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HYMENOPTEROUS PARASITOIDS (HYMENOPTERA: BRACONIDAE, EULOPHIDAE, PTEROMALIDAE) OF THE ALFALFA LEAFMINERS IN IRAN AND THEIR DIVERSITY

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Alfalfa leafminers and their parasitoids were studied during 2008–2010 in the Northwest Iran. Alfalfa leafminers include five species that *Liriomyza trifolii* (Burgess), *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) and *Phyllonorycter medicaginella* (Gerasimor) (Lepidoptera: Gracillariidae) were the most important. An illustrated key to leafminers based on symptoms is given. These species were parasitized with 15 hymenopterous parasitoids including three braconid, 10 eulophid and two pteromalid specis. Of which, two species of Braconidae (*Chorebus calthae* Griffiths, *Dacnusa heringi* Griffiths), four Eulophidae (*Chrysocharis albicoxis* Erdös, *Chrysocharis crassiscapus* (Thomson), *Sympiesis xanthostoma* (Nees), *Diglyphus pachyneuron* Graham) and one species of Pteromalidae (*Sphegigaster ineus* Mitroiu) are reported for first time from Iran. An identification key to reared parasitoid species is given. The diversity of hymenopterous parasitoids is discussed.

KEY WORDS: *Medicago sativa*, leafminers, natural enemies, Hymenoptera, Chalcidoidea, Ichneumonoidea, new records, diversity, Iran.

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В 2008-2010 гг. на северо-западе Ирана были изучены листовые минеры люцерны и их паразитоиды. Выявлено 5 видов минеров, из которых наиболее важными являются *Liriomyza trifolii* (Burgess) и *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) и *Phyllonorycter medicaginella* (Gerasimor) (Lepidoptera: Gracillariidae). Дана определительная таблица минеров, основанная на повреждении ими растений. На минерах обнаружено 15 видов паразитических перепончатокрылых, в т.ч. 3 вида браконид, 10 – эулофид и 2 – птеромалид. Из них *Chorebus calthae* Griffiths, *Dacnusa heringi* Griffiths (Braconidae), *Chrysocharis albicoxis* Erdös, *Chrysocharis crassiscapus* (Thomson), *Sympiesis xanthostoma* (Nees), *Diglyphus pachyneuron* Graham (Eulophidae) и *Sphegigaster ineus* Mitroiu (Pteromalidae) впервые указываются для Ирана. Приведена определительная таблица выведенных паразитических перепончатокрылых и обсуждается их разнообразие.

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INTRODUCTION

Alfalfa (Medicago sativa L.) has an economic importance with special place among the feed plants and is grown in extensive area in Iran (Karimi, 1999). This plant damages by different groups of pests that leafminers of different orders have been reported on alfalfa in Iran (Pourhaji et al., 2011). Leafminers larvae live and feed within plant leaves, consuming mesophyll tissue without damaging the leaf epidermis (Salvo & Valladares, 2007). Their habit has been developed by a group of over 10,000 species of holometabolous insects, concentrated in four orders: Diptera, Coleoptera, Hymenoptera, and Lepidoptera (Connor & Taverner, 1997). These pests reduce the photosynthetic capacity of leaves with their excavated galleries. Different factors caused these groups become serious pests: 1) insecticide resistance development, 2) elimination of their natural enemies, 3) the use of agrochemicals, 4) allowing them to go unnoticed until reaching high densities, 5) the protection of their immature stages inside plant tissue, especially against the effects of contact insecticides, and 6) the increase in monocultivation (Murphy & LaSalle, 1999; Salvo & Valladares, 2007). Hence, some species have developed resistance and presented these pests as primary pests such as Liriomyza trifolii (Burgess) (Reitz et al., 1999). Six Diptera miners, the agromyzids Agromyza frontella (Rondani), Agromyza nana Meigen, Chromatomyia horticola (Goureau), Liriomyza congesta

(Becker) and *Ophiomyia curvipalpis* (Zetterstedt), are recorded or tentatively recorded on *Medicago* and 3 non-Diptera miners are recorded on *Medicago* in Britain (Pitkin *et al.*, 2012), while it includes five species in Iran (Pourhaji *et al.*, 2011).

Within the leafminers, Agromyzidae is known as one of the most serious pests of many vegetable and horticultural crops worldwide (Spencer, 1973). Agromyzid fly, *L. trifolii* is a common serpantine leafminer on cut and pot chrysanthemums in California (Parrella, 1982; Hara, 1986). Venette *et al.* (2007) reported little resistance to alfalfa blotch leafminer damage in over 80 alfalfa varieties. Its damage has been estimated 7% yield loss in USA and about 4.5% reduction in Iran (Pourhaji *et al.*, 2011). Five eulophid parasitoids have been recorded on alfalfa leafminers from Turkey (Gençer, 2004).

Parasitoids of *Liriomyza* (Dip.: Agromyzidae) has been studied by Talebi *et al.* (2005), Asadi *et al.* (2006) and Dousti *et al.* (2008) in Iran. Noyes (2013) listed over 300 species of agromyzid parasitoids, and over 80 species that are known to attack different *Liriomyza* species. Several studies have dealt with the simultaneous use of parasitoids together with chemical and cultural control (Salvo & Valladares, 2007). After several times release of *Dacnus dryas* Nixon (Hymenoptera: Braconidae) in Canada between 1974 and 1978, 98% reduction of leafminer population has been reported

Medicago, Melilotus and *Trifolium* species attack by Lepidopterous leafminers such as *Phyllonorycter medicaginella* (Gerasimor) (Lepidoptera: Gracillariidae) (Šefrová, 2002). This species was reported from some European countries such as Poland to Belgium, Denmark, Bulgaria and Ukraine (Kuchlein *et al.*, 2002). It is widely distributed in East-Azarbaijan province, Iran (Pourhaji *et al.*, 2011). De Prins and Steeman (2012) believe this moth is a very small species that easily overlooked because it is almost invisible on the upperside. Their larva lives in a tentiform mine on the underside of the leaf. It pupates inside the mine and hibernates as a pupa (Šefrová, 2002).

Lepidopterous and dipterous leafminers are attacked by several types of natural enemies, especially hymenopterous parasitoids. Most species of leafminers parasitoids belong to the Eulophidae, Pteromalidae and Encyrtidae (Chalcidoidea), and the Braconidae and Ichneumonidae (Ichneumonoidea). These play role of primary, secondary and tertiary parasitoids of leafminers (Csóka, 2003).

