Ratzeburg, 1844); *Tetrastichus cyclogaster obscurata* Ruschka, 1924; *Tetrastichus ecus* (Walker, 1839)

Diagnosis: Female: Body length 0.8–0.9 mm; head whitish to yellow; antennae 8-segmented, funicle 3-segmented, clava 3-segmented (Fig. 10), space between ocelli dark brown, antennae pale brown, dorsal surface of pedicel dark brown; thorax metallic green, length of thorax including propodeum 0.72x as long as abdomen, length of thorax 1.6x as long as wide (Fig. 15); first three segments of abdomen whitish to yellow, but dorsal surface of three terminal segments is dark brown; legs white; length of fore wings 2.19x as long as wide, postmarginal vein considerably reduced (Fig 4); **Male**: Body length 0.67–0.8 mm; antennae 9-segmented, funicle 4-segmented with branch–like projection (Fig. 9).

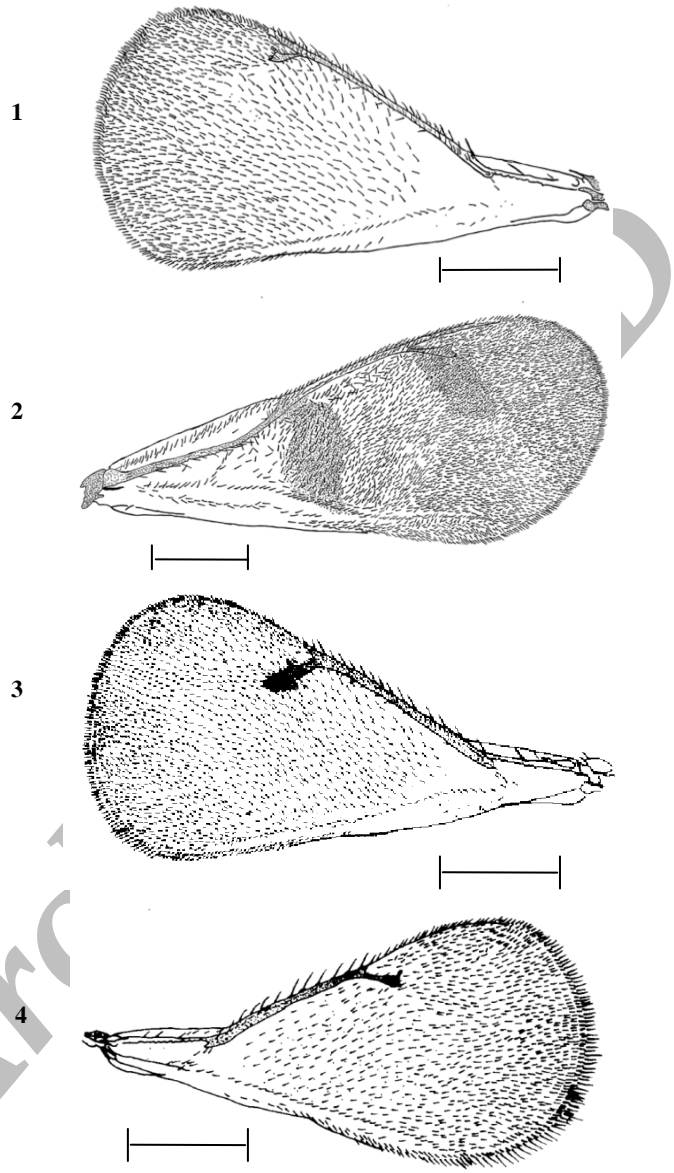

Fig. 1-4. Forewings, 1. *Achrysocharoides suprafolius* ; 2. *Sympiesis acalle*;
3. *Neochrysocharis longiventris*; 4. *Minotetrastichus frontalis*;
Scale bars=50 micrometers (original)