Considering that natural enemies (especially parasitoids) are the most frequent source of leafminers guild mortality (Salvo & Valladares, 2007) in this research we focused on biological control strategies that should be compatible with IPM programs, therefore, in the first step we tried to determine native biocontrol agent especially parasitic wasps that attack alfalfa leafminers in Iran. Furthermore, some ecological parameters were estimated for parasitoids in studied areas.

MATERIALS AND METHODS

Collection and rearing

The infested leaves of alfalfa that contains leafminers larvae and pupae were collected from field localities and placed into plastic bags and labeled with the locality information. Samplings were made at 15 days intervals in the period from April to October during the years 2008-2010. Collections were made from different areas of East-Azarbaijan province in the northwest of Iran belong Ajabshir, Jolfa, Khosro-Shahr, Maragheh, Marand, Sarab, Shabestar and Osku. The infested leaves transferred to the laboratory. Also dark pupae inside a mine, surrounded by small meconial (fecal) pellets, the leaf sniped off and placed it into a plastic containers separately and rearing was conducted at 25±2°C and %60±10 RH. The containers were daily checked for the emergence of leafminers and parasitoids.

Systematic study

Emerged parasitoids were collected daily using an aspirator and preserved in 75% ethyl-alcohol. Identification of Chalcidoidea were made using Bouček (1959, 1965), Hansson (1985), Graham (1959, 1987, 1991), LaSalle & Parella (1991), Gates *et al.* (2002), Whitfield & Wagner (1991), Mitroiu (2008) and Zhu *et al.* (2000). Previously reported parasitoids on alfalfa leafminers in Iran were reviewed. The morphological characteristics of each species are given in brief. Finally all of reported parasitoids on alfalfa related leafminers were tabulated.

Abbreviations

Abbreviations used in the text include:

F1, F2, etc.: first funicle segment, second funicle segment, etc.

OCL: occipital-ocellar line (=the shortest distance between each posterior ocellus and occipital margin)

OOL: ocular-ocellar line (=the shortest distance between posterior ocellus and adjacent eye margin)

POL: posterior ocellar line (=the shortest distance between the posterior ocelli)

T1, T2, etc.: first metasomal tergite, second metasomal tergite, etc.

Diversity analysis

The Shannon-Weiner diversity index uses the following formula:

Shannon's H' =
$$-\sum_{i=1}^{N_o} [p_i * \log p_i]$$
 where p_i is the proportion of the total number

of individuals belonging to a morphotype, and N_o is the total number of morphotypes seen in that sample. Also, The Simpson's diversity index is calculated using the following formula:

Simpson's D = 1 -
$$\sum_{i=1}^{N} \frac{n_i(n_i - 1)}{N(N - 1)}$$
 where n_i is the number of individuals of a

particular morphotype and N is the total number seen in the sample (Magurran, 2004).

Evenness, the other information-statistical index, is affected by both the number of species and their equitability or evenness compared to a community's actual diversity, and the value of E is constrained to 0- 1.0. Shannon's evenness is calculated by the formula: H'/Hmax.

LEAFMINER SPECIES

According to Pourhaji et al. (2011), there are three lepidopterous *Coleophora* vicinella (Zeller) (Coleophoridae), *Phyllonorycter medicaginella* (Gerasimor) (Gracillariidae) and *Aproaerema anthyllidella* (Hübner) (Gellechidae), and two dipterous leafminers of the family Agromyzidae *Liriomyza trifolii* (Burgess) and *Chromatomyia horticola* (Goureau) in the northwest of Iran.

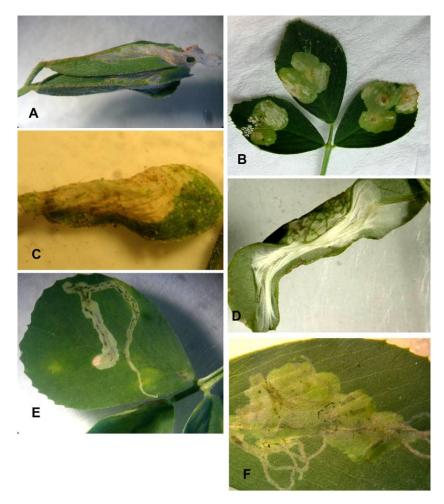


Fig. 1. Alfalfa leafminers symptoms: A – *Aproaerema anthyllidella*, B – *Coleophora vicinella*, C – *Phyllonorycter medicaginella*, upper side, D – *P. medicaginella*, lower side, E & F – *Chromatomyia horticola* & *Liriomyza trifolii*.

Key to species of alfalfa leafminers based on symptoms

ALFALFA LEAFMINERS PARASITOIDS

These five alfalfa leafminers were parasitised by 15 hymenopterous parasitoids in larval and pupal stages. These parasitoids belong to three families Braconidae (3 species), Eulophidae (10 species) and Pteromalidae (2 species) (see Table 1). Including previously reported species, totally 25 species from 15 genera were listed in Table 1. This wide range of the biocontrol agents can establish natural control of these pests in the studied areas.

Most of reared parasitoids were obtained from Agromyzidae and within lepidopterous alfalfa leafminers, parasitoids were only reared on *P. medicaginella*. Twelve and four parasitic wasps were reared on larval stage of Agromyzidae and *P. medicaginella*, respectively (Table 1). Most species of parasitoids that attack *P. medicaginella* belong to the Eulophidae and Petromalidae (Chalcidoidea), several genera of which have radiated widely on leafminers.

Reared parasitoids in this study could be separate by presented key.

Key to species of alfalfa leafminers parasitoids in East-Azarbaijan province

- 1. Forewing venation complete, with at least 2 closed cells (Fig. 6D). (**Braconidae**)
- Forewing venation reduced, without closed cells (Fig. 3C). (Chalcidoidea) 4

- - 6

Superfamily	Family	Genus	Species	Host	References
Ichneumonoidea	Braconidae	Apanteles	Apanteles sp.	L. trifolii / C. horticola	This paper
			(circumscriptus species-group)		
-		Chorebus	C. calthae Griffiths*	L. trifolii / C. horticola	This paper
		Dacnusa	D. heringi Griffiths	L. trifolii / C. horticola	This paper
Chalcidoidea	Eulophidae	Baryscapus	B. impeditus (Nees)	L. trifolii	Talebi et al. (2011)
			Ch. albicoxis Erdös*	L. trifolii / C. horticola	This paper
		Chrysocharis	Ch. crassiscapus (Thomson)*	L. trifolii / C. horticola	This paper
		•	Ch. submutica Graham	P. medicaginella	This paper
			C. talitzkii Bouček	L. trifolii / C. horticola	This paper
		Cirrospilus	Contraction Wollson	L. trifolii / C. horticola	This paper
		,	C. VIIIalus Walker	L. sativae	Asadi et al. (2006)
			D onaccinamic Brdãc	L. sativae	Asadi et al. (2006)
			D. CLU3311161 113 121003	L. trifolii	Dousti et al. (2008)
			(Included and a second se	L. trifolii / C. horticola	This paper
		Diglyphus	D. Isaea (waikei)	L. sativae	Asadi et al. (2006)
			D. pachyneuron Graham*	L. trifolii / C. horticola	This paper
			D autobainan (Caniford)	I twifelii	Dousti et al. (2008)
			D. puich ipes (Clawiolu)	L. utjou	Talebi et al. (2011)
			H zilahisahassi Erdös	L. sativae, L. trifolii, C.	Zahiri et al. (2003),
				horticola	Asadi et al. (2006)
		TT	H. wailesellae Nowicki	L. sativae	Zahiri et al. (2003)
		nemipiarsenus	H. waterhousii Westwood*	P. medicaginella	This paper
			H. varicornis (Girault)	L. trifolii	Yefremova et al. (2007)
			V. C. Moren	L. sativae	Asadi et al. (2006)
		Neocurysocnaris	IV. JOLMOSUS (W CSIWOOU)	L. trifolii	Zahiri et al. (2003)
		Pnigalio	Pnigalio sp. aff. pectinicornis	L. sativae, L. trifolii	Asadi et al. (2006)
		Sympiesis	S. xanthostoma (Nees)*	P. medicaginella	This paper
	Pteromalidae	Cyrtogaster	C. vulgaris Walker	C. horticola,	Charalizadeh,
				D madianaturalla	This noner
		Pteromalus	Pteromalus sp.	r. meaicagineita L. trifolii /C. horticola	I IIIS paper
		Sphegigaster	S. ineus Mitroiu*	P. medicaginella	This paper

Table 2. Reared hymenopterous parasitoids of alfalfa agromyzid leafminers in this research with number of each species in different localities of East-Azarbaijan province during 2008–2010

Parasotoids	Ajabshir	Khosro-Shahr	Marand	Sarab	Shabestar	Total
Apanteles sp.	•	Jun. (42), Jul. (13, 12)	•	-	-	9
Chorebus calthae Griffiths	0ct. (1 🆓)	May (1♀), Sep. (1♀), Jul. (2♀)	-	Sep. (1 \mathcal{J}), Aug. (1 \mathcal{I})	Oct. (1♂), Sep. (1♀)	6
Chrysocharis albicoxis Erdös	ı	May (5♀), Jul. (5♀)	Oct. (4⊋)	•	•	14
Ch. crassiscapus (Thomson)	Oct. $(3\delta, 2\varphi)$	•	Jun. (92)	Sep. (1♀), Oct. (1♂)	Oct. (1 🆓)	17
Ch. submutica Graham	Aug. $(5^{\circ}, 2^{\circ})$	Jul. (5 ♀, 5♂), May (9 ♀, 1♂)	-	•	I	27
Cirrospilus talitzkii Bouček	•	-	•	Oct. (1♀)	-	1
Ci. vittatus Walker		Jun. (1 2)	•	-	-	1
Dacnusa heringi Griffiths	Oct. (1♂), Aug. (2♂), Sep. (1♀)	Jul. $(I \circlet, I \circlet)$, Oct. $(1 \circlet)$	Oct. (4♀)	•	1	11
Diglyphus isaea (Walker)	Aug. (72, 13)	Jun. (2♀), Jul. (11♀, 3♂)	•	Oct. $(6$, 3 $\mathcal{J})$	Oct. $(21$, 1 , 1 , 1 , $Aug. (2$, 1),	58
D. pachyneuron Graham	•	May (1δ) , Jul. (1δ)	•	Oct. (2 ♀♀)	•	4
Hemiptarsenus waterhousii Westwood	Aug. (2♀), Oct. (1♂)	Sep. (2♀), Jun. (1♀)	ı	•	Sep. (2 ♀)	8
Neochrysocharis formosus (Westwood)	Jun. (6⊋)	Aug. (2♀), Jun. (1♀, 1♂), Sep. (1♂)	1	Oct. (5♀, 1♂)	Sep. (1?)	18
Pteromalus sp.	•	Jun. (3δ) , Sep. (2δ)	•	-	Aug. (1δ)	6
Sphegigaster ineus Mitroiu		Jun. (2 2)	-	-	-	2
Sympiesis xanthostoma (Nees)	3	May (12), Jun. (62, 2♂), Jul. (2♀), Sep. (4♀, 1♂)	I	ſ	I	16
Total:	34	94	17	18	31	198

5. Scutellum with 1 pair of setae (Fig. 3D), submarginal vein with 2 or more setae
dorsally, notauli usually absent. (Subfamily Entedoninae)
- Scutellum with 2 pairs of setae (Fig. 2B), submarginal vein with 1 or more setae
dorsally, notauli present or absent. (Subfamily Eulophinae)
6. Postmarginal vein shorter than stigmal vein (Figs. 2D, E)
- Postmarginal vein longer than stigmal vein (1.5-2 times) (Fig. 3C). (Chrysocha-
<i>ris</i>)
7. Occipital margin carinate, mesonotum strongly reticulated (large and dense reti-
culations) (Fig. 3D) Chrysocharis crassiscapus (Thomson)
- Occipital margin rounded, mesonotum weekly reticulated (fine and sparse reticu-
lations)
8. Temple relatively large, propodeum anteriorly with a raised Y-shaped carina
Chrysocharis submutica Graham
- Temple relatively small, propodeum without a Y-shaped carina
9- Funicle 2-segmented (Fig. 4C)
– Funicle more than 3-segmented (Fig. 5C)
10. Notauli incomplete, postmarginal vein longer than stigmal vein, body with
metallic reflection (Fig. 4). (<i>Diglyphus</i>)
- Notauli complete, postmarginal vein as long as stigmal vein or shorter, body
partly yellow (Figs. 2A, B, C). (<i>Cirrospilus</i>)
11 Cubital using surged baselly toward enterior magning of using an automatic
11- Cubital vein curved basally toward anterior margin of wing, speculum very
narrow, tibia metallic with basal and apical yellow band (Fig. 4A)
Diglyphus isaea (Walker)
- Cubital vein not distinctly curved basally, speculum present and extended
posteriorly to cubital vein, fore and middle tibia yellow (Fig. 4B)
Diglyphus pachyneuron Graham
12- Speculum small, mesosoma without lateral dark bands (Fig. 2C)
<i>Cirrospilus vittatus</i> Walker
- Speculum wide, mesosoma with two lateral dark bands (Fig. 2A)
<i>Cirrospilus talitzkii</i> Bouček
13-Torulus high on head, above lower eye margin, thus apex of scape extends
beyond level of vertex. Forewing and costal cell narrow, forewing at least 2.6
times as long as broad and costal cell 10-15 times as long as broad
Hemiptarsenus waterhousii Westwood
- Torulus at or below lower eye margin, thus apex of scape not extending beyond
level of vertex. Forewing and costal cell not so narrow, forewing less than 2.6
times as long as broad and costal cell less than 10 times as long as broad
14. Metasoma sessile, clypeal margin shallowly emarginate
Pteromalus sp.
– Metasoma distinctly petiolate (petiole longer than propodeum) clypeal margin
sharply 2-toothed (Fig. 6B)