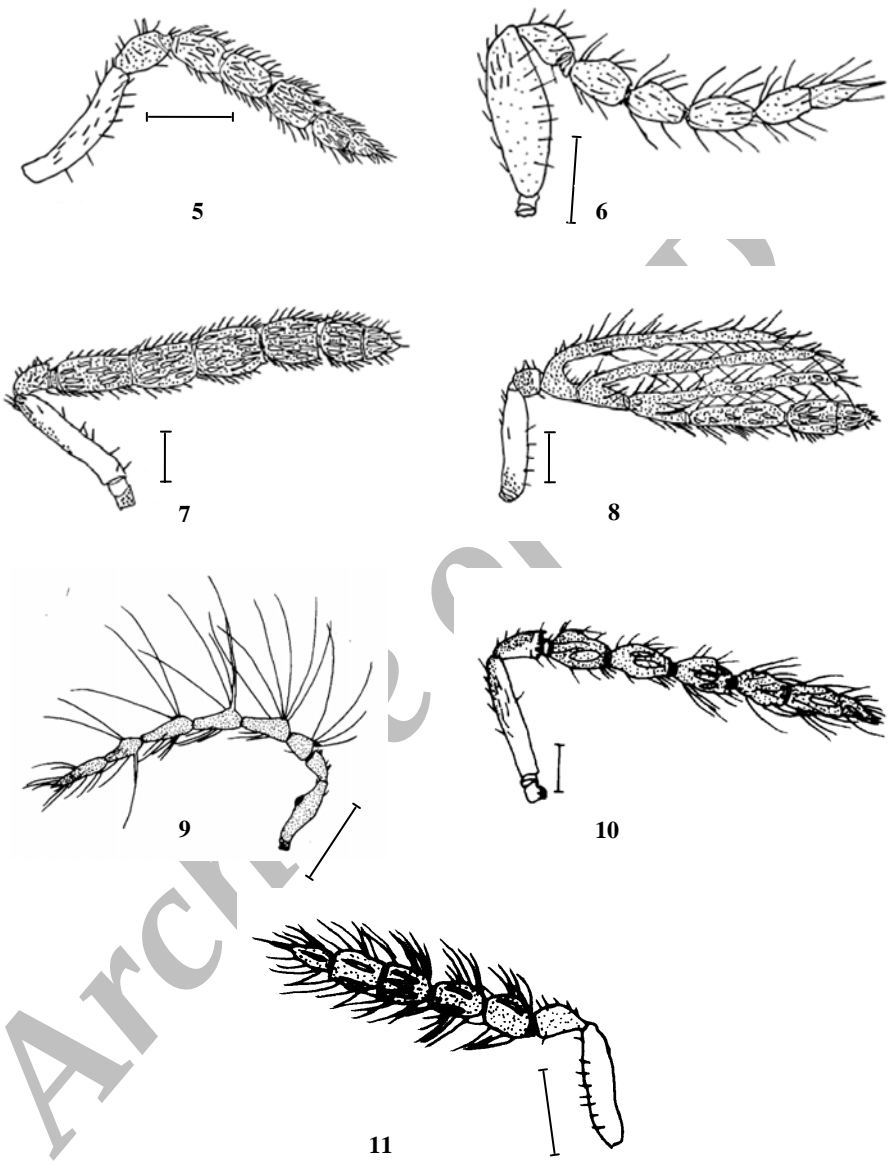

Fig. 5-11- Antennae, 5. *Achrysocharoides suprafolius* ♀, 6. ♂; 7. *Sympiesis acalle* ♀, 8. ♂; 9. *Minotetrastichus frontalis*, ♂, 10. ♀; 11. *Neochrysocharis longiventris* ♀; scale bars=50 micrometers (original)