List of identified species of alfalfa leafminers parasitoids

Family Braconidae

Twelve subfamilies including 40 genera of braconids have been reported on leafmining lepidopterous species in Holarctic region (Whitfield & Wagner, 1991). We reared two subfamilies Alysiinae and Microgastrinae.

Alysiinae is a cosmopolitan subfamily of endoparasitoids of cyclorrhaphous Diptera. It can be recognized by the typically curved vein 1-M of fore wing, which is straight among Alysiinae and first and second metasomal tergites have no spiracles (van Achterberg, 1993). Two species of this subfamily were reared on alfalfa leafminers.

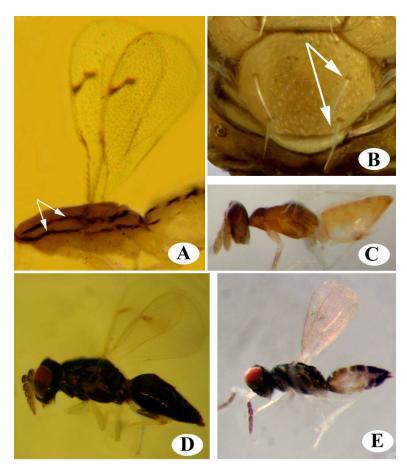


Fig. 2. A – *Cirrospilus talitzki*, mesosoma in lateral view and fore wings; B – *Cirrospilus* sp., scutellum in dorsal view; C – *Cirrospilus vittatus*, female in lateral view; D, E – *Neochrysocharis formosus*: D – female in lateral view, E – male in lateral view.

Microgastrinae is very large cosmopolitan subfamily of endoparasitoids of larval Lepidoptera. This group is very common; among the earliest Braconidae in spring. These wasps have 18-segmented antennae, vein 2-SR of fore wing connected to vein r, scutellar sulcus more or less developed (van Achterberg, 1993). Only one species of this subfamily was reared on alfalfa leafminers.

Apanteles sp.

MATERIAL EXAMINED. East-Azarbaijan province, Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, VI 2010, 4 \bigcirc ; 7.VII 2008, 1 \bigcirc ; 9.VII 2008, 1 \bigcirc (A. Pourhaji leg.).

NOTES. This genus belonges the subfamily Microgastrinae and contains several members that are important larval parasitoids of lepidopteran pests. The adult *Apanteles* wasp is about 3 to 5 mm long with a black body and long antennae. The female has a short ovipositor. Reared species belonges the *circumscriptus* species-group of *Apanteles* (V. Zikic, personal communications). This group parasitise different species of *Phyllonorycter* (Lepidoptera: Gracillaridae). Our reared specimens were obtained from *P. medicaginella*.

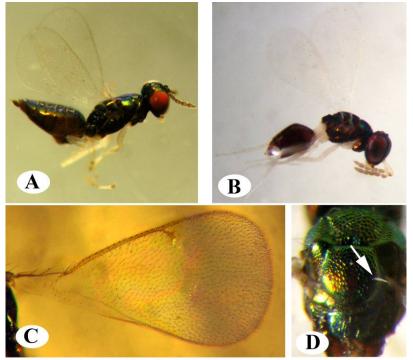


Fig. 3. A – *Chrysocharis submutica*, female in lateral view. – *C. albicoxis*, female in lateral view. C, D – *C. crassiscapus*: C – fore wing, D – mesosoma in dorsal view.

11

Chorebus calthae Griffiths, 1967

MATERIAL EXAMINED. East-Azarbaijan province: Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, 29.V 2008, 1 \bigcirc ; 18.IX 2008, 1 \bigcirc ; 7.VII 2008, 1 \bigcirc ; VII 2010, 1 \bigcirc (A. Pourhaji leg.); Ajabshir, 1.X 1009, 1 \bigcirc (A. Pourhaji leg.); Shabestar, 1.X 2009, 1 \bigcirc , 8.IX 2009, 1 \bigcirc (A. Pourhaji leg.); Sarab, 1.IX 2009, 1 \bigcirc ; 5.viii.2009, 1 \bigcirc (A. Pourhaji leg.).

NOTES. This species belongs to the subfamily Alysiinae and tribe Dacnusiini. It is widely distributed in Europe (de Jong, 2012). We reared *C. calthae* on alfalfa agromyziid leafminers and has been recorded from *Phytomyza calthivora* Hendel (Dipera: Agromyzidae) (Pitkin *et al.*, 2013). *Chorebus calthae* can distinguish with 25-segmented antennae and 4 non-equal teeth mandibles. This is first record of *C. calthae* from Iran.

Dacnusa (Dacnusa) heringi Griffiths, 1967

MATERİAL EXAMİNED. East-Azarbaijan province: Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, 18.VII 2010, 1 \bigcirc , 1 \bigcirc ; 14.X 2008, 1 \bigcirc (A. Pourhaji leg.); Ajabshir, 18.X 2009, 1 \bigcirc (A. Pourhaji leg.); Ajabshir, 31.VIII 2009, 2 \bigcirc , 28.IX 2009, 1 \bigcirc (A. Pourhaji leg.); Marand, 19.X 2009, 4 \bigcirc (A. Pourhaji leg.).

NOTES. This species belongs to the subfamily Alysiinae and tribe Dacnusiini. It is widely distributed in Europe (de Jong, 2012). *Dacnusa heringi* were reared on alfalfa agromyziid leafminers and characterized by 21-segmented female antennae, 20- segmented male antennae and 4 equal teeth mandibles. This is first record of *C. calthae* from Iran.

Family Eulophidae

We reared nine species in five genera on alfalfa leafminres. Including ten reported species from Iran, there are 19 species on alfalfa leafminers (Table 1).

Chrysocharis albicoxis Erdös, 1958

Fig. 3B

MATERIAL EXAMINED. East-Azarbaijan province: Khosro-shahr, 1346m, 37°58'28"N, 46°02'55"E, 3.VII 2009, 5; 26.V 2009, 4; (A. Pourhaji leg.); Marand, 19.X 2009, 4; (A. Pourhaji leg.); Khosro-Shahr, 22.V 2008, 1; (A. Pourhaji leg.).

NOTES. The genus *Chrysocharis* can be identify by following characters: antenna with apical two segmented fused, or with all flagellar segments free, clypeus not delimited, postmarginal vein longer than stigmal vein, without a sulcus surrounding ocellar triangle. *Chrysocharis albicoxis* was reared from Agromyziidae and *P. medicaginella* on alfalfa. According to Hansson (1985) it belongs to the *mediana* species group. It has small temples; coxae and femora usually pale (coxae occasionally

dark and metallic, and with femora darkened); anteromedian part of propodeum with weak and irregular reticulation or with a shallow and indistinct fovea; petiole small with raised surface narrow and smooth, gaster short; ratio length of thorax+ propodeum/ length of gaster about 0.9 in female and about 1.0 in male.

Chrysocharis crassiscapus (Thomson, 1878)

Figs 3C, D

MATERIAL EXAMINED. East-Azarbaijan province: Ajabshir, 18.X 2009, 3 $\stackrel{\circ}{\bigcirc}$, 2 $\stackrel{\circ}{\bigcirc}$ (A. Pourhaji leg.); Marand, 12.VI 2010, 9 $\stackrel{\circ}{\bigcirc}$ (A. Pourhaji leg.); Shabstar, 1.X 2009, 1 $\stackrel{\circ}{\bigcirc}$ (A. Pourhaji leg.); Sarab, 1.IX 2008, 1 $\stackrel{\circ}{\bigcirc}$; 4.X 2009, 1 $\stackrel{\circ}{\bigcirc}$ (A. Pourhaji leg.).

NOTES. This species was reared mostly on Agromyziidae of alfalfa but some specimens were reared on *P. medicaginella* in Marand. According to Graham (1959) and Hansson (1985) this species has following characters: the thorax green to blue or golden; legs white, femora faintly brownish proximally; occiput slightly margined medially; funicle 3-segmented, clava 2-segmented; postminarginal vein $2\times$ stigmal vein, costal cell with row of hairs in lower surface; petiole with 1-2 lateral hairs, petiole wider than long (about $2\times$ as long as broad) and as long as propodeum + scutellum; hind part of mesoscutm with a weak median groove, dorsellum anteriorly on the sides with two quite large fovea and with median part impressed; anteromedian part of propodeum with a raised anchor- or Y-shaped structure.

Chrysocharis submutica Graham, 1963

Fig. 3A

MATERIAL EXAMINED. East-Azarbaijan province: Ajabshir, VIII 2010, 5 \bigcirc , 2 \bigcirc ; 8.VI 2010, 2 \bigcirc (A. Pourhaji leg.); Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, VII 2010, 5 \bigcirc , 5 \bigcirc ; V 2010, 9 \bigcirc , 1 \bigcirc (A. Pourhaji leg.).

NOTES. *Chrysocharis submutica* was reared on *P. medicaginella* but it was reported on Agromyzidae (Dipera) and Elachistidae and Gracillariidae (Lepidoptera) (Noyes, 2013). This species was charactersied by following feature: Frons below fork usually purple reticulation with quite high and narrow septae and with small meshes, temples relatively large, entire vertex reticulated with very low and very narrow septae, head in dorsal view with relatively large compound eyes and relatively small vertex, POL (distance between hind pair of ocelli) about 2×OD (ocellar diameter), occipital margin completely rounded, pronotal carina weak or missing, hind margin of pronotum smooth and shiny, reticulation on thoracic dorsum usually weak especially on scutellum.

Cirrospilus talitzkii (Bouček, 1961)

Fig. 2A

MATERIAL EXAMINED. East-Azarbaijan province, Sarab, 4.X 2010, 1 \bigcirc (A. Pourhaji leg.).

NOTES. Cirrospilus can be distinguished from other Eulophidae by the presence of the following character states: funicle two segmented in both sexes; notauli complete and straight, reaching or nearly so the transscutal line posteriorly; scutellum with longitudinal submedian grooves, grooves sometimes indicated by change in sculpture only; posterior pair of scutellar setae close to the hind margin of the scutellum; submarginal vein with 3 or more setae dorsally, postmarginal vein present, subequal in length to stigmal vein, uncus at the apex of this latter vein (Lotfalizadeh & Delvare, 2006). Lotfalizadeh & Delvare (2006) reviewed this genus in Iran. We reared two species of Cirrospilus on alfalfa leafminers. Cirrospilus talitzkii was recently reported from Iran as parasitoid of Phyllonorycter pupulifoliella (Treitschke) (Lepidoptera: Gracillaridae) in West-Azarbaijan, Shahin-Dezh (Sadeghi et al., 2011). Its body length 1.9-2.3 mm; body mainly yellow, gasteral tergites (T1-T5) with one median longitudinal dark cross-line connected with a basal and apical cross-line, the latter more or less bent forward sublaterally and there connected with lateral dot, in lateral view seen as an oblique comma; the line often partly broken into dots, T1 usually lacks the basal cross-line, T2 mostly without the median line, T5-6 with a reduced pattern consisting of transverse row of three dots; ovipositor sheaths black; antenna very stout, yellow, pedicellus dorsally with a black spot and short black hairs which extend also dorsally on F1, F1 slightly and F2 hardly longer than wide; abdomen oval-acuminate, as long as head+ thorax or nearly so.

Cirrospilus vittatus Walker, 1838

Fig. 3C

MATERIAL EXAMINED. East-Azarbaijan province, Khosro-Shahr, 15.VI 2008, $1 \bigcirc$ (A. Pourhaji, leg.).