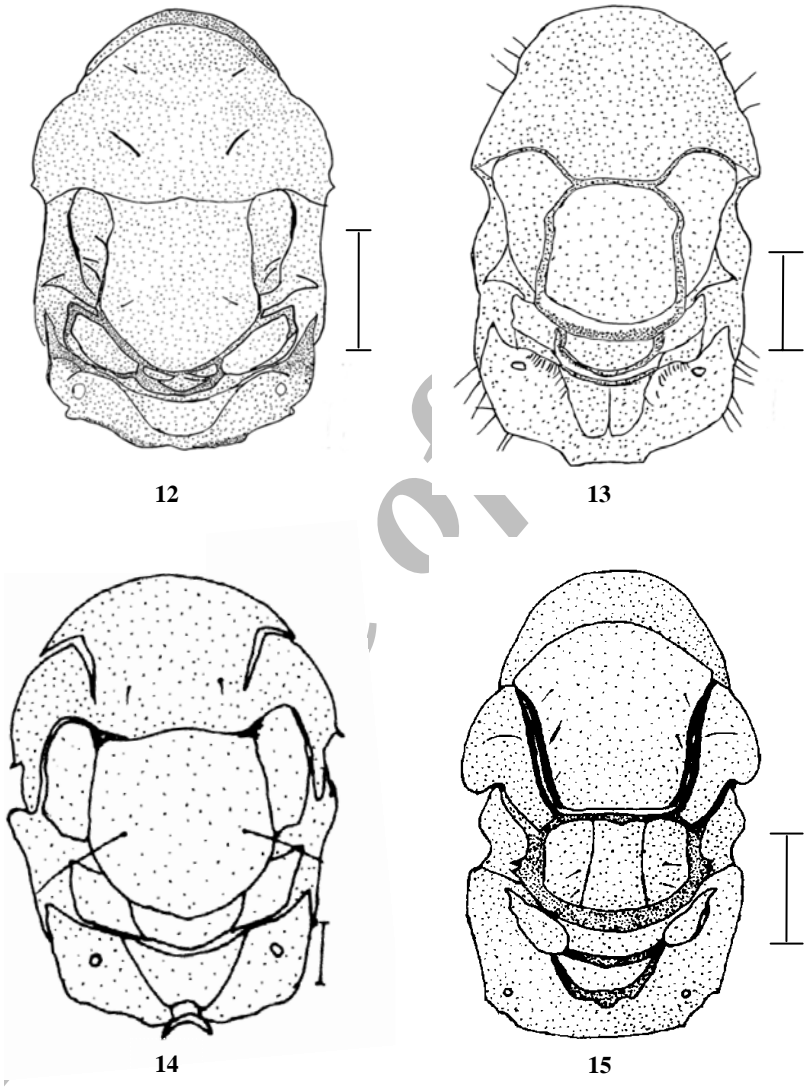

Fig. 12-15. Thorax, 12. *Achrysocharoides suprafolius*; 13. *Sympiesis acalle*; 14. *Neochrysochaeis longiventris* 15. *Minotetrastichus frontalis*; Scale bars=50 micrometers (original)

Distribution and host range: *M. frontalis* is recorded here for the first time from Iran. It is one of the most common and polyphagous parasitoids of various groups of leafminers. It is distributed in Europe, Canada and United States of America (Noyes, 2007). It was reared from *P. corylifoliella*, *P. blancardella* (Tomov, 2002), *P. robiniella* and *C. ohridella* (Grabenweger, 2003).

Z. talitzkii and *P. agraules* have recently been reported from Iran (Yefremova *et al*., 2007). *P. agraules* was also reported from Austria, Bosnia, Bulgaria, France, Germany, Greece, Italy, North Africa, Russia, Spain, Turkey and Ukraine (Noyes, 2007). It is a solitary ectoparasitoid and a common species with a wide host range (Boucek & Askew, 1968). This parasitoid is one of the larger parasitoids attacking *Cameraria ohridella* (Grabenweger, 2003; Freise *et al*., 2002). This species has already been reported as parasitoid of *P. coryliofoliella* (Noyes, 2007) and *P. robiniella* (Grabenweger, 2003).

Z. talitskii was the second dominant species in the parasitoid complex. This species is distributed in Italy, Kazakhstan, Moldova, Russia, Turkmenistan, Ukraine, eastern USSR (Noyes, 2007). This parasitoid has also been reported as parasitoid of HRMM in Moldavia and Ukraine (Boucek & Askew 1968). It is a common primary ectoparasitoid of leafminer moths (Gracillariidae, Lyonetiidae, Phyllocnistidae) and may also attack other lepidopterous and dipterous leafminers (Noyes, 2007; Yefremova, 2007).

In conclusion it should be noted that the reduction of broad-spectrum insecticides applications in apple orchards led to the rich diversity of parasitic wasps. Several studies have shown that parasitoid populations are much larger in untreated orchards than in orchards sprayed with insecticides (Cross *et al*., 1999). It can be asserted that parasitoids have a great effect on population density of the leaf miner moths, but more attention should be given to the knowledge of the biology and ecology of parasitoid species to better use them in biological control programs.

Acknowledgments

The financial support for this work was provided by Tarbiat Modares University. The authors wish to thank to Prof. Dr. K. Kamali (Tarbiat Modares University, Tehran) and two anonymous reviewers for their valuable comments and suggestions on the manuscript and Dr. A. Lozan (Institute of Entomology, Ceske Budejovice, Czech Republic) for identification of *P. bicolor*. We also thank to Mr. Ahmad Amiri for valuable assistance with the collections of specimens.

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