NOTES. This species was reared on Agromyziidae species while it has a wide host range and known as parasitoid of Coleoptera (Curculionidae), Diptera (Agromyziidae, Cecidomyiidae), Hemiptera (Aleyrodidae, Asterolecaniidae), Hymenoptera (Argidae, Braconidae, Cimbicidae, Diprionidae, Tenthredinidae) Lepidoptera (Bucculatricidae, Coleophoridae, Elachistidae, Eriocraniidae, Gelechiidae, Gracillariidae, Heliozelidae, Lyonetiidae, Momphidae, Nepticulidae, Tischeriidae, Yponomeutidae) (Noyes, 2013). Its body length 1.3 to 1.5 mm in the female and 1 to 1.2 mm in the male; body mainly yellow, with dark metallic strips on head and thorax; notauli complete and weakly curved, reaching scutellar margin; scutellum flat, with parallel submedian grooves that may be difficult to discern, dorsellum as long as propodeum medially; scape cylindrical in lateral view, pedicel longer than F1 segment; forewing without fuscous markings, cubital line strongly curved at base, postmarginal vein shorter than stigmal vein.

Diglyphus isaea (Walker, 1838)

Figs 4A, C

MATERIAL EXAMINED. East-Azarbaijan province: Ajabshir, 16.VIII 2010, 4° ; 31.VIII 2010, 3° , 1° (A. Pourhaji leg.); Shabstar, 1.X 2009, 18° , 1° ; 11.X

2009, 3 \bigcirc ; 25.VIII 2009, 2 \bigcirc (A. Pourhaji leg.); Sarab, 4.X 2009, 6 \bigcirc , 3 \bigcirc (A. Pourhaji leg.); Khosro-Shahr, 10.VI 2008, 1 \bigcirc ; 17.VI 2008, 1 \bigcirc ; 6.VII 2008, 2 \bigcirc ; 9.VII 2008, 9 \bigcirc , 3 \bigcirc (A. Pourhaji leg.).

NOTES. In the genus *Diglyphus* following morphological characters can be observed: body with metallic coloration; tarsi 4-segmented; funicle 2-segmented in both sexes; submarginal vein with 3 or more setae dorsally; postmarginal vein at least as long as stigmal vein; notauli incomplete (if complete, superficial posteriorly

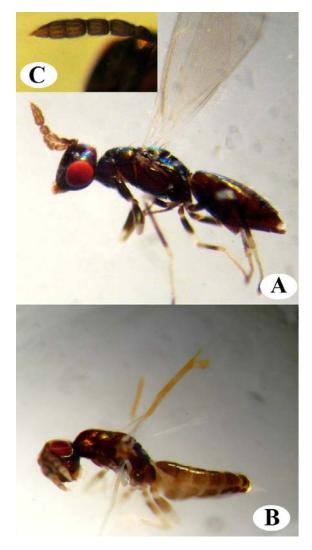


Fig. 4. A, C – *Diglyphus isaea*; A – female in lateral view, C – female antenna; B – D. *pachyneuron*, male in lateral view.



and curved); scutellum with lateral lines; propodeum without median carina or plicae; submarginal vein with more than 3 dorsal setae. This genus is most easily confused with *Cirrospilus*. However, *Cirrospilus* species have complete notauli which continue to the posterior margin of the mesoscutum and without metallic coloration.

Two species of the genus have been reared on alfalfa leafminers in Iran. *Diglyphus isaea* is an important parasitoid of agromyzid leafminers, which includes the economically important genus *Liriomyza*. We reared this species on dipterous alfalfa leafminers (*L. trifolii* and *C. horticola*) and lepidopterous leafminer (*P. medicaginella*). *Diglyphus isaea* is recorded from nine species of *Liriomyza* (Noyes, 2013) and commercialized to control of these leafminers. It is common in Iran and has been reported on *L. sativae*. This species was reported as an important biocontrol agent of *L. trifolii* and *C. horticola* on Bean, Tomato and Squash in Turkey (Gençer, 2004).

Body length of D. *isaea* 1.5 to 1.7 mm in the female and 1.3 to 1.5 mm in the male; antenna dark brown with scape metallic; all coxae, trochanter, basal 3/4 of femora, tibiae (except basally and apically), last tarsomer dark with metallic shine; scutellum with violet shine; scape cylindrical in lateral view, funicle 2-segmented, clava 3-segmented; pronotum triangle shaped in dorsal view, mesoscutum with incomplete notauli, scutellum with parallel submedian grooves; postmarginal vein as long as stigmal vein, cubital vein strongly curved at base, speculum rather narrow, sometime disappeared.

Diglyphus pachyneurus Graham, 1963 Fig. 4B

MATERIAL EXAMINED. East-Azarbaijan province, Sarab, 4.X 2008, 2 \bigcirc (A. Pourhaji leg.); Khosro-Shahr, 30.V 2008, 1 \Diamond ; 9.VII 2008, 1 \Diamond (A. Pourhaji leg.).

NOTES. This is first report of *D. pachyneurus* from Iran as parasitoid of alfalfa leafminer (Agromyzidae). Gençer (2009) reported *D. pachyneurus* on *C. horticola* from Turkey. Its body length 1.5-1.8 mm; body green, antenna brown with basal half of scape yellow, scape, pedicle legs with metallic ting, all tibiae and tarsi (except black 4th) white with a dark subbasal ring on the hind tibiae; short flagellum which slightly clavate, relatively short funcular segments; scutellum with superficial reticulations, notauli incomplete; the postmarginal vein short to equal to the stigmal vein, male with wing veins thickened, cubital vein straight at base, speculum large and closed on lower side.

Hemiptarsenus waterhousii Westwood, 1833 Figs 5B–D

MATERIAL EXAMINED. East-Azarbaijan province: Ajabshir, VIII 2010, 2; 19.X 2010, 13 (A. Pourhaji leg.); Shabestar, 8.IX 2009, 2 9 (A. Pourhaji leg.); Khosro-Shahr, 6.IX 2010, 2 9; 25.VI 2008, 1 9 (A. Pourhaji leg.).

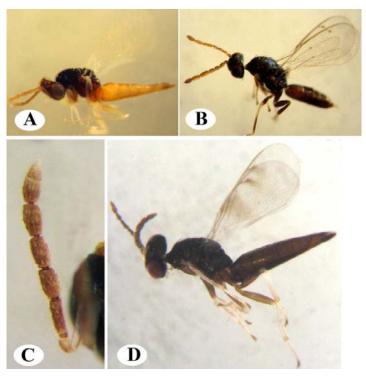


Fig. 5. A – *Symplesis xanthostoma*, female in lateral view; B-D – *Hemiptarsenus waterhousii*: B – male in lateral view, C – female antenna, D – female in lateral view.

NOTES. Diagnostic characters of the genus *Hemiptarsenus* is as follow: antennae inserted near middle of face and toruli located well above lower margin of eyes, scape well exceeding front ocellus and reaching well beyond vertex, female funicle 4-segmented, in male with 3 long branches; pronotum about as long as broad or transverse, costal cell of forewing extremely narrow (at least 10 times as long as wide). We reared two species of *Hemiptarsenus* from *P. medicaginella* that identified as *Hemiptarsenus waterhousii* Westwood and an unknown species. Undetermined species was included some male specimens, therefore the identification was not possible. This species was reared on *Phyllonorycter medicaginella* and has been reported on Diptera (Agromyzidae), Lepidoptera (Bucculatricidae, Gracillariidae, Momphidae, Nepticulidae) (Noyes, 2013).

Hemiptarsenus waterhousii is characterized by body completely dark, all coxae at least partly dark, hind tibiae at least partly dark; fore and mid femora completely non-metallic dark; scape completely yellow; clava concolorous with funicles; mid lobe of mesoscutum violet; scutellum green; forewing with at least some dark markings; cubital vein bare/open below basal cell; reticulations on vertex raised; frontal groove absent; toruli well above lower eye margin; antennal scrobe sculptured; funicle broadened toward clava; clava distinctly clavate; scutellum reticulate; reticulations on



mid lobe of mesoscutum elongate, at least 1.5 times as long as wide; setae on scutellum with anterior pair as strong as or as long as posterior ones; reticulations on propodeum raised, but much weaker than those on scutellum.

Neochrysocharis formosus (Westwood, 1833)

Figs 2D, E

MATERIAL EXAMINED. East-Azarbaijan province: Ajabshir, VI 2010, 5 \bigcirc ; VI 2010, 1 \bigcirc (A. Pourhaji leg.); Sarab, 4.X 2009, 5 \bigcirc , 1 \bigcirc (A. Pourhaji leg.); Shabestar, 8.IX 2009, 1 \bigcirc (A. Pourhaji leg.); Khosro-Shahr, 19.VIII 2008, 2 \bigcirc ; 2.VI 2008, 1 \bigcirc , 1 \bigcirc ; 6.IX 2010, 1 \bigcirc (A. Pourhaji leg.).

NOTES. The genus Neochrysocharis Kurdjumov has following characters: antennal flagellumers strongly compressed, fusiform, all funiclar segments transverse; tarsi 4-segmented; submarginal vein with 2 setae, postmarginal vein at most as long as stigmal vein; forewing with a single row of setae extending from stigmal, and often with transverse dark bands; scutellum with one pair of setae; propodeum without median carina or plica; transepimeral suture curved. This genus includes 49 valid species (Noyes, 2013) while only three species have recorded from Iran: Neochrysocharis aratus (Walker), N. formosus Westwood and N. longiventris (Askew) (Talebi et al., 2011). We reared this species on agromyzid leafminers of alfalfa and Gençer (2004) reported this species as parasitoid of L. trifolii on Bean and Tomato. Different hosts from several insect orders have been listed for N. formosus: Coleoptera (Chrysomelidae, Curculionidae), Diptera (Agromyzidae, Cecidomyiidae, Drosophilidae, Tephritidae), Hemiptera (Aleyrodidae, Aphididae), Hymenoptera (Cimbicidae, Diprionidae, Pamphiliidae, Tenthredinidae) and Lepidoptera (Bucculatricidae, Coleophoridae, Elachistidae, Gelechiidae, Gracillariidae, Heliozelidae, Lasiocampidae, Lyonetiidae, Momphidae, Nepticulidae, Pyralidae, Yponomeutidae) (Noves, 2013).

Sympiesis xanthostoma (Nees, 1834) Fig. 5A

MATERIAL EXAMINED. East-Azarbaijan province, Khosro-Shahr, 29.V 2008, 1 \bigcirc ; 25.VI 2008, 6 \bigcirc , 2 \bigcirc ; VII 2008, 1 \bigcirc ; 29.VII 2008, 1 \bigcirc ; 6.IX 2010, 1 \bigcirc , 1 \bigcirc , 18.IX 2008, 1 \bigcirc ; Khosro-Shahr, 7.IX 2008, 2 \bigcirc (A. Pourhaji leg.).

NOTES. The genus *Sympiesis* has following characters: tarsi 4-segmented; funicle 4-segmented in both sexes; submarginal vein with 3 or more setae dorsally; postmarginal vein present, longer than stigmal vein; notauli incomplete; scutellum sculptured, not shiny, without lateral lines; propodeum with or without median carina but without plica or costula. We reared some male specimens of another unknown species of *Sympiesis* that its morphological characters are not fitted to available keys. *Sympiesis xanthostoma* has been reported on Lepidoptera (Gracillariidae, Tortricidae) (Noyes, 2013) but for first time reported on *Phyllonorycter medicaginella*.

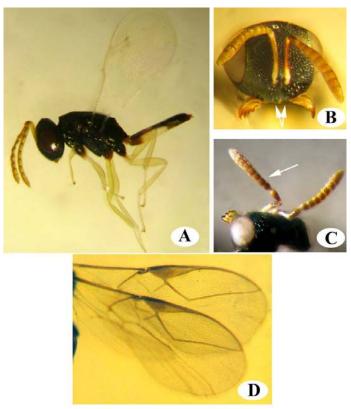


Fig. 6. A – *Pteromalus* sp., male in lateral view; B-C – *Sphegigaster ineus*: head and antennae in frontal view, C – head an antennae in dorsal view. D – fore wings of Braconidae.

It has completely dark body, scape dark dorsally along all of segments and $2.5 \times$ as long as pedicel; metasoma completely dark brown with metallic shine; upper face metallic green; hind coxae completely dark brown, metallic green, or mostly yellow; reticulations on face engraved; antennal scrobe smooth; mesosoma completely dark; femura and tibia whitish; reticulations on hind coxae raised reticulations on vertex raised; frontal groove angulated; V-shaped; notauli incomplete; scutellum green; setae on mid lobe of mesoscutum and scutellum yellow; costal cell with 1 line of setae on lower surface; mid lobe of mesoscutum with paired setae only; median carina of propodeum fine; basal cell of forewing closed below, abdomen longer than head + thorax, more than $2 \times$ as long as wide.

Family Pteromalidae

The family Pteromalidae with 618 genera is one of the largest families among parasitic Hymenoptera of the superfamily Chalcidoidea. It includes important natural enemies of many harmful insects widely distributed in major insect orders such as Coleoptera, Diptera, Lepidoptera, Hymenoptera and Homoptera. The genus *Pteromalus* includes 502 species that have not a reliable identification key.

Pteromalus sp.

Fig. 6A

MATERIAL EXAMINED. East-Azarbaijan province: Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, 25.VI 2008, 3 ♂, 6.IX 2010, 2 ♂ (A. Pourhaji leg.); Shabestar, 25.VIII 2009, 1♂. (A. Pourhaji leg.).

NOTES. Only male specimens were collected that did not let us to identify species of these specimens.

Sphegigaster ineus Mitroiu, 2008

Fig. 6B, C

MATERIAL EXAMINED. East-Azarbaijan province, Khosro-Shahr, 1346m, 37°58'28"N, 46°02'55"E, 25.VI 2008, 2 \bigcirc (A. Pourhaji leg.).

NOTES. With 52 species worldwide (Noyes, 2013) that only five species have been recorded from Iran. *Sphegigaster ineus* with having short antennae and broad funicular segments nearly allied to *S. stepicola* Boucek. Mitroiu (2008) discussed its differentiated characters with all of species that have broad funicular segments. *S. ineus* is known only from Romania without any biological report. Therefore, this the second record of *S. ineus* with it's biological association with *P. medicaginella*.

PARASITOIDS DIVERSITY

Diglyphus isaea had the highest frequency in all of the investigated areas (except for Khosro-Shahr) (Table 3). The species was not observed in Marand. The species of *Apanteles* sp., *Sphegigaster ineus*, *Sympiesis xanthostoma* and *Ci. vittatus* were only observed in Khosro-Shahr. The *Cirrospilus talitzkii* species was only seen in Sarab and the parasitoid species of *Ch. crassiscapus* was collected from all the investigated areas except for Khosro-Shahr. The presence or absence of parasitoid species in each area requires consistent samplings and examining their stability in each of the areas.

The maximum amount of Shannon diversity index of parasitoid wasps was 2.23 in Khosro-Shahr which is caused by the highness of the species richness rate related to the parasitoid wasps in the area (which is an indicator of the appropriateness of the environmental conditions for the parasitoids' activity in this area in comparison to other areas). The lowest rate of Shannon diversity index was 0.85 that belongs to Shabestar district. Although there were a lot of parasitoid wasp species in that area, the low frequency of the gathered species have led to the decrease of Shannon diversity index.

The minimum Simpson index of the parasitoid wasps was estimated to be 0.41 in Shabestar (Table 4). It demonstrates that it is 41% probable that two parasitoid wasps that were randomly chosen in Shabestar belong to two different species of parasitic wasps.

The most evenness index (0.93) was related to parasitoid wasps in Ajabshir district and this amount of estimated evenness indicates that the frequency of various species of collected wasps in the area is 93% similar (Table 4). The maximum species richness of parasitoid wasps belongs to Khosro-Shahr and the minimum richness was observed in Marand with three species. The highest amount of parasitoid wasps' frequency was also recorded in Khosro-Shahr due to the high species richness.

Parasitoids	Ajabshir	Khosro- Shahr	Marand	Sarab	Shabe- star
Apanteles sp.	0	6.5	0	0	0
Chorebus calthae Griffiths	2.9	4.3	0	9.1	6.5
Chrysocharis albicoxis Erdös	0	10.6	23	0	0
Ch. crassiscapus (Thomson)	14.7	0	53	9.1	3.2
Ch. submutica Graham	20.6	21.4	0	0	0
Cirrospilus talitzkii Bouček	0	0	0	4.6	0
Ci. vittatus Walker	0	1.1	0	0	0
Dacnusa heringi Griffiths	11.8	3.2	23	0	0
Diglyphus isaea (Walker)	23.5	17.2	0	40.1	77.4
D. pachyneuron Graham	0	2.2	0	9.1	0
Hemiptarsenus waterhousii Westwood	8.8	3.3	0	0	6.5
<i>Neochrysocharis formosus</i> (Westwood)	17.7	5.4	0	27	3.2
Pteromalus sp.	0	5.4	0	0	3.2
Sphegigaster ineus Mitroiu	0	2.2	0	0	0
Sympiesis xanthostoma (Nees)	0	17.2	0	0	0

Table 3. Abundance of hymenopterous parasitoids of alfalfa agromyzid leafminers in different localities of East-Azarbaijan province during 2008-2010.

DISCUSSION

Based on literatures, *P. medicaginella* is just parasitized by one eulophid species (Noyes, 2013), therefore, its association with *Ch. submutica*, *H. waterhousii*, *Sphegigaster ineus* and *Sympiesis xanthostoma* are new.

Most of the leafminer parasitoid species correspond taxonomically to the superfamilies Chalcidoidea (family Eulophidae), Ichneumonoidea (family Braconidae). Askew & Shaw (1986) believe these parasitoids have been classified as idiobionts (permanently paralyze their host when ovipositing) or koinobionts (only temporarily paralyzes the host that continue its development). Most of them belong to the family Eulophidae (93%) including two subfamilies (Eulophinae and Entedoninae). While, Csóka (2003) mentioned Eulophidae as the most species rich family of chalcids with endoparasitoids (70 %) and ectoparasitoids species (30%) that there are either polyphagous or oligophagous.

In this study we didn't rear the genus *Pnigalio*, while it was reported on leafminers of alfalfa in Iran. Amongst reared parasitoid species *D. isaea* was the most common species. Within the reared parasitic wasps, *Ch. albicoxis*, *Ch. crassiscapus*, *D. pachyneuron* are new for Iranian fauna. Biological association of all species except *D. isaea* and *C. vittatus* with alfalfa leafminers (*L. trifolii* and *C. horticola*) is new.

		Heterogeneity	1	Number of	Total
Sites	Shannon's H' index	Simpson's index	Shannon's E index	species	abundance
Ajabshir	1.82	0.85	0.93	7	34
Khosro-Shahr	2.23	0.88	0.87	13	93
Marand	1.01	0.65	0.92	3	17
Sarab	1.51	0.77	0.85	6	22
Shabestar	0.85	0.41	0.49	6	6

Table 4. Species diversity indexes of hymenopterous parasitoids of alfalfa agromyzid leafminers in different localities of East-Azarbaijan province during 2008-2010.

Salvo & Valladares (2007) believe parasitoids of leafminers are predominantly generalists, and can thus rapidly include in their host ranges newly introduced leafminer species, frequently achieving effective regulation a few years after the pest becomes established.

In an ecosystem, the diversity and stability factors are interrelated (each of them is required for the existence of the other one) and have complementary effects. The truth is that a system must initially have a relative stability (balance) so that its diversity increases. Now, under such conditions and with relative stability, the increase of diversity will change the circumstances in a way that it will maintain stability. Therefore, the highness of species richness and indices, including Shannon index, in an area is primarily indicative of that area's stability.

